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Research Article
Anti-obesity Effect of a Novel Potent Synthetic Steroidal Liver X Receptor α (LXRα)-selective Agonist in Male ob/ob C57BL/6 Mice

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Abstract

Background: Reduced body weight and fat mass, especially subcutaneous white adipose tissue, were observed in liver X receptor β knockout (LXRβ$^{-/-}$) compared with Wild Type (WT) mice. The similar results were also observed in liver X receptor α and β double knockout (LXRαβ$^{-/-}$) mice. However, liver X receptor α knockout (LXRα$^{-/-}$) mice had normal body weight and fat mass. Conversely, brown adipose tissue, an energy expenditure-related thermogenic tissue, was reduced only in LXRα$^{-/-}$ mice compared with WT mice. Furthermore, specific knockout of LXRα in mouse adipose tissue gained more weight and fat mass on a high-fat diet compared with WT controls. **Objective:** This study was aimed to explore the role of LXRα activator on its anti-obesity effect. **Materials and Methods:** The ATI-111, a novel synthetic LXRα-selective agonist, was orally administrated to male ob/ob mice once a day for 6 weeks. Animal body weight, dietary intake and blood glucose were measured during the treatment period. Measured values were presented as Mean±Standard Error of the Mean (SEM). Differences between means were analyzed for statistical significance ($p<0.05$) by using unpaired student t-test. **Results:** A significant weight loss was observed in ATI-111-treated mice compared with vehicle-treated mice after 4 weeks treatment. In the meanwhile, blood glucose levels were remarkably declined in ATI-111-treated mice compared with vehicle-treated mice after 6 weeks treatment. The average daily dietary intake by each mouse, measured every 2 weeks, was tremendously lower in ATI-111-treated mice compared with vehicle-treated mice after 6 weeks treatment. **Conclusion:** This study indicates that the activation of LXRα by ATI-111 may regulate mouse appetite by inhibiting hunger via a leptin-independent mechanism. It also suggests the potential pharmaceutical development to target LXRα for the treatment of obesity and type 2 diabetes.

Key words: Obesity, LXRα, agonist, appetite, diabetes, energy, glucose, pharmaceutical

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Obesity is becoming the major worldwide public health issue associated with a number of diseases, such as cardiovascular diseases, type 2 diabetes and certain type of cancers[1,2]. It is attributable to the chronic imbalance between energy intake and Energy Expenditure (EE) and there is no effective therapy so far. To identify the target that can either decrease energy intake or increase EE is always the major challenge in battling this epidemic[3,4].

In mammals, the adipose tissue is at least divided into White Adipose Tissue (WAT) and Brown Adipose Tissue (BAT) with different morphology, distribution, gene expression and function. The WAT is the main energy reservoir and secretes a huge number of hormones and cytokines to regulate metabolism and insulin sensitivity[3]. The BAT is specialized in EE through nonshivering thermogenesis via mitochondrial uncoupling protein 1 (UCP-1)[5]. It has been recognized that LXRs regulate energy metabolism as well as glucose and fat metabolism[6-10]. Recent studies from Gustafsson's group showed the reduced body weight, fat mass and subcutaneous white adipose tissue (SAT), whereas, increased lean mass in LXRβ$^{-/-}$ and LXRαβ$^{-/-}$ but not LXRα$^{-/-}$ mice compared with Wild Type (WT) mice[11]. In the meanwhile, significantly reduced ($p<0.05$) BAT weight was only observed in LXRα$^{-/-}$ mice compared with WT mice[11]. The LXRβ preferential activator GW3965, a weaker LXRα activator, lowered EE and UCP-1 expression level in the BAT of WT mice. Surprisingly, GW3965 treatment increased triglyceride (TG) content in the BAT of WT and LXRα$^{-/-}$ but not LXRβ$^{-/-}$ mice[5]. The studies from another group also found that LXRα deficiency in mouse adipose tissue gained more weight and fat mass on high-fat diet compared with WT controls[12]. Those results indicate that LXRα and LXRβ may have different effects on the regulation of EE metabolism as well as glucose and fat metabolism and activation of LXRα may exhibit more benefits against obesity.

