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Liver X receptor regulates expression of MRP2 but not that of MDR1 and BCRP in the
liver

Ikumi Chisaki, Masaki Kobayashi, Shirou Itagaki, Takeshi Hirano, Ken Iseki^{*}

Laboratory of Clinical Pharmaceutics & Therapeutics, Division of Pharmasciences,
Faculty of Pharmaceutical Sciences, Hokkaido University

Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo 060-0812, Japan

^{*}To whom correspondence should be addressed.

Ken Iseki, Ph. D., Laboratory of Clinical Pharmaceutics & Therapeutics, Division of
Pharmasciences, Faculty of Pharmaceutical Sciences, Hokkaido University

Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo 060-0812, Japan

Tel/Fax: +81-11-706-3770

e-mail: ken-i@pharm.hokudai.ac.jp

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Abstract

Liver X receptors (LXRs) belong to the nuclear hormone receptor superfamily. Multidrug resistance-associated protein 2 (MRP2), multidrug resistance 1 (MDR1) and breast cancer resistance protein (BCRP) play an important role in the efflux of a broad range of endogenous and xenobiotic compounds from hepatocytes. Since the effects of LXR activation on these transporters have been obscure, we investigated the effects of LXR agonists, TO901317 and 25-hydroxycholesterol, on MRP2, MDR1, BCRP expression in HepG2 cells and the rat liver. In an *in vitro* study, TO901317 increased ABCA1, an LXR target gene, and MRP2 mRNA and protein levels. On the other hand, TO901317 had little effect on MDR1 and BCRP mRNA levels. In an *in vivo* study, Abca1 and Mrp2 mRNA and protein levels were increased by TO901317, but TO901317 had no effect on Mdr1a and Bcrp mRNA levels in the rat liver. Moreover, TO901317-induced MRP2 mRNA expression was blocked by LXR α knockdown. In this study, we demonstrated that LXR activation induced expression of MRP2 but not that of MDR1 and BCRP in hepatocytes. The results suggest that agonists for LXR activate transcription of the MRP2 gene in order to promote excretion of endogenous and xenobiotic compounds from hepatocytes into bile.

Introduction

Multidrug resistance-associated protein 2 (MRP2, ABCC2), multidrug resistance 1 (MDR1, ABCB1) and breast cancer resistance protein (BCRP, ABCG2) belong to the ATP-binding cassette (ABC) superfamily of transporters. These transporters are localized to the apical membrane of hepatocytes and mediate efflux of a broad range of endogenous and xenobiotic compounds. MRP2 is responsible for the transport of a wide variety of organic anions such as bilirubin glucuronide and glutathione conjugates [1]. MDR1 plays a critical role in drug absorption, biliary excretion, renal secretion, and central nervous system (CNS) entry of a broad range of hydrophobic substrates [2-3]. BCRP has been implicated in multidrug resistance during chemotherapy, secretion of drugs into breast milk [4], and limitation of the oral bioavailability of sulfasalazine [5]. It has been reported that these transporters are regulated by various nuclear receptors. MRP2 has been shown to be regulated by pregnane X receptor (PXR), farnesoid X receptor (FXR) and constitutive androstane receptor (CAR). Increased MRP2 mRNA expression and transactivation has been found following treatment with ligands for PXR, FXR and by the CAR activator [6]. MDR1 has been shown to be regulated by PXR and CAR [7-9] and BCRP has been shown to be regulated by peroxisome proliferator activated receptor gamma (PPAR γ) in human dendritic cells [10]. The liver X receptors

(LXR α and LXR β) are transcription factors that belong to the nuclear hormone receptor superfamily. LXRs form heterodimers with the retinoid X receptor (RXR) to bind to specific response elements in promoters or enhancers of the target gene. The LXR β isoform is ubiquitously expressed in adults, whereas the expression of LXR α is predominantly restricted to tissues such as the liver, adipose tissue, kidney and small intestine. The formation of HDL by disposal of excess cholesterol and phospholipid from peripheral tissues and the liver is mediated by LXR α , which increases expression of genes encoding ATP-binding cassette proteins, ABCA1 and ABCG1 [11-14], bile acid metabolic enzymes [15], and apolipoproteins [16]. However, the effects of LXR activation on MRP2, MDR1 and BCRP expression have been obscure.

The aim of this study was to determine the effects of LXR agonists on MRP2, MDR1 and BCRP expression in HepG2 cells and the rat liver.

2. Materials and methods

2.1. Cell culture

HepG2 cells were kept in Dulbecco's modified Eagle's medium (Sigma Aldrich Japan, Tokyo) with 10% fetal bovine serum (ICN Biomedicals, Inc., Aurora, OH) and 1% penicillin-streptomycin at 37°C under 5% CO₂ as described previously [17].

2.2. Animals

Male Wistar rats, aged 7 to 8 weeks (260-305 g in weight), were obtained from Jla (Tokyo, Japan). The housing conditions were described previously [18]. The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the “Guide for the Care and Use of Laboratory Animals”. The dosage of TO901317 suspended with methylcellulose was 10 mg/kg for rats. Rats were starved for 6 or 12 hours after administration and euthanized.

2.3. Immunocytochemistry

HepG2 cells were washed once with PBS and fixed with methanol for 5 min. After the fixed cells had been washed three times with PBS, 10% FBS was added and the cells were incubated for 1 h at room temperature. The cells were then reacted with

LXR α (P-20) (Santa Cruz Biotechnology, Santa Cruz, CA) (diluted 1:100) for 24 h at room temperature. Then the cells were washed three times with PBS and treated with donkey anti-goat IgG-FITC secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:400. Nuclei were stained with DAPI. The samples were then visualized by using a confocal microscope (Zeiss LSM-510; Carl Zeiss Inc., Thornwood, NY).

2.4. Nuclear extraction

Nuclear extraction was carried out as described in a previous report with some modification [19]. HepG2 cells were seeded on 6-well plastic plates. Following cell attachment (24 h), 1 or 10 μ M TO901317 was added for 24 h. Total protein extracts were prepared from the cells. The cells were scraped and centrifuged at 1,300 g for 1 min at 4°C. The pellet was suspended in 1 mL of PBS and centrifuged at 1,300 g for 1 min at 4°C. The resulting pellet was suspended in 100 μ L Solution 1 containing 0.6% Nonidet[®] P-40, 150 mM NaCl, 10 mM HEPES (pH 7.9), 1 mM EDTA, 0.5 mM PMSF. The suspension was allowed to stand for 5 min on ice and centrifuged at 2,100 g for 5 min at 4°C, and the pellet was suspended in 50 μ L Solution 2 containing 25% glycerol, 20 mM HEPES (pH 7.9), 420 mM NaCl, 1.2 mM MgCl₂, 0.2 mM EDTA, 0.5 mM

dithiothreitol, 0.5 mM PMSF, 2 mM benzamidine, 5 µg/mL pepstatin and leupeptin. The suspension was sonicated for 20 min at 4°C and centrifuged at 3,000 g for 15 sec at room temperature. The protein concentration in the clear supernatant was determined by the method of Lowry et al. [20].

