# Metabolic regulation of magnolol on the nuclear receptor, liver X receptor

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**Abstract.** The aim of the present study was to investigate whether magnolol, the essential component of the traditional Chinese medicine, *Magnolia officinalis*, can pass through liver X receptor α (LXRα), to subsequently play an important role in the lipid metabolic balance. Using a HepG2 human hepatoma cell line, mammalian cellular one-hybridization and mammalian cell transcriptional activation experiments were performed to detect the combination degree of magnolol at different concentrations with LXRα, and assess the transcriptional activity. In addition, using a THP-1 human monocytic cell line, quantitative polymerase chain reaction was performed to assess the effect on the expression levels of downstream genes. Magnolol was shown to dose-dependently combine with LXRα, and subsequently regulate the transcriptional activity of LXRα. In addition, magnolol was found to adjust the expression of associated LXRα downstream genes in the macrophages. In conclusion, magnolol was demonstrated to affect LXRα, which may outline a new molecular mechanism through which magnolol exerts a lipid-lowering function.

## Introduction

Due to the growth and improvement in living standards, the changes in eating habits and lifestyles and the increasingly aging population, the incidence of metabolic diseases, such as diabetes and atherosclerosis (AS), is increasing each year. According to statistics from the World Health Organization, ischemic heart disease ranks first among the top ten fatal diseases in 2011 (1). Ischemic heart disease is pathologically and largely based on AS, and the majority of AS cases are caused by a metabolic disturbance in glucolipids. Previous studies have shown that nuclear receptors (NRs) are closely associated with glucolipid metabolism *in vivo* (2,3). Liver X receptor (LXR), as an important member of the NR superfamily, participates in the regulatory processes of a number of physical activities *in vivo*, including the metabolism of cholesterol, glucose and fat, inflammation and the maintenance of the metabolic balance. LXR has attracted increasing attention due to its important function in glucolipid metabolism (4). There are two subtypes of LXR: LXRα and LXRβ. The expression of LXRα is tissue-specific, with LXRα highly expressed in the liver, intestine, kidney, adrenal gland, spleen, adipose tissue and macrophages, while LXRβ is extensively expressed in tissue cells. As previously demonstrated, application of a LXR agonist can reduce the intracellular cholesterol content by decreasing the rate of intestinal cholesterol absorption, increasing the level of bile acid and decreasing the rate of reverse cholesterol transfer, thereby ameliorating AS (5,6).

Due to their structural diversity, small natural product molecules have become an essential source of small molecular lead compounds. Magnolol is one of the key monomer components of the traditional Chinese medicine, *Magnolia officinalis* extract (7). *Magnolia officinalis* has a long history as a traditional Chinese medicine and is largely applied for dispelling a cold, relieving headaches, inhibiting anxiety, treating diarrhea and apoplexia, removing chest stuffiness, composing central nerves, and as an anti-fungus and anti-ulcer treatment (8-10). In addition, Jiangzhi Ninggan capsules, which exhibit lipid-lowering effects, contain magnolol (11). Magnolol has been demonstrated to interact with the retinoid X receptor α (RXRα) and the peroxisome proliferator-activated receptor γ, amongst other NRs associated with glucolipid metabolism (12). Therefore, in the present study, whether magnolol interacts with the LXR to regulate LXR-targeted expression of downstream genes was investigated. The aim of the study was to provide further understanding into the lipid-lowering mechanism of magnolol, in order to offer a novel idea for the research and development of anti-AS drugs.

## Materials and methods

*Cell culture*. All the cells were cultured in an environment with 5% $CO_2$ at 37˚C. The HepG2 human hepatoma cell line (American Type Culture Collection, Manassas, VA, USA) was cultured in Minimal Essential Medium (MEM; Gibco

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Life Technologies, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (FBS; Gibco Life Technologies), 100 U/ml penicillin and 100 $\mu$g/ml streptomycin (Sigma-Aldrich, St. Louis, MO, USA). The THP-1 human monocyte cell line (American Type Culture Collection) was cultured in RPMI-1640 medium (Gibco Life Technologies) containing 10% FBS with 10 mmol/l HEPES, 100 U/ml penicillin and 100 $\mu$g/ml streptomycin (Sigma-Aldrich).