The LXRβ is expressed ubiquitously throughout many organs and tissues, whereas, LXRα is concentrated in metabolically active tissues, such as liver, intestine and macrophages, as well as in adipose tissue[13,14]. However, activation of LXRs, especially LXRα, can cause hepatic steatosis and hypertriglyceridemia by triggering the expression of genes related to liver de novo lipogenesis, such as sterol regulatory element binding protein 1c (SREBP-1c), acetyl-coenzyme A carboxylase (ACC) and Fatty Acid Synthase (FAS)[10,15]. Those side effects shadow the LXRα as a target for the pharmaceutical development. A potent LXRα-selective agonist ATI-111, which inhibited the conversion of SREBP-1c precursor form to its active form and in turn avoided altering the levels of hepatic lipids and liver-secreted nascent lipoproteins, has been recently developed[16]. Because LXRα activation-induced hepatic steatosis and hypertriglyceridemia might potentially affect body mass buildup, LXRβ-selective compounds such as GW3965 have usually been used to address their pharmaceutical development against obesity[5]. Here, side effects less or free LXRα-selective agonist ATI-111 has firstly been used in male ob/ob mice (Obesity animal model) to explore the role of activating LXRα signaling pathway on body mass buildup and its potential pharmacological effect against obesity.

MATERIALS AND METHODS

All of experimental chemicals were purchased from Sigma-Aldrich or synthesized at the University of Chicago between February, 2009 and March, 2010. Animal study design, performance and data collection were carried out at the University of Chicago between June, 2010 and April, 2011. Data analysis was carried out at JiShuiTan Hospital, Beijing between November, 2016 and January, 2017.

Chemicals: The ATI-111, 3α, 6α, 24-trihydroxy-22-en-24, 24-di(trifluoromethyl)-5b-cholane, was synthesized from 3α,6α-dihydroxy-23,24-bisnor-cholanic acid. Its structure and purity (>99%) were confirmed by ^{1}H, ^{13}C nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry. Details on the synthesis of ATI-111 were described by Peng *et al.*[16] in its Fig. 1 (Reference 16 can be found and free downloaded from PubMed). All of chemicals used for ATI-111 synthesis were commercial grade or above and purchased from Sigma-Aldrich (Chemicals company).

Experimental animals: All work with animals followed National Institutes of Health guidelines for care and use of animals in experimentation. Animal work was reviewed and approved by the University of Chicago Institutional Animal Care and Use Committee. Eight weeks old male ob/ob mice on a C57BL/6 background were obtained from Jackson Laboratory (Bar Harbor, ME, USA) and housed at pathogen-free facility at the University of Chicago. Mice in each treatment group (5 mice per group) were individually housed in cages (one mouse in one cage) and fed a normal chow diet (Harlan TEKLAD, Madison, WI, USA). Mice were treated by gavage daily with either vehicle (20% microemulsion-Coconut oil: Cremophor EL: Transcutol: Water = 10:5:4:81 in volume) or with the LXR agonist ATI-111 dissolved in a 20% microemulsion at dose of

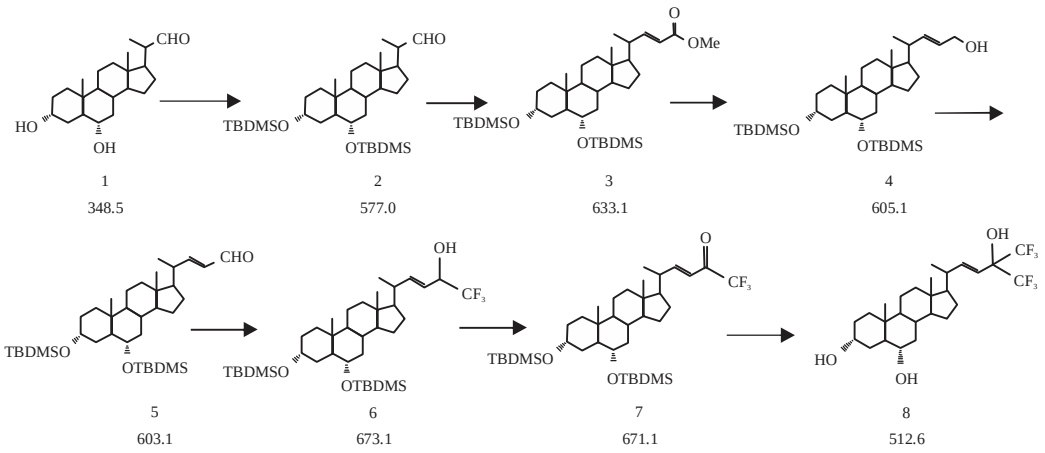

Fig. 1: Procedure of synthesis of 3α, 6α-24-trihydroxy-22-en-24-di-trifluoromethyl-5β-cholane (ATI-111) (8)