2.5. Western blot analysis

Western blot analysis was performed as described previously [21]. HepG2 cells were seeded on 6-well plastic plates. Following cell attachment (24 h), various concentrations of reagents were added for 24 h. Total protein extracts were prepared from the cells. The cells were scraped and centrifuged at 1,300 g for 1 min at 4°C. The pellet was suspended in 1 mL of PBS and centrifuged at 1,300 g for 1 min at 4°C. The resulting pellet was suspended in 100 µL lysis buffer containing 1% Triton X-100, 0.1% SDS and 4.5 M urea. The suspension was allowed to stand for 5 min on ice and was sonicated for 15 min at 4°C. The suspension was then centrifuged at 12,000 g for 15 min at 4°C. The liver crude membrane was used for Western blot analysis. The sample was prepared as described previously [22]. The protein concentration in the clear supernatant was determined by the method of Lowry et al. [20]. The samples were denatured at 100°C for 3 min in a loading buffer containing 0.1 M Tris-HCl, 4% SDS,

10% 2-mercaptoethanol, 20% glycerol, 0.004% BPB and 9 M urea and separated on 4.5% stacking and 10% SDS polyacrylamide gels. Proteins were transferred electrophoretically onto nitrocellulose membranes at 15 V for 90 min. The membranes were blocked with PBS containing 0.05% Tween 20 (PBS/T) and 10% non-fat dry milk for 1 h at room temperature. After being washed with PBS/T, the membranes were incubated with mouse anti-Nucleoporin p62 monoclonal antibody (BD Biosciences, Wobarn, MA) (diluted 1:1,000), Insulin R β (C-19) (Santa Cruz Biotechnology, Santa Cruz, CA) (diluted 1:200), LXR α (P-20) (Santa Cruz Biotechnology, Santa Cruz, CA) (diluted 1:200), mouse monoclonal antibody to MRP2 (Abcam, Cambridge, UK) (diluted 1:50) or mouse anti-actin monoclonal antibody (Chemicon, Temecula, CA) (diluted 1:500) for 24 h at room temperature and washed three times with PBS/T for 10 min each time. The membranes were subsequently incubated for 1 h at room temperature with horseradish peroxidase-conjugated goat anti-mouse secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA), horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA), horseradish peroxidase-conjugated donkey anti-goat secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:2,000 or 4,000 and washed three times with PBS/T for 10 min each time. The bands were visualized by

enhanced chemiluminescence according to the instructions of the manufacturer (Amersham Biosciences Corp., Piscataway, NJ).

2.6. Quantitative real-time PCR

Quantitative real-time PCR was performed using an ABI PRISM 7700 Sequence Detector (Applied Biosystems, Foster City, CA) with Platinum[®] SYBR[®] Green qPCR SuperMix UDG (Invitrogen, Carlsbad, CA) following the manufacturer's protocol as described previously [23]. PCR was performed using human ABCA1-specific primers through 40 cycles of 94°C for 15 s, 56°C for 30 s and 72°C for 30 s, using human MRP2-specific primers through 40 cycles of 94°C for 15 s, 60°C for 30 s and 72°C for 30 s, using human MDR1-specific primers through 40 cycles of 94°C for 15 s, 58°C for 30 s and 72°C for 30 s, using human BCRP-specific primers through 40 cycles of 94°C for 15 s, 58°C for 30 s and 72°C for 30 s, using human LXR α -specific primers through 40 cycles of 94°C for 15 s, 55°C for 30 s and 72°C for 30 s, or using human GAPDH-specific primers after pre-incubation at 95°C for 15 min. The primers specific to hABCA1-, hMRP2-, hMDR1-, hBCRP-, hLXR α - and hGAPDH were designed on the basis of sequences in the GenBankTM database (accession no.: AF285167, NM_000392, NM_000927, NM_004827, NM_005693 and NM_002046, respectively).

The sequences of the specific primers were as follows: the sense sequence was 5'-AAC AGT TTG TGG CCC TTT TG-3' and the antisense sequence was 5'-AGT TCC AGG CTG GGG TAC TT-3' for hABCA1, the sense sequence was 5'-ACA GAG GCT GGT GGC AAC C-3' and the antisense sequence was 5'-ACC ATT ACC TTG TCA CTG TCC ATG A-3' for hMRP2, the sense sequence was 5'-TGC TCA GAC AGG ATG TGA GTT G-3' and the antisense sequence was 5'-AAT TAC AGC AAG CCT GGA ACC-3' for hMDR1, the sense sequence was 5'-TTA TCC GTG GTG TGT CTG GA-3' and the antisense sequence was 5'-TTC CTG AGG CCA ATA AGG TG-3' for hBCRP, the sense sequence was 5'-TTC ATC AAC CCC ATC TTC-3' and the antisense sequence was 5'-ACC AGT TTC ATT AGC ATC C-3' for hLXR α , and the sense sequence was 5'-AAG GTC ATC CCT GAG CTG AA-3' and the antisense sequence was 5'-TTC TAG ACG GCA GGT CAG GT-3' for hGAPDH. PCR was performed using rat Abca1-specific primers through 40 cycles of 94°C for 15 s, 61°C for 30 s and 72°C for 30 s, using rat Mrp2-specific primers through 40 cycles of 94°C for 15 s, 52°C for 30 s and 72°C for 30 s, using rat Mdr1a-specific primers through 40 cycles of 94°C for 15 s, 50°C for 30 s and 72°C for 30 s, using rat Bcrp-specific primers through 40 cycles of 94°C for 15 s, 50°C for 30 s and 72°C for 30 s or using rat Gapdh-specific primers after pre-incubation at 95°C for 15 min. The primers specific to rAbca1-, rMrp2-, rMdr1a-,

rBcrp- and rGapdh were designed on the basis of sequences in the GenBankTM database (accession no.: NM_178095, NM_012833, NM_133401, AB094089 and AF106860, respectively). The sequences of the specific primers were as follows: the sense sequence was 5'-CAG GCT GAT GTC AGT CTC CA-3' and the antisense sequence was 5'-GGC TTC AGG ATG TCC ATG TT-3' for rAbca1, the sense sequence was 5'-TGA TCG GTT TCG TGA AGA GCT-3' and the antisense sequence was 5'-ACG CAC ATT CCC AAC ACA AA-3' for rMrp2, the sense sequence was 5'-GCA GGT TGG CTG GAC AGA TT-3' and the antisense sequence was 5'-GGA GCG CAA TTC CAT GGA TA-3' for rMdr1a, the sense sequence was 5'-GTT TGG ACT CAA GCA CAG CA-3' and the antisense sequence was 5'-TGA GTT TCC CAG AAG CCA GT-3' for rBcrp, and the sense sequence was 5'-ATG GGA AGC TGG TCA TCA AC-3' and the antisense sequence was 5'-GTG GTT CAC ACC CAT CAC AA-3' for rGapdh. The PCR products were normalized to amplified GAPDH, which was the internal reference gene (housekeeping gene). Standard curves were prepared for each target and housekeeping gene. A standard curve was established between the threshold cycles (Ct) and the log₁₀ (copy numbers) by using Applied Biosystems sequence detection system software, version 1.9.1. The software calculates the relative amount of the target gene and the housekeeping gene based on the Ct.

2.7. LXR α small interfering RNA (siRNA) and siRNA transfection

Two kinds of Silencer[®] Validated siRNA (ID: #5370 and #5458) targeted to different regions of the LXR α gene and nontargeting siRNA as a Silencer[®] Negative control #1 siRNA were purchased from Ambion (Austin, TX). Delivery of siRNAs into HepG2 cells was performed by reverse transfection methods as per the manufacturer's protocol. Six μ L nontargeting siRNA or LXR α siRNA (2 μ M) and 194 μ L OPTI-MEM[®] I Reduced Serum Medium (GIBCO, Grand Island, NY) were mixed in 12-well plate and incubated at room temperature for 10 min after addition of 2 μ L Lipofectamine[™] RNAiMAX (Invitrogen, Carlsbad, CA). Then 1 mL of suspended HepG2 cells (1.0×10^5 cells/mL) in growth medium without antibiotics was added. Following siRNA transfection (24 h), the medium was replaced with fresh normal growth medium and then the cells were used for analysis and experimentation at the times indicated.