*Transient cellular transfection and luciferase reporter gene assay*. Following HepG2 cell growth to a density of 30-50%, the cells were seeded in a 24-well plate with non-serum MEM. Lipofectamine 2000 transfection reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) was applied for the transfection of the plasmids into the cells. The mammalian one-hybridization experiment is a method extensively applied to identify transcription factors at a molecular level. As an agonist of LXR, T0901317 (J&K Scientific Ltd., Shanghai, China) is known to effectively activate the transcriptional activity of the LXRα-ligand binding domain (LBD) (13). Using the mammalian one-hybridization method and T0901317 as a positive control, the effect of various concentrations of magnolol (J&K Scientific Ltd.) on the transcriptional activity of the LXRα-LBD was analyzed in order to identify whether magnolol was able to directly combine with the LXRα-LBD.

In the mammalian one-hybrization experiment, 400 ng pCMX-Gal4-DBD-LXRα-LBD, 400 ng UAS-TK-Luc, and 100 ng reference pRL-SV40 plasmid were transfected (Promega Corporation, Madison, WI, USA). In the transcriptional activation experiment, 400 ng pcDNA3.1a-LXRα, 400 ng pcDNA3.1a-RXRα, 400 ng pGL2-basic-LXRE-Luc plasmids and 100 ng reference plasmid pRL-SV40 were transfected (Promega Corporation). pRL-SV40 was used as the negative control. After cellular transfection for 6 h, the medium was changed to complete medium, 10, 20 or 40 $\mu$M magnolol was added and incubated for 18-24 h. The positive control received 50 nM T0901317 and the negative control contained 10 $\mu$M DMSO. Following incubation, the cells were washed with phosphate-buffered saline (PBS), and 100 $\mu$l lysate was added to each well for cell lysis at 37˚C for 20 min. Subsequently, the activity of the firefly luciferase and reference luciferase were detected, according to the manufacturer's instructions (Promega Corporation, Madison, WI, USA).

*Quantitative polymerase chain reaction (PCR)*. Prior to the PCR experiment, 160 nmol/l phorbol ester (Sigma-Aldrich) was incubated with the THP-1 cells for 24 h to induce cell differentiation into macrophages. The cells were subsequently cultured in non-serum medium containing 50 $\mu$g/ml oxidized low-density lipoprotein (Jingmei Biotechnology Co., Nanjing, China) for 48 h, and lipids were phagocyted, then formed foam cells. After 24 h of treatment, the medium in the Petri dish was removed and the cells were washed with PBS. The total RNA was extracted using TRIzol reagent (Takara Biotechnology Co., Ltd., Dalian, China), according to the manufacturer's instructions. A reverse transcription kit (Takara Biotechnology Co., Ltd.) was used to convert the extracted RNA into cDNA, after which quantitative PCR detection was conducted. The primer sequences were as follows: Human ATP-binding cassette transporter A1 (ABCA1) forward, 5'-GATTGGCTT CAGGATGTCCATGTTGGAA-3' and reverse, 5'-GTATTT TTGCAAGGCTACCAGTTACATTTGACAA-3'; human ATP binding cassette transporter G1 (ABCG1) forward, GCCACTTTCGTGGGCCCAGTGA-3' and reverse, 5'-TCT CATCACCAGCTGTGTTGCA-3'. β-Actin was used as the reference gene in the genetic expression experiments, and the β-actin primers were as follows: Forward, 5'-GCGGGAAAT CGTGCGTGAC-3' and reverse, 5'-CGTCATACTCCTGCT TGCTG-3'.

Sample cDNA underwent quantitative PCR amplification using a SYBR fluorescent probe kit (Takara Biotechnology Co., Ltd.). The amplification procedure was as follows: Initial denaturation at 95˚C for 10 sec, followed by 40 cycles of 95˚C for 5 sec, 60˚C for 20 sec, 72˚C for 10 sec and a final extension at 72˚C for 10 min.

*Statistical analysis*. All experimental data are expressed as the mean ± standard deviation. Statistical analysis was performed using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA). The statistical significance of differences was analyzed by one way analysis of variance and Dunnett's post hoc test, where $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*Magnolol is an agonist of LXRα*. At 6 h after HepG2 cell transfection with the corresponding plasmid, magnolol was applied at different concentrations, as well as T0901317. After 18-24 h, the detection of luciferase activity was performed. T0901317 was shown to markedly stimulate the transcriptional activity of the LXRα-LBD, while magnolol was shown to dose-dependently increase luciferase activity. These results indicated that magnolol was able to interact with the LXRα-LBD directly (Fig. 1). NRs are ligand-dependent transcription factors, and magnolol was shown to combine with the LXRα-LBD at a molecular scale, demonstrating that magnolol may affect the expression of LXR downstream genes by combining with LXR response elements (LXRE).