1: Synthesis of 3α, 6α-di-t-butyldimethylsiloxy-23,24-dinor-5β-cholan-22-al (2): Compound 3α, 6α-dihydroxy-23, 24-dinor-5β-cholan-22-al (1) was synthesized by following Shen's procedure (Shen, Z., Zhou, W. Science in China, Series B: Chemistry, Life Sciences and Earth Sciences, 1992, 35 (10), 1181-6). To a solution of 1 (4.44 g, 12.7 mmol) in pyridine (100 mL) was added TBDMS-Cl (4.21 g, 2.2 equiv) and the mixture was stirred at room temperature for 24 h. Then methanol (1 mL) was added to quench the reaction. After 15 min, the solvents were removed under reduced pressure and the residue was co-evaporated with toluene (2×50 mL). The resulting residue was dissolved in ethyl ether (300 mL) and washed with water (150 mL), saturated $NaHCO_3$ (150 mL) and brine (150 mL), respectively. After drying over $MgSO_4$ and filtration, ethyl ether was removed under reduced pressure. The residue was purified by silica gel chromatography to give 2 as white foam (6.25 g, 85%). ^{1}H NMR (500 MHz) (CD_3Cl) d: 0.01 (s, 6H), 0.06 (s, 6H), 0.72 (s, 3H), 0.98 (s, 18H), 1.01-2.45 (m, 28H), 3.54 (m, 1H), 3.98 (m, 1H), 9.57 (d, J = 3.0 Hz, 1H).

2: Synthesis of methyl (22E)-3α, 6α-di-t-butyldimethylsiloxy-5β-chol-22-en-24-oate (3): To dry 2,2-dimethoxypropane (100 mL) was added sodium hydride (95%, 300 mg). After stirring for 15 min under argon, methyl diethylphosphonate (2.49 g, 11.9 mmol) was added and the mixture was stirred at room temperature for 1 h. Then 23,24-dinor-22 aldehyde (2, 6.24 g, 10.8 mmol) dissolved in THF and 2,2-dimethoxypropane (200 mL, 1:1 v/v) was added dropwise and the reaction solution was stirred at room temperature overnight. Saturated ammonium chloride (300 mL) was then added to neutralize the reaction followed by adding water and ethyl acetate. After extraction, the organic phase was washed with water, 5% $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. After removing ethyl acetate under reduced pressure, the resulting residue was purified by silica gel chromatography to give product 3 (6.17 g, 90%). ^{1}H NMR (400 MHz) (CD_3Cl) d: 0.05 (s, 6H), 0.07 (s, 6H), 0.69 (s, 3H), 0.90 (s, 18H), 1.01-2.26 (m, 28H), 3.54 (m, 1H), 3.74 (s, 3H), 4.00 (m, 1H), 5.75 (d, J = 15.6 Hz, 1H), 6.85 (dd, J = 15.6 and 9.0 Hz, 1H). ^{13}C NMR (400 MHz) (CD_3Cl) d: 167.9, 155.4, 119.0, 73.3, 69.0, 56.3, 55.4, 49.9, 43.5, 40.2, 40.1, 40.0, 36.4, 35.8, 35.3, 31.4, 30.2, 28.5, 26.4, 26.3, 24.6, 23.9, 21.1, 19.6, 18.8, 18.5, 12.7, -4.2, -4.4.