2.8. MTT assay

The 3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was performed as described previously [24] with a certain modification. MTT is normally reduced by dehydrogenases of viable cells and transformed to formazan. The

assay detects living, but not dead, cells and the signal generated is dependent on the degree of activation of the cells. For the MTT assay, HepG2 cells were seeded on 96-well plastic plates and various concentrations of nontargeting siRNA or LXR α siRNA were added for the times indicated. At 1 h before the end of treatment, 10 μ L of PBS-containing MTT solution (0.5%) was added, and the cells were incubated for a further 1 h. The MTT medium was then replaced with 0.2 ml dimethylsulfoxide, and absorbance was read at 590 nm. Absorbance measured in MTT assays was expressed as percent of the control (defined as 100%).

2.9. Statistical Analyses

Student's t-test was used to determine the significance of differences between two group means. Statistical significance among means of more than two groups was determined by one-way analysis of variance (ANOVA). Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Effect of LXR activation on expression of ABC transporters in HepG2 cells

First, we confirmed that whether TO901317, a nonsteroidal synthetic LXR agonist, activates LXR. TO901317 (1, 10 μ M) promoted LXR α translocation from the cytosol to nuclei (Fig. 1A). Moreover, nucleoporin, localized to the nuclei, existed in greater abundance in the nuclear extract than in the cytosol, and insulin R β , localized to the plasma membrane, existed in the cytosol fraction (Fig. 1B(a, b)). In addition, TO901317 increased LXR α protein level in the nuclear extract (Fig. 1B(c)). These results suggest that TO901317 activated LXR α in HepG2 cells. Next, to clarify the effect of LXR α activation on ABC transporters, we examined changes in the mRNA levels of ABCA1, which is an LXR target gene, MRP2, MDR1 and BCRP induced by TO901317 in HepG2 cells. TO901317 up-regulated ABCA1 and MRP2 mRNA levels in a concentration-dependent manner (Fig. 2A, B). On the other hand, TO901317 had little effect on MDR1 and BCRP mRNA levels (Fig. 2C, D). These results suggest that MRP2 mRNA expression, but not that of MDR1 and BCRP, is regulated via an LXR-dependent pathway. Next, we examined time courses of the effects of TO901317 on ABCA1 and MRP2 mRNA levels in HepG2 cells. ABCA1 and MRP2 mRNA levels were significantly increased from 12 to 72 h (Fig. 3A). Moreover, we investigated whether

MRP2 mRNA expression induced by TO901317 correlated with the protein expression in HepG2 cells. As shown in Fig. 3B, TO901317 increased ABCA1 and MRP2 protein levels in a concentration-dependent manner. Naturally occurring oxysterols, mainly derivatives of cholesterol, function as agonists for LXR. 25-hydroxycholesterol (25-OHC), one of the oxysterols, also increased ABCA1 and MRP2 mRNA and protein levels (Fig. 4A, B).

3.2. In vivo effects of LXR activation on mRNA levels of ABC transporters

To confirm the above-mentioned findings *in vivo*, TO901317 was given to rats at 10 mg/kg as described previously with a certain modification [25]. Abca1 and Mrp2 mRNA levels were significantly increased in the rat liver at both 6 and 12 h after oral administration of 10 mg/kg TO901317 (Fig. 5A(a, b)). On the other hand, TO901317 had no effect on Mdr1a and Bcrp mRNA levels (Fig. 5A(c, d)). Moreover, TO901317 up-regulated Abca1 and Mrp2 protein levels at both 6 and 12 h after oral administration of 10 mg/kg TO901317 (Fig. 5B). These results were completely in agreement with the results of the *in vitro* study (Fig. 2).

3.3. Effect of LXR α knockdown on MRP2 mRNA level in HepG2 cells

To clarify whether regulation of MRP2 expression is associated with an LXR α -dependent pathway, we examined the effect of LXR α knockdown on TO901317-induced MRP2 expression. First, quantitative real-time RT-PCR was performed to quantify LXR α mRNA levels at 72 h after transfection of HepG2 cells with siRNA. In this study, different siRNA constructs targeted to the LXR α gene, LXR α siRNA #5370 and #5458, were used. The negative control siRNA did not affect LXR α mRNA level at 72 h after transfection compared with that in cells not transfected with siRNA (data not shown). Therefore, we used this negative control siRNA as a control in this study. Two siRNAs, #5370 and #5458 (1, 10 nM), for LXR α significantly decreased LXR α mRNA level in HepG2 cells at 72 h after transfection (Fig. 6A). The effect of 10 nM siRNAs was stronger than that of 1 nM siRNAs. On the other hand, these LXR α siRNAs (1, 10 nM) had little effect on viability of HepG2 cells (Fig. 6B). Thus, we selected subsequently used LXR α siRNA at a concentration of 10 nM. TO901317-induced MRP2 mRNA alteration (Fig. 7A) was blocked by LXR α siRNAs, #5370 and #5458, transfected to HepG2 cells (Fig. 7B, C). These results suggest that MRP2 expression is regulated by an LXR α -dependent pathway.

4. Discussion

In this study, we showed that LXR activation induced expression of the ABC transporter MRP2 in HepG2 cells and the rat liver. This is first study to examine the effect of LXR activation on the expression of MRP2.

LXR are members of the nuclear hormone receptor superfamily represented by two subtypes, LXR α and LXR β [26-28]. Oxysterols have been identified as endogenous agonists, and TO901317 is known to be a nonsteroidal synthetic agonist for both subtypes [29-30]. These receptors play significant roles in cholesterol homeostasis [31]. LXR forms a heterodimer with the RXR to promote transcription activity of target genes. The genes encode several important proteins including ABCA1 that facilitate cholesterol removal from the liver and macrophages are targets of LXR. We used ABCA1 expression alteration as a positive control of LXR activation in this study. MRP2, which is a member of the ABC superfamily of transporters, consists of two ATP-binding domains and 12 membrane-spanning regions. MRP2 is a 190-kDa phosphoglycoprotein localized in the apical membrane of hepatocytes and is involved in the transport of a wide variety of organic anions such as bilirubin glucuronide and glutathione conjugates. The transport of organic anions via MRP2 is a major driving force in bile salt-independent bile flow. Notably, MRP2 plays a role in the transport of