*Magnolol increases the transcriptional activation of LXRα*. To function as transcription factor, LXR is required to form a heterodimer with the RXR, with the combination in the LXRE, in order to initiate downstream genetic expression. A mammalian transcriptional activation experiment was conducted to detect whether magnolol was able to promote the combination of the LXR/RXR heterodimer and LXRE. At 6 h after HepG2 cell transfection with the corresponding plasmids, the cells were treated with magnolol at various concentrations or T0901317 (positive control) for 24 h. As shown in Fig. 2, T0901317 significantly increased the luciferase activity, promoted the combination of the LXR/RXR heterodimer and LXRE, and increased the transcriptional activity of the LXRE. Furthermore, magnolol was shown to dose-dependently increase the transcriptional activity of LXRE. These results indicated that magnolol was able to regulate the combination of the LXR/RXR heterodimer and LXRE, thereby affecting the expression of LXR downstream genes.

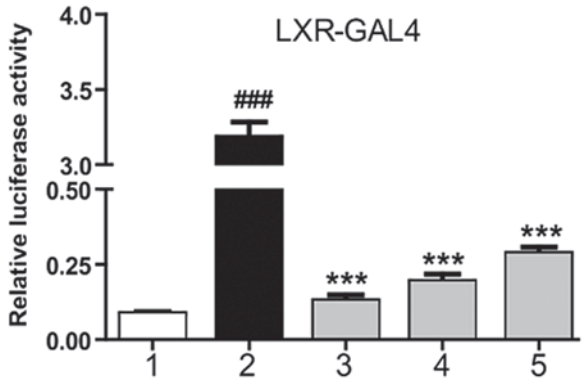


Figure 1. Effect of magnolol on LXR-GAL4 one-hybridization activity. 1, dimethyl sulfoxide negative control group; 2, T0901317 (50 nM) positive control group; 3, low-dose magnolol group (10 $\mu$M), 4, medium-dose magnolol group (20 $\mu$M); 5, high-dose magnolol group (40 $\mu$M). $^{###}$P<0.001, vs. negative control groups; $^{***}$P<0.05, vs. negative control group. LXR, liver X receptor.

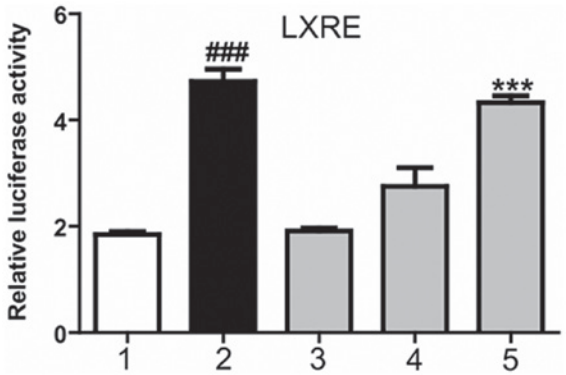


Figure 2. Effect of magnolol on LXRE transcriptional activity. 1, dimethyl sulfoxide negative control group; 2, T0901317 (1 $\mu$M) positive control group; 3, low-dose magnolol group (10 $\mu$M), 4, medium-dose magnolol group (20 $\mu$M); 5, high-dose magnolol group (40 $\mu$M). $^{###}$P<0.001, vs. negative control groups; $^{***}$P<0.001, vs. negative control group. LXRE, liver X receptor response elements.