3: Synthesis of (22E)-3α, 6α-di-t-butyldimethylsiloxy-5β-chol-22-en-24-ol (4): To the solution of 3 (6.16 g, 9.73 mmol) in benzene (200 mL) at -78°C under argon was added diisobutylaluminum hydride (1 M in THF, 38.92 mL) dropwise. After stirring at -78°C for 3 h and then room temperature for 2 h, HCl (5%) was added to quench the reaction. Ethyl acetate (300 mL) and water (200 mL) were added to extract the product. Organic phase was then washed with water, 5% $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. After removing ethyl acetate under reduced pressure, the resulting residue was purified by silica gel chromatography to give product 4 (4.7 g, 80%). ^{1}H NMR (400 MHz) (CD_3Cl) d: 0.05 (s, 6H), 0.08 (s, 6H), 0.66 (s, 3H), 0.89 (s, 9H), 0.91 (s, 9H), 1.01-2.09 (m, 28H), 3.55 (m, 1H), 3.98 (m, 1H), 4.09 (d, J = 4.4 Hz, 2H), 5.56 (m, 2H). ^{13}C NMR (400 MHz) (CD_3Cl) d: 139.7, 126.3, 73.0, 68.6, 64.0, 56.2, 55.7, 49.6, 42.8, 39.9, 39.7, 39.6, 36.0, 35.9, 35.4, 34.9, 31.0, 29.8, 28.4, 26.0, 25.9, 24.2, 23.5, 20.8, 20.2, 18.4, 18.1, -4.3, -4.5.

4: Synthesis of (22E)-3α, 6α-di-t-butyldimethylsiloxy-5β-chol-22-en-24-al (5): A solution of oxalyl chloride (2.7 mL, 32 mmol) in dichloromethane (100 mL) was cooled to -50°C and then dimethylsulfoxide (4.5 mL, 63 mmol) was added dropwise and the mixture was stirred at -50°C for 1 h. A solution of 4 (4.7 g, 7.76 mmol) in dichloromethane (70 mL) was added dropwise and the reaction mixture was stirred at -50°C for 30 min. Triethylamine (11 mL, 79 mmol) mL) was added and the resulting mixture was stirred at 0-25°C for 1 h. Then saturated ammonium chloride (20 mL) was added. After stirring for 15 min, dichloromethane (200 mL) was added to extract the product. The organic phase was washed with $NaHCO_3$ and brine and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The resulting residue was subjected to silica gel chromatography to give product 5 (3.32 g, 71%). ^{1}H NMR (500 MHz) (CD_3Cl) d: 0.06 (s, 6H), 0.07 (s, 6H), 0.70 (s, 3H), 0.88 (s, 9H), 0.89 (s, 9H), 1.01-2.45 (m, 28H), 3.54 (m, 1H), 3.96 (m, 1H), 6.05 (dd, J = 15.5, 8.0 Hz, 1H), 6.72 (dd, J = 15.5 and 9.0 Hz, 1H). ^{13}C NMR (400 MHz) (CD_3Cl) d: 194.6, 164.2, 130.8, 72.9, 68.5, 55.9, 54.9, 49.5, 43.3, 40.3, 39.8, 39.6, 36.0, 35.9, 35.4, 34.9, 31.0, 29.8, 28.1, 26.0, 25.9, 24.3, 23.5, 20.7, -4.4, -4.6.

5: Synthesis of (22E)-3α, 6α-di-t-butyldimethylsiloxy-5β-chol-22-en-24-trifluoromethyl-ol (6): To a solution of 5 (3.32 g, 5.5 mmol) in THF (20 mL) was added a catalytic amount of CsF followed by trimethyl(trifluoromethyl)silane. The mixture was stirred overnight at room temperature and then the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (150 mL), washed with 5% $NaHCO_3$ and brine and dried over $MgSO_4$. After the solvent was removed under reduced pressure, the resulting residue was subjected to silica gel chromatography to give product 6 (3.26 g, 88 %). ^{1}H NMR (500 MHz) (CD_3Cl) d: 0.03 (s, 6H), 0.08 (s, 6H), 0.67 (s, 3H), 0.88 (s, 9H), 0.92 (s, 9H), 1.01-2.17 (m, 28H), 3.55 (m, 1H), 4.00 (m, 1H), 4.39 (m, 1H), 5.55 (m, 1H), 5.85 (m, 1H). ^{13}C NMR (400 MHz) (CD_3Cl) d: 145.1, 144.6, 119.5, 72.9, 68.5, 56.0, 55.3, 49.4, 42.8, 39.9, 39.8, 39.7, 39.5, 35.9, 35.8, 35.3, 34.8, 30.9, 29.7, 25.9, 25.8, 24.1, 23.4, 20.6, 19.7, 18.4, 18.0, 12.1, -4.7, -4.9.