xenobiotics, including the anti-cancer drugs cisplatin, anthacyclines, vinca alkaloids, and methotrexate [32-37]. MRP2 has been shown to be regulated by the nuclear receptors PXR, FXR and CAR. Increased MRP2 expression has been noted following treatment with agonists for PXR (rifampicin, hyperforin), agonists for FXR (chenodeoxycholic acid, GW4064), and a CAR activator (phenobarbital) [6]. However, the involvement of LXR in MRP2 regulation is unknown. TO901317, a nonsteroidal synthetic LXR agonist, up-regulated ABCA1 and MRP2 mRNA and protein levels in HepG2 cells in a concentration-dependent manner (Fig. 2A, B, 3). Moreover, TO901317-induced MRP2 mRNA alteration was blocked by LXR α knockdown (Fig. 7). An endogenous LXR agonist, 25-hydroxycholesterol (25-OHC), also increased ABCA1 and MRP2 mRNA and protein levels (Fig. 4). Moreover, administration of TO901317 to rats increased Abca1 and Mrp2 mRNA and protein levels in the liver (Fig. 5A(a, b), 5B). On the other hand, TO901317 had no effect on MDR1 and BCRP mRNA levels in HepG2 cells and the rat liver (Fig. 2C, D, Fig. 5A(c, d)). These results suggest that LXR activation induced expression of MRP2 but not that of MDR1 and BCRP. Further investigations to clarify the alteration of transport function and localization of MRP2 induced by LXR activation using MRP2 over-expressed cells are in progress. Recently, Argmann et al. previously reported that treatment with 3-hydroxy-3-methylglutaryl

coenzyme A (HMG-CoA) reductase inhibitors (statins) increased hepatic ABCA1 expression via an LXR-dependent pathway [38]. Therefore, endogenous and exogenous compounds such as statins that affect LXR activation possibly increase MRP2 expression and promote excretion of MRP2 substrates. Moreover, recent studies have shown that the transcription of LXR α was induced by PPAR γ , a member of the nuclear hormone receptor superfamily [39]. Three subtypes of PPAR, PPAR α , PPAR β/δ and PPAR γ , have been identified. We examined the effects of treatment with each PPAR agonist and LXR α knockdown on MRP2 expression in HepG2 cells. In this study, increased MRP2 expression was noted following treatment with agonists for PPAR α (clofibrate), PPAR β/δ (GW0742) and PPAR γ (troglitazone) (supplemental figure). In addition, MRP2 mRNA alteration induced by the three PPAR agonists was blocked by LXR α knockdown. Therefore, drugs that affect PPAR activation such as fibrates (PPAR α agonists) and thiazolidinediones (PPAR γ agonists) may also increase MRP2 expression in hepatocytes.

In this study, we demonstrated that LXR activation induced expression of MRP2 but not that of MDR1 and BCRP in hepatocytes. Finally, endogenous and exogenous compounds that induced LXR activation can lead to alter pharmacokinetics of MRP2 substrates.

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Figure Captions

Fig. 1. Nuclear translocation of LXR α induced by TO901317 in HepG2 cells (A), existence of nucleoporin (a) and insulin R β (b) and effect of TO901317 on LXR α protein level (c) in the nuclear extract of HepG2 cells (B).

(A) HepG2 cells were treated with TO901317 (1, 10 μ M) for 24 h. Localization of LXR α was determined using an antibody against LXR α (green). Nuclei were stained with DAPI (blue). Scale bar shows 10 μ m. Data shown are typical results of three independent experiments.

(B) Nuclei and cytosol were separated by the method of Deryckere et al. [19].

(c) HepG2 cells were treated with TO901317 (1, 10 μ M) for 24 h.

Data shown are typical results of three independent experiments.

Fig. 2. Effects of TO901317 on ABCA1 (A), MRP2 (B), MDR1 (C) and BCRP (D) mRNA levels in HepG2 cells.

HepG2 cells were treated with TO901317 (0.1-10 μ M) for 24 h. Each column represents the mean with S.D. of 6 determinations.

**; significantly different from control at $p < 0.01$.

Fig. 3. Time courses of TO901317 effects on ABCA1 (a) and MRP2 (b) mRNA levels (A) and effects of TO901317 on ABCA1 (a) and MRP2 (b) protein levels (B) in HepG2 cells.

(A) HepG2 cells were treated with TO901317 (1 μ M) for 1-72 h. Each point represents the mean $\pm$ S.D. of 5-6 determinations.

*; significantly different from control at $p < 0.05$.

(B) HepG2 cells were treated with TO901317 (0.1-10 μ M) for 24 h.

Data shown are typical results of three independent experiments.

Fig. 4. Effects of 25-hydroxycholesterol (25-OHC) on ABCA1 (a) and MRP2 (b) mRNA levels (A) and protein levels (B) in HepG2 cells.

(A) HepG2 cells were treated with 25-hydroxycholesterol (25-75 μ M) for 24 h. Each column represents the mean with S.D. of 3-8 determinations.

*; significantly different from control at $p < 0.05$.

(B) HepG2 cells were treated with 25-hydroxycholesterol (25-75 μ M) for 24 h.

Data shown are typical results of three independent experiments.

Fig. 5. Effects of TO901317 on Abca1 (a), Mrp2 (b), Mdr1a (c) and Bcrp (d) mRNA

levels (A) and Abca1 (a) and Mrp2 (b) protein levels (B) in the rat liver.

(A) Male Wistar rats were administered the methyl cellulose with or without 10 mg/kg TO901317 for 6 or 12 h. Each column represents the mean with S.D. of 3-4 determinations.

*; significantly different from control at $p < 0.05$.

(B) Male Wistar rats were administered the methyl cellulose with or without 10 mg/kg TO901317 for 6 or 12 h.

Data shown are typical results of three independent experiments.

Fig. 6. siRNA-mediated silencing of LXR α mRNA level in HepG2 cells (A) and effect of LXR α siRNA on viability of HepG2 cells (B).

(A) HepG2 cells were transfected with negative control or LXR α siRNAs (1, 10 nM) for 72 h. Each column represents the mean with S.D. of 3 determinations.

*; significantly different from negative control at $p < 0.05$.

(B) Cell viability was measured by the MTT assay. HepG2 cells were transfected with negative control or LXR α siRNAs (1, 10 nM) for 72 h. Each column represents the mean with S.D. of 6 determinations.

Fig. 7. Effect of LXR α siRNA on TO901317-induced MRP2 mRNA level in HepG2 cells.

HepG2 cells were transfected with negative control or LXR α siRNAs (10 nM) for 72 h and treated with TO901317 (1, 10 μ M) for 24 h. Each column represents the mean with S.D. of 3-6 determinations.

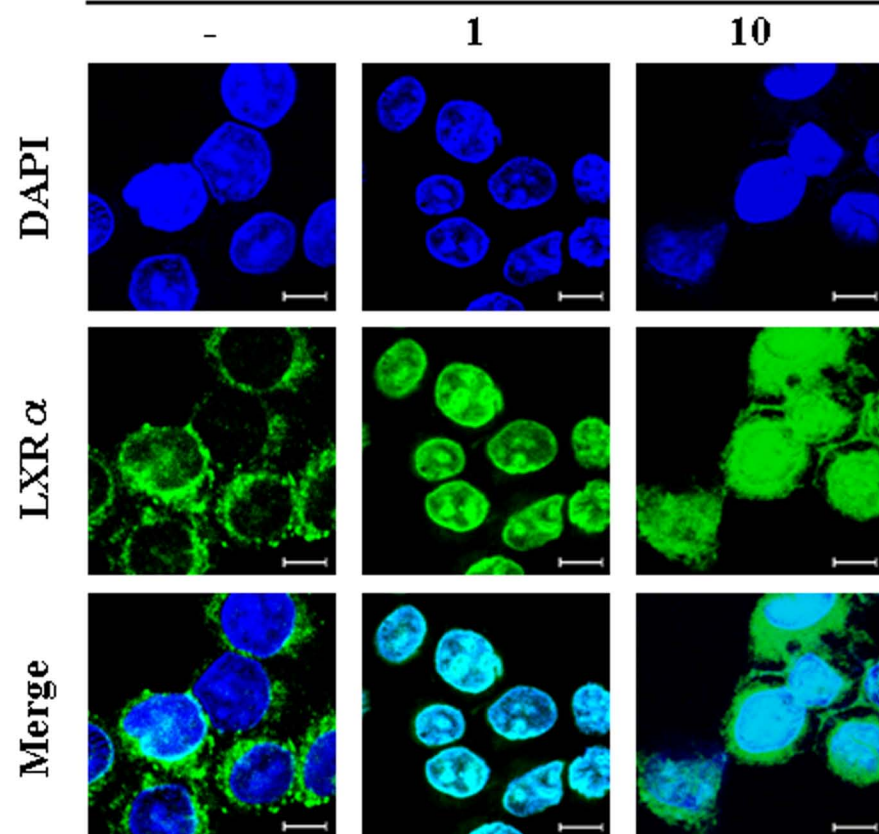
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Supplemental figure. Effects of LXR α siRNA on PPAR agonist-induced MRP2 mRNA levels in HepG2 cells.