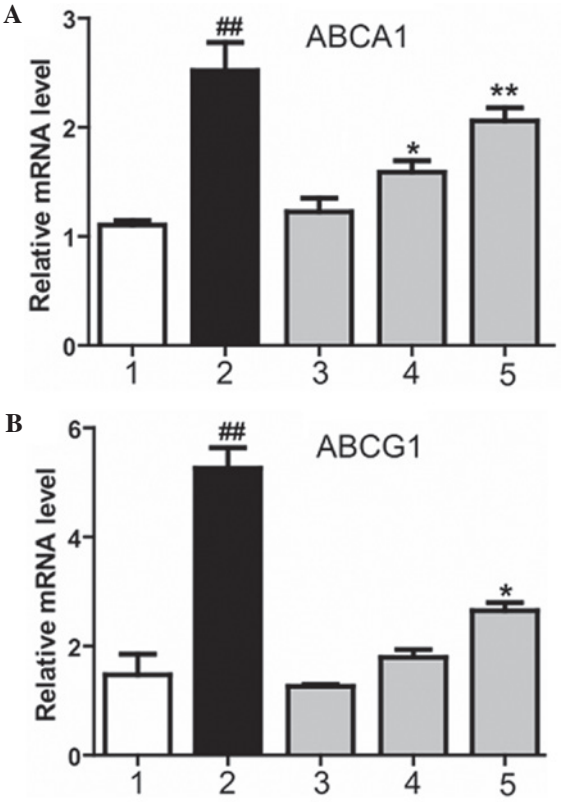


Figure 3. Effect of magnolol on the mRNA expression levels of (A) ABCA1 and (B) ABCG1, downstream genes of the liver X receptor. 1, dimethyl sulfoxide negative control group; 2, T0901317 (2 $\mu$M) positive control group; 3, low-dose magnolol group (10 $\mu$M), 4, medium-dose magnolol group (20 $\mu$M); 5, high-dose magnolol group (40 $\mu$M). $^{##}$P<0.01, vs. negative control group; $^{**}$P<0.01 and $^{*}$P<0.05, vs. negative control group. ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP binding cassette transporter G1.

*Magnolol increases the expression levels of the LXRα downstream genes, ABCA1 and ABCG1.* ABCA1 and ABCG1 are two essential proteins that mediate cholesterol efflux in macrophages (14). In order to investigate whether magnolol affects the expression of ABCA1 and ABCG1 via the LXR, and subsequently ameliorates AS, the effect of magnolol on the expression of ABCA1 and ABCG1 was investigated in THP-1 macrophage-derived foam cells using quantitative PCR. Magnolol was applied at various concentrations to treat the cells. Magnolol was demonstrated to markedly increase the mRNA expression levels of ABCA1 and ABCG1, and the effect became more significant as the magnolol concentration increased (Fig. 3). Therefore, the results indicated that magnolol dose-dependently regulated the mRNA expression levels of ABCA1 and ABCG1.

## Discussion

In clinical practice, as a result of good pharmacological effects, magnolol is widely applied in drugs with *Magnolia officinalis* as the major component, such as Huoxiang Zhengqi Shui, Banxia Houpo Tang, Dachengqi Tang, Tongfusan and Jiangzhining liver capsules. Numerous studies have investigated the pharmacological effects of magnolol, of which a number have been demonstrated, including the inhibition of inflammation to protect endothelial cells from damage, relieving acute inflammatory pain, inhibiting cellular mutation by suppressing mutant enzyme activity, and increasing the combination degree of tranquilizers and the γ-aminobutyric acid (GABA) receptor by increasing the expression of the GABA receptor; thus, magnolol ultimately achieves central sedative and anxiolytic effects (14-16). In addition, magnolol has been shown to inhibit skin photoaging by constraining the nuclear transcription factor, nuclear factor-κB (17). However, there are comparatively less studies investigating the lipid-lowering mechanism of magnolol. One of the research areas in AS is the regulatory systems of LXRα; thus, the present study hypothesized that magnolol interacts with LXRα to aid lipid-lowering.

In the present study, using a HepG2 cell line, magnolol was shown to directly combine with the NR, LXRα-LBD, and increase LXR transcriptional activation. In addition, using a THP-1 cell line, magnolol was demonstrated to regulate the expression of ABCA1 and ABCG1, which are downstream genes of LXR. Previous studies have indicated that the expression of ABCA1, ABCG1 and ABCG5/8, LXR downstream genes, can affect the formation of high-density lipoprotein

(HDL) (18-21), while the latest treatment method for AS is to increase the level of HDL cholesterol in the serum (22,23). Therefore, the results of the present study indicate that magnolol may promote cholesterol efflux by increasing the expression levels of ABCA1 and ABCG1, inhibiting the formation of foam cells and regulating the HDL level, to subsequently ameliorate AS. However, whether magnolol can ameliorate AS in a high fat-induced model or AS gene knock-out model requires further investigation.

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