6: Synthesis of (22E)-3α, 6α-di-*t*-butyldimethylsiloxy-5β-chol-22-en-24-trifluoromethyl-one (7): To a solution of 6 (3.26 g, 4.84 mmol) in dichloromethane (150 mL) was added Dess-Martin reagent (1.2 equiv) under argon. The reaction mixture was stirred overnight at room temperature and then ethyl ether (100 mL) was added. After filtration, the filtrate was washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. After the solvent was removed under reduced pressure, the resulting residue was purified by silica gel chromatography to give product 7 (2.44 g, 75%). ^{1}H NMR (500 MHz) (CD_3Cl) d: 0.02 (s, 6H), 0.06 (s, 6H), 0.68 (s, 3H), 0.88 (s, 18H), 1.01-2.39 (m, 28H), 3.52 (m, 1H), 3.98 (m, 1H), 6.34 (d, J = 16 Hz, 1H), 7.19 (dd, J = 16 and 9 Hz, 1H). ^{13}C NMR (500 MHz) (CD_3Cl) d: 180.2, 161.6, 119.1, 72.8, 68.4, 55.7, 54.7, 49.4, 43.3, 40.7, 39.7, 39.5, 35.9, 35.8, 35.3, 34.8, 30.9, 29.7, 27.8, 25.9, 25.8, 24.2, 23.4, 20.6, 18.6, 18.3, 18.0, 12.2, -4.8, -4.9.

7: Synthesis of 3α, 6α-24-trihydroxy-22-en-24-di-trifluoromethyl-5β-cholane (8): To a solution of 7 (2.43 g, 3.62 mmol) in glycol dimethyl ether was added catalytic amount of CsF. After stirring at room temperature for 15 min, trimethyl(trifluoromethyl)silane (716 mL, 1.2 equiv) was added and the reaction mixture was stirred overnight at room temperature . After removing the solvent, the residue as dissolved in ethyl acetate (300 mL) and washed with water, saturated sodium bicarbonate and brine and dried over $MgSO_4$. After the removal of the solvent, the residue was dissolved in ethanol (100 mL) and HCl (1 mL, 37%) was added dropwise. The mixture was stirred at room temperature for 1 h and then all solvents were removed at reduced pressure to give a residue, which was purified by silica gel chromatography to give 8 (1.01 g, 54.5%) as white solid. ^{1}H NMR (CD_3OD): 0.79 (s, 3H), 0.98 (s, 3H), 1.14-2.41 (m, 25H); 3.44 (br. 1H), 3.54 (m, 1H), 3.64 (br., 1H), 4.07 (m, 1H), 5.68 (d, J = 15.5 Hz, 1H), 6.29 (dd, J = 15.5 and 9.5 Hz, 1H). ^{13}C NMR: 145.3, 116.0, 70.8, 66.7, 56.1, 55.2, 48.7, 42.7, 39.9, 39.8, 39.7, 35.7, 35.6, 34.9, 34.7, 30.4, 27.8, 23.9, 23.1, 20.6, 19.2, 11.6.

10 mg kg^{-1} for 6 weeks[16]. In detail, ATI-111 was dissolved into 20% microemulsion with the concentration as 5 mg mL^{-1}. Mouse body weight was measured daily before the treatment. A mouse with 50 g in body weight was gavaged either by 100 µL vehicle in control group or 100 µL ATI-111-containing 20% microemulsion in compound-treated group to reach the dose of 10 mg kg^{-1} (10 mg compound for 1 kg b.wt.). Therefore, the volume of vehicle or 5 mg mL^{-1} ATI-111-containing 20% microemulsion used in both control and compound-treated group was calculated by 2 µL g^{-1} (2 µL vehicle or 5 mg mL^{-1} ATI-111-containing 20% microemulsion for 1 g b.wt.). Actually, it was very difficult to accurately gavage mouse with 100 µL volume. The vehicle and 5 mg mL^{-1} compound-containing 20% microemulsion were performed the 1:2 dilution with water before gavage. Therefore, the treatment volume was calculated by 6 µL g^{-1} (6 µL for 1 g b.wt. and 300 µL for 50 g b.wt.) in diluted microemulsion. All of chemicals used for animal study were analytical grade or above and purchased from Sigma-Aldrich.