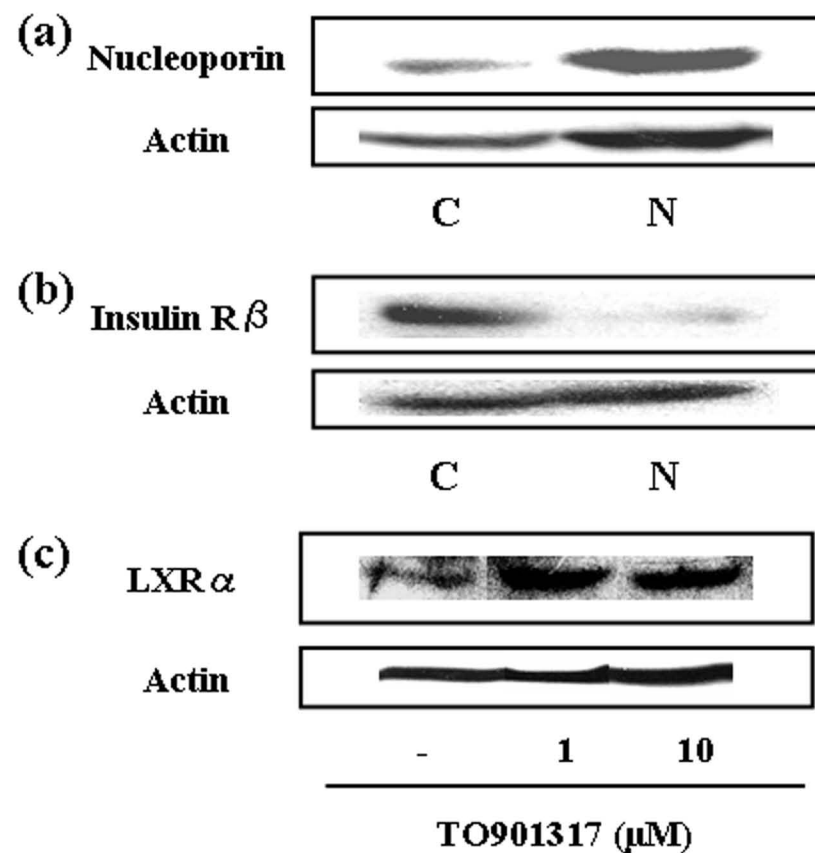
HepG2 cells were transfected with LXR α siRNA (10 nM) for 72 h and treated with various concentrations of PPAR agonists for 24 h. Each column represents the mean with S.D. of 3-6 determinations.

*; significantly different from control at $p < 0.05$. †; significantly different from LXR α siRNA control at $p < 0.05$. N.S.; not significant.

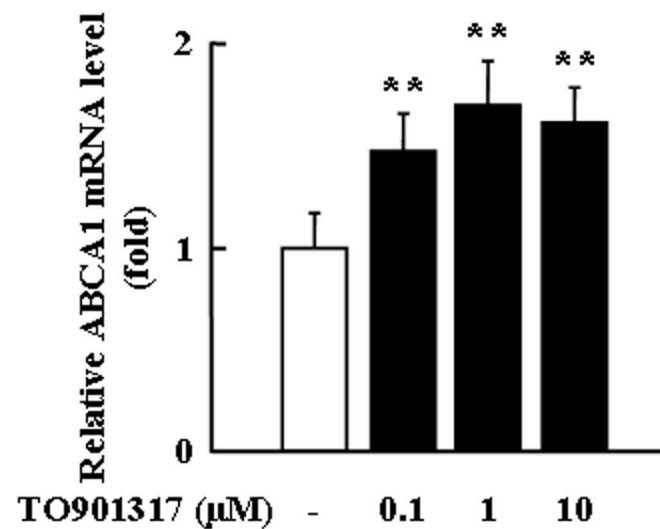
(A)

TO901317 (μM)