Body weight, dietary intake and blood glucose analysis: Mouse body weight measured at week 0, 2, 4 and 6 was used in analytic study. Before the animal study, a special request to feed the mice only by the researchers was filed to animal facility. During the animal study period, animal dietary administration was performed only by the researchers to exclude this part as the routine work from the facility staffs. This special request allowed the researchers to accurately monitor the animal dietary intake. Animal diet in each cage was weighted at week 0, 2, 4 and 6. The differences between the consecutive measure points were divided by 14 and the results were the average daily dietary intake for this mouse between the consecutive time points. Mouse glucose concentration in blood obtained from a tail vein was measured using AlphaTRAK strips (Abbott Diagnostics, Lake Forest, IL, USA) at week 0, 2, 4, 6. In detail, the end of mouse tail was cut with sterilized surgery scissors by 0.5-1 mm and squeezed out a drop of blood to the strip. Then the strip was immediately inserted into the glucose meter for the measurement.

Statistical analysis: Values were presented as Mean±Standard Error of the Mean (SEM) for five animals of each group. Differences between means were analyzed for statistical significance by using unpaired student t-test. A statistically significant difference was set at $p<0.05$[16].

RESULTS

Effect on body weight: Eight weeks old male ob/ob mice were divided into two groups with the average body weight 51.06±0.34 g in control group vs. 51.14±0.91 g in compound treatment group. In comparison with vehicle-treated mice, ATI-111 treatment affected male ob/ob mice body weight. After 2 weeks treatment, the body weight in ATI-111-treated mice didn't go up and kept flat (Fig. 2). The average body weight was 53.70±0.57 g after 2 weeks compound treatment, 53.62±0.70 g after 4 weeks compound treatment and 53.80±0.67 g after 6 weeks compound treatment. In the meanwhile, the average body weight was 54.84±0.47 g after 2 weeks vehicle treatment, 56.60±0.51 g after 4 weeks vehicle treatment and 58.78±0.66 g after 6 weeks vehicle treatment. The average body weight was 5.3% less in 4 weeks ATI-111-treated mice compared with 4 weeks vehicle-treated mice. The average body weight was 8.5% less in 6 weeks ATI-111-treated mice compared with 6 weeks vehicle-treated mice. The differences of body weight between vehicle-treated and compound-treated mice were statistically significant ($p<0.05$) after 4 weeks treatment (Fig. 2).

Effect on blood glucose level: Before the treatment, the average blood glucose level of mice was 156.20±15.09 mg dL^{-1} in control group vs. 172.40±33.14 mg dL^{-1} in compound treatment group. Blood glucose levels were constantly dropped down in ATI-111-treated mice compared with stable levels of blood glucose in vehicle-treated mice during the 6 weeks treatment (Fig. 3). After 2 weeks treatment, the average blood glucose level of mice was 143.60±19.26 mg dL^{-1} in vehicle treatment group vs. 132.40±21.58 mg dL^{-1} in compound treatment

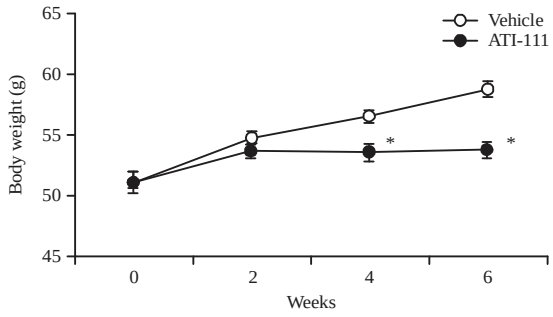

Fig. 2: Effect of ATI-111 on the body weight of ob/ob mice
Values are presented as Mean±Standard Error of the Mean (SEM). Significant differences between vehicle and ATI-111 treatment groups are indicated as *$p<0.05$, n = 5