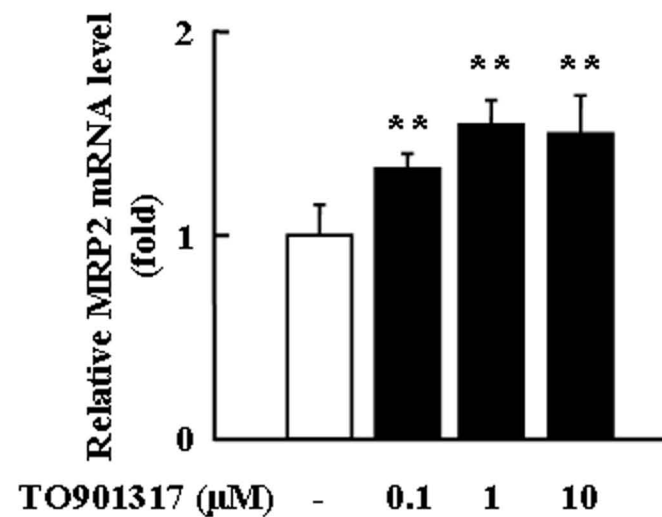
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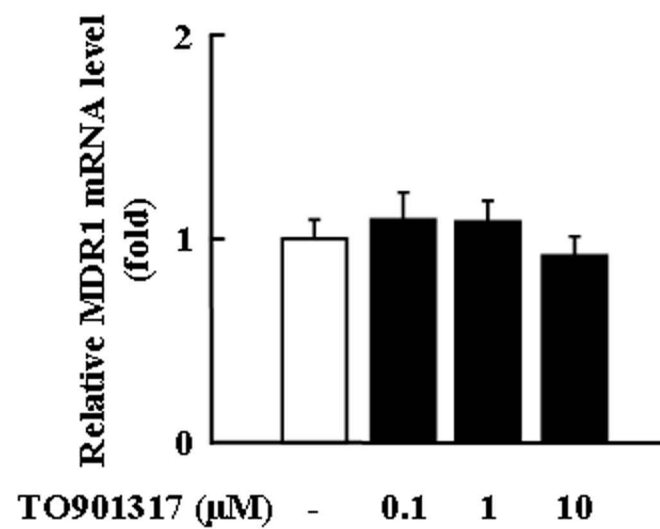
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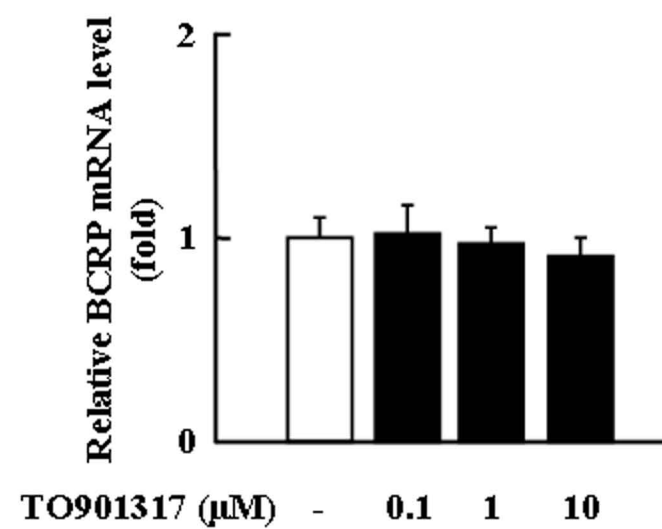
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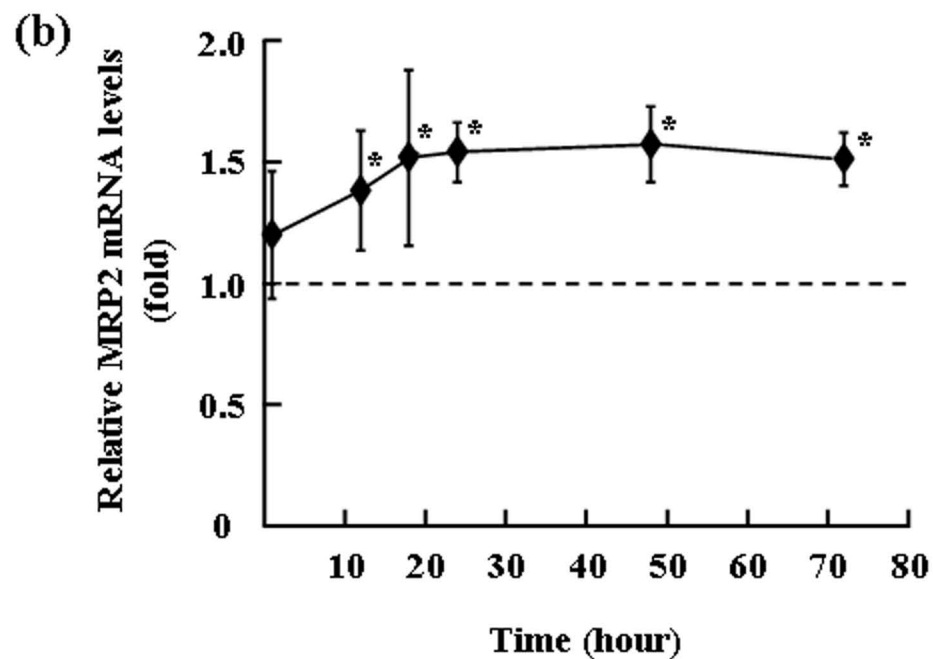
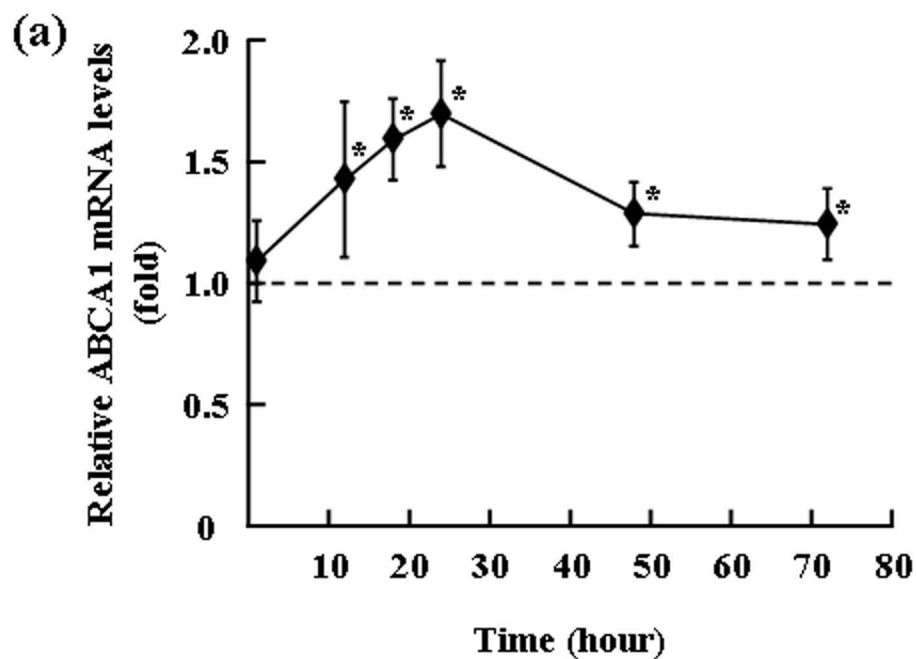
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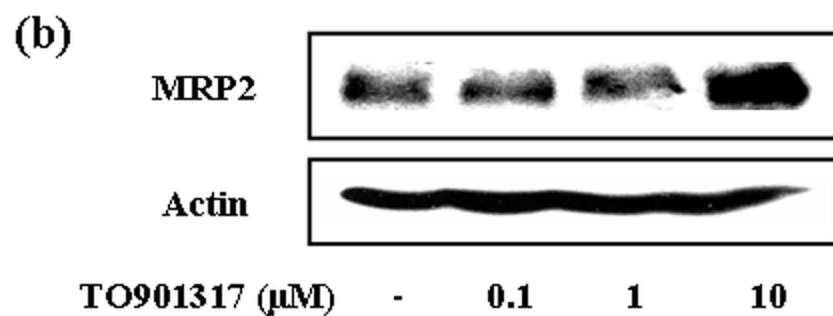
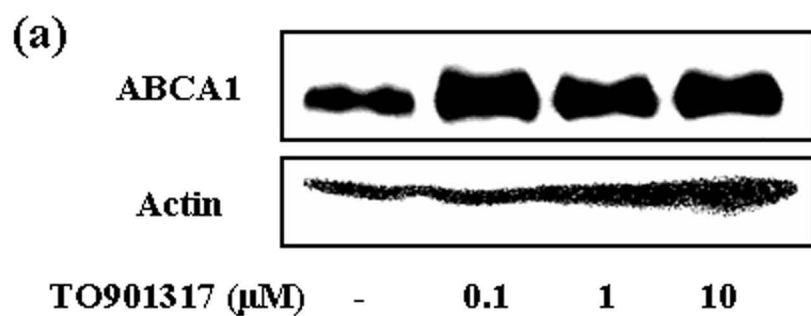
(D)



(A)