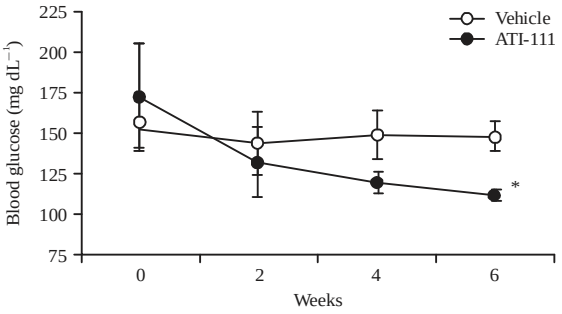

Fig. 3: Effect of ATI-111 on blood glucose levels of ob/ob mice
Values are presented as Mean±Standard Error of the Mean (SEM). Significant differences between vehicle and ATI-111 treatment groups are indicated as *p<0.05, n = 5

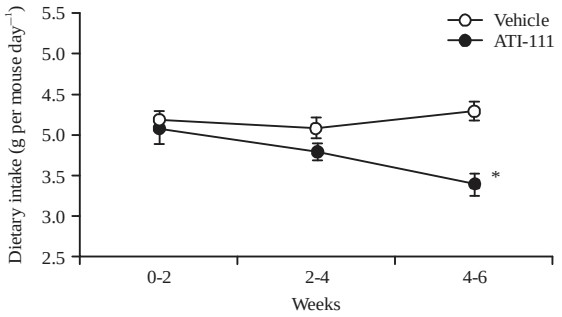

Fig. 4: Effect of ATI-111 on the food intake of ob/ob mice
Values are presented as Mean±Standard Error of the Mean (SEM). Significant differences between vehicle and ATI-111 treatment groups are indicated as *p<0.05, n = 5

group. After 4 weeks treatment, the average blood glucose level of mice was 148.80±14.93 mg dL^{-1} in vehicle treatment group vs. 119.20±6.34 mg dL^{-1} in compound treatment group. The average blood glucose level was 19.9% lower in compound treatment group compared with vehicle treatment group. However, the differences of blood glucose levels between vehicle-treated and compound-treated mice were not statistically significant (p>0.05) (Fig. 3). After 6 weeks treatment, the average blood glucose level of mice was 147.40±9.12 mg dL^{-1} in vehicle treatment group vs. 111.80±3.25 mg dL^{-1} in compound treatment group. The average blood glucose level was 24.2% lower in compound treatment group compared with vehicle treatment group. The differences of blood glucose levels between vehicle-treated and compound-treated mice became statistically significant (p<0.05) (Fig. 3).

Effect on dietary intake: Dietary intake by each mouse was measured every 2 weeks during the treatment period. During the first 2 weeks treatment (weeks 0-2), the average dietary intake was 4.20±0.10 g $mouse^{-1}$ day^{-1} in vehicle treatment group vs. 4.10±0.20 g $mouse^{-1}$ day^{-1} in compound treatment group. During the second contiguous 2 weeks treatment (weeks 2-4), the average dietary intake was 4.10±0.13 g $mouse^{-1}$ day^{-1} in vehicle treatment group vs. 3.80±0.11 g $mouse^{-1}$ day^{-1} in compound treatment group. The average dietary intake was 7.3% less in compound-treated mice compared with vehicle-treated mice. However, the differences were not statistically significant (p>0.05) (Fig. 4). During the third contiguous 2 weeks treatment (weeks 4-6), the average dietary intake was 4.30±0.11 g per mouse day^{-1} in vehicle treatment group vs. 3.40±0.14 g per mouse day^{-1} in compound treatment group. The average dietary intake was 20.9% less in compound-treated mice compared with vehicle-treated mice. The differences were statistically significant (p<0.05) (Fig. 4).

DISCUSSION

In this study, up to 8.5% reduced body weight was found in LXRα-selective activator ATI-111-treated male ob/ob mice compared with vehicle-treated male ob/ob mice, which was similar to the LXRβ-selective activator GW3965-treated WT mice[5]. The ob/ob mice are typically obesity animals with the triple or even more body weight compared with WT mice at the same age. The treatment dose of ATI-111 was 10 mg kg^{-1} day^{-1} in this study vs. 50 mg kg^{-1} day^{-1} of GW3965 in WT mice study[5]. Therefore, the LXRα-selective activator ATI-111 was more efficient on its effect against obesity compared with the LXRβ-selective activator GW3965. In addition, LXRβ is expressed ubiquitously and LXRα is concentrated in metabolically active tissues[13,14]. Therefore, the activation of LXRα signaling pathway, especially using the side effects less or free compound ATI-111[16], might have more benefits on the pharmaceutical safety compared with the activation of LXRβ signaling pathway.

The ATI-111-treated male ob/ob mice had up to 20.9% less dietary intake compared with vehicle-treated male ob/ob mice, which was also similar to GW3965-treated WT mice[5]. Those results suggest that both LXRα and LXRβ signaling pathways affect animal energy intake. Previously, an increased leptin gene expression was found in the subcutaneous fat of $LXR\alpha^{-/-}$ mice[5]. In this study, ob/ob mice were leptin deficient mice. Therefore, a leptin independent way might be responsible for ATI-111-induced decline of dietary intake in ob/ob mice. Brain hypothalamus area, which integrates peripheral signals such as insulin and leptin, has been found to modulate appetite. The LXRβ has also been found to control hormone feedback in mouse brain, especially the neurons in the area of hypothalamus[11]. The potential roles of

LXRα on brain are still not clear. However, an increase in LXRα expression in the hypothalamus of fructose-fed rats has been found[17]. Therefore, this study and others indicate that both LXRα and LXRβ signaling pathways might affect animal appetite through the central nervous system and the further studies are required to dissect the mechanism in detail.

It was most likely that the activation of LXRα signaling pathway by ATI-111 was able to decline animal energy intake and in turn reduced the animal body weight. However, 4 weeks ATI-111 treatment was able to statistic significantly ($p<0.05$) reduce mice body weight. In the meanwhile, the decrease of dietary intake from those 4 weeks ATI-111-treated mice was not statistically significant. Therefore, other mechanisms such as the regulation of EE beyond the energy intake might also play a role on its anti-obesity effect. Adipose tissue-specific LXRα$^{-/-}$ mice have been shown a decrease both in WAT lipolytic and oxidative capacities. In addition, another LXRα preferential activator T1317, which induces hepatic steatosis and hypertriglyceridemia, has been found to decrease adipocyte size in WAT and increase glycerol release from primary adipocytes[12]. There was no direct evidence in this study to show the regulation of ATI-111 on animal EE. But it will be very interesting to perform more experiments in the future for the investigation of regulating EE by using side effects less or free LXRα-selective agonist.

In this study, up to 24.2% lower blood glucose level was found in LXRα-selective activator ATI-111-treated male ob/ob mice compared with vehicle-treated male ob/ob mice. This anti-diabetic effect was at least partially attributable to the reduced dietary intake in those ATI-111-treated mice. However, other mechanisms such as the suppression of hepatic gluconeogenesis, normalization of glycemia and improvement of insulin sensitivity by activating LXR signaling pathway also should be considered[18-20]. The ob/ob mice are typically an animal model for obesity with a moderate high blood glucose level. A typical type II diabetic animal model with aggressive high blood glucose level might be required to further validate the anti-diabetic effect of ATI-111.

In the past 5 years, activation of LXRα signaling pathway in adipose tissue has been becoming a promising strategy against obesity. In addition to the raise of adipose tissue lipolytic and oxidative capacities and in turn the increase of EE in adipose tissue[12], it also inhibited adipocyte differentiation[21]. Furthermore, LXRα-mediated ABCA1/ABCG1 expression in adipocytes might be involved into the mechanisms of using GLP-1 mimetics or DPP-4 inhibitors against obesity[22,23]. Activation of LXRβ signaling pathway might also have the anti-obesity effect[24]. However, it had the risk to induce metabolic disorders[25]. Therefore, targeting LXRα instead of LXRβ is becoming more popular.

CONCLUSION

The LXRα preferential activator without triggering hepatic steatosis and hypertriglyceridemia, such as ATI-111, will have the pharmaceutical potential against obesity by regulating energy intake and energy expenditure. In addition, activation of LXRβ signaling pathway may bring more safety issues due to its ubiquitous expression through the whole body. Locally expressed LXRα, especially in adipose tissue, is a better target for pharmaceutical development with the potential benefit in drug safety.

SIGNIFICANCE STATEMENTS

This study discovers the possible anti-obesity effect of LXR α-selective activator. It will help the researcher to uncover the critical area of obesity that many researchers were not able to explore. Thus, a new theory on pharmaceutical development against obesity may be arrived at targeting LXRα signaling pathway.

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