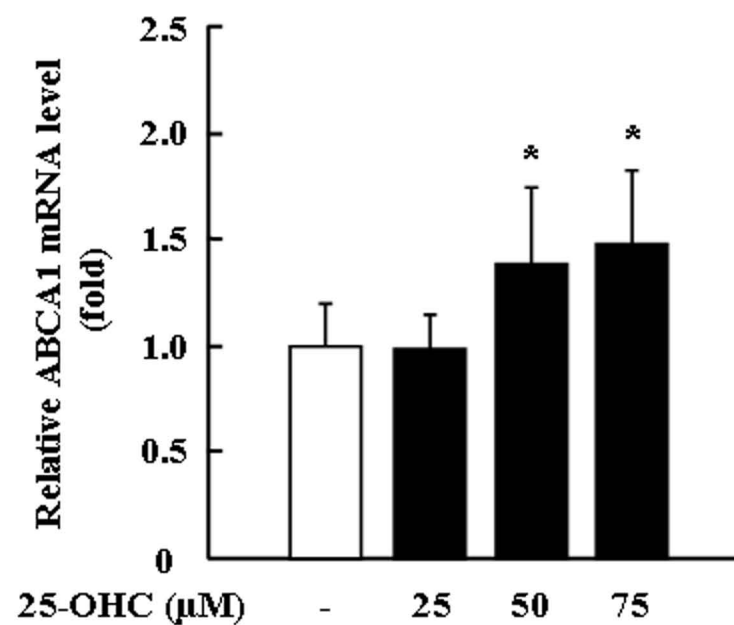


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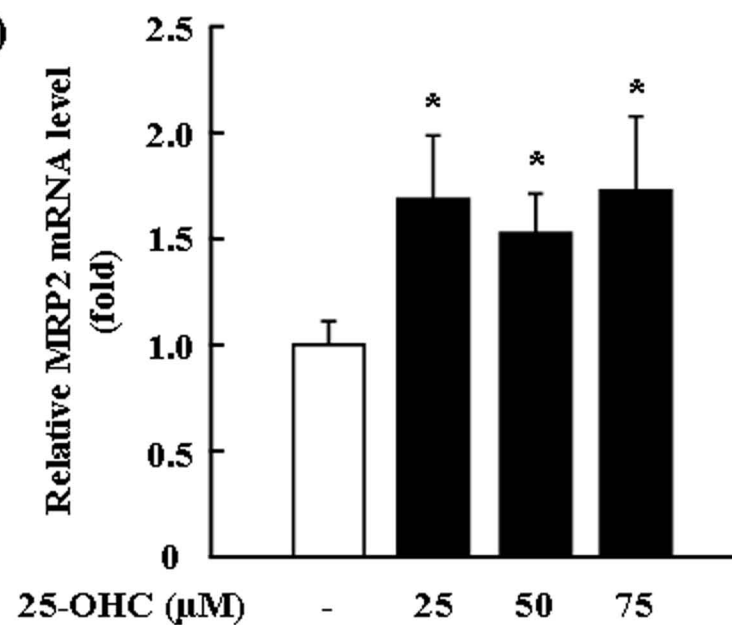


(A)

(a)

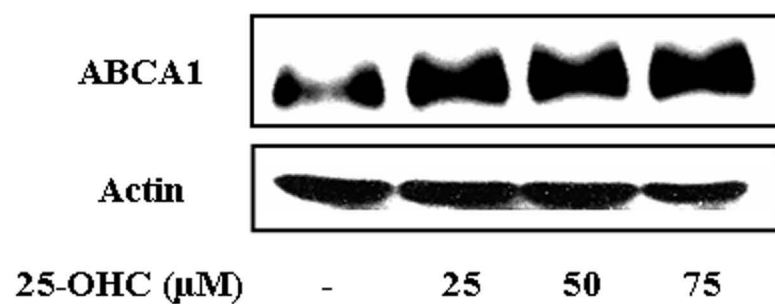


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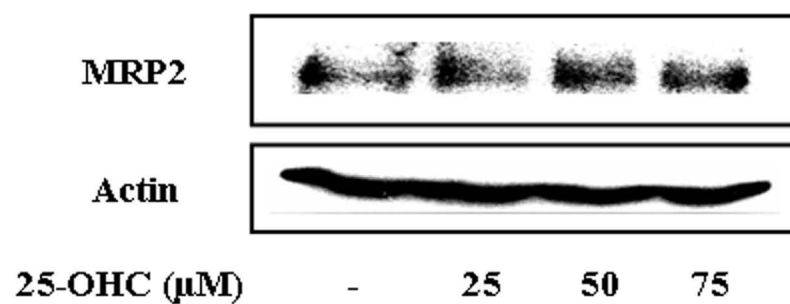


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(a)

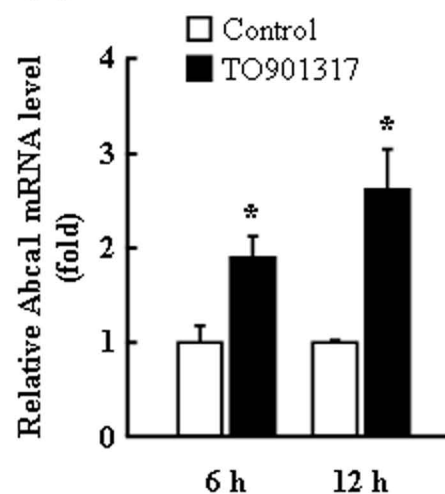


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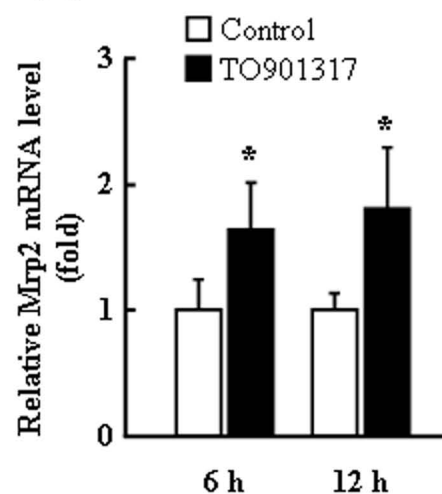


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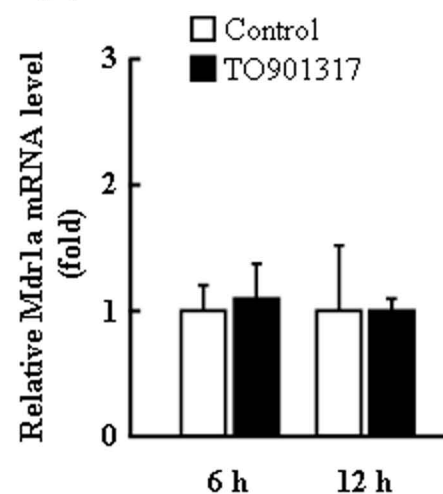
(a)



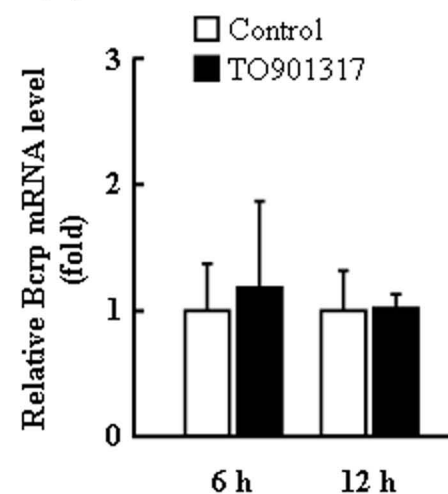
(b)



(c)



(d)



(B)

(a)

Control TO901317 Control TO901317

Abca1



Actin



6 h

12 h

(b)

Control TO901317 Control TO901317

Mrp2



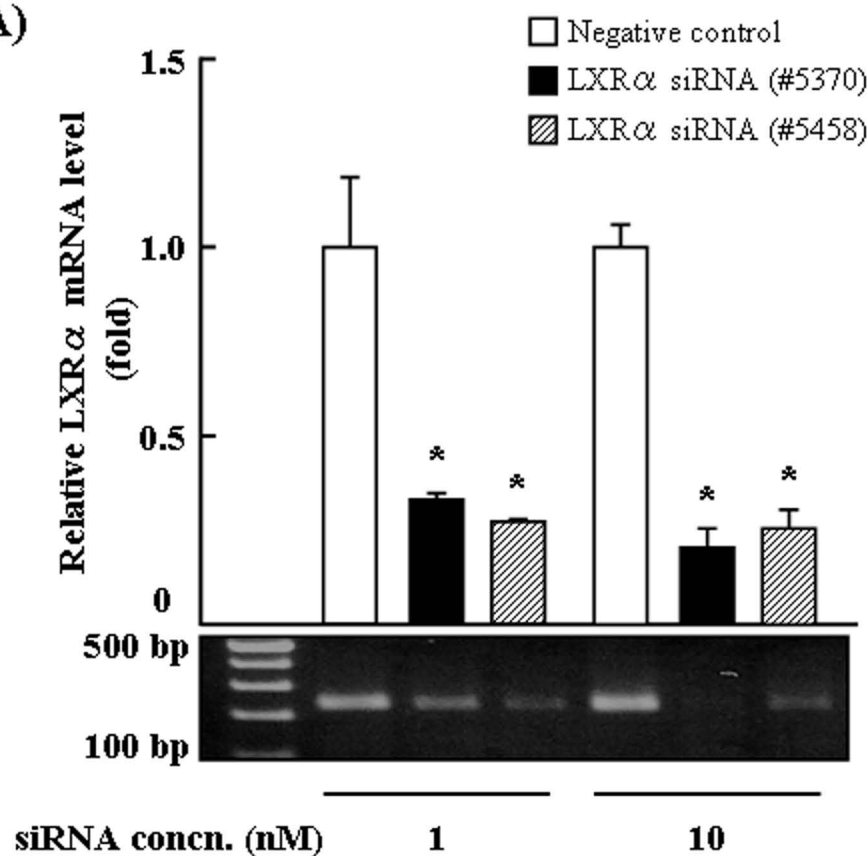
Actin



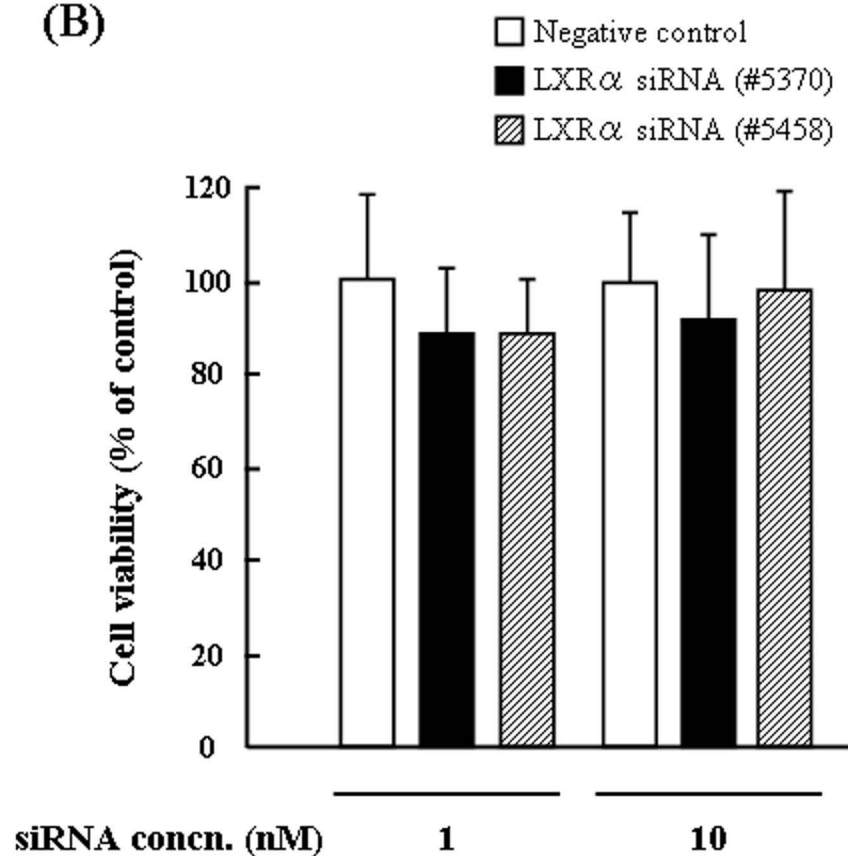
6 h

12 h

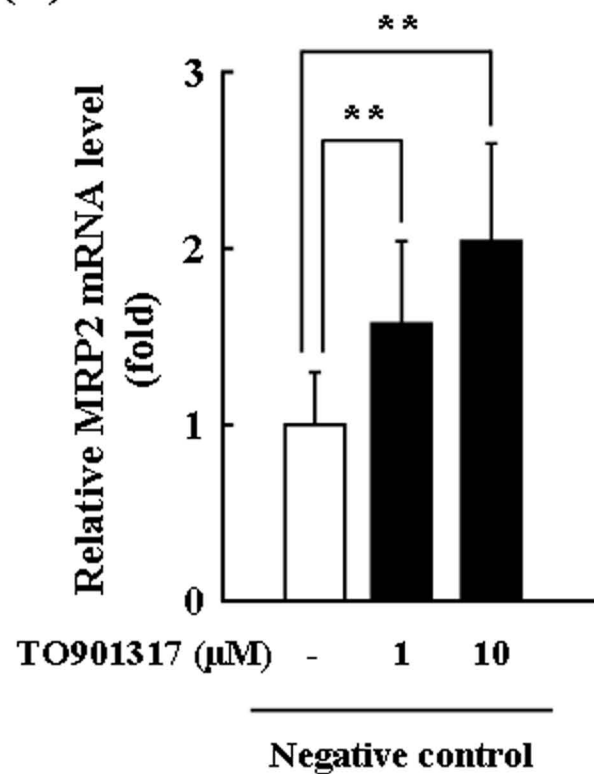
(A)



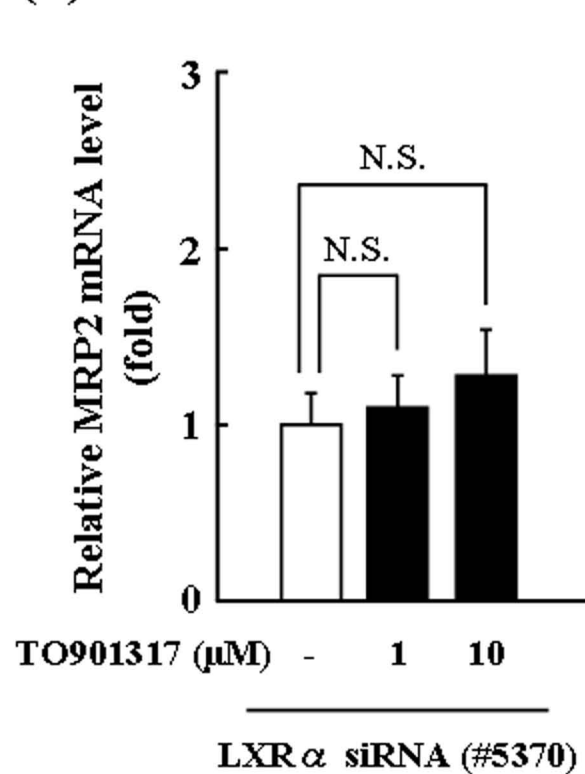
(B)



(A)



(B)



(C)

