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Synthetic LXR Agonist Suppresses Endogenous Cholesterol Biosynthesis and Efficiently Lowers Plasma Cholesterol

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Abstract

The liver X receptors (LXRs) are key regulators of genes involved in cholesterol homeostasis. Natural ligands and activators of LXRs are oxysterols. Numerous steroidal and non-steroidal synthetic LXR ligands are under development as potential drugs for individuals suffering from lipid disorders. N,N-dimethyl-3 β -hydroxycholeamide (DMHCA) is a steroidal ligand of LXRs that exerts anti-atherogenic effects in apolipoprotein E-deficient mice without causing negative side effects such as liver steatosis or hypertriglyceridemia. In this report, we investigated the consequences of DMHCA treatment on cholesterol homeostasis *in vivo* and *in vitro*. Despite its hydrophobicity, DMHCA is readily absorbed by C57BL/6 mice and taken up by intestinal cells, the lung, heart and kidneys, but is undetectable in the brain. DMHCA significantly reduces cholesterol absorption and uptake in duodenum and jejunum of the small intestine and in turn leads to a reduction of plasma cholesterol by 24%. The most striking finding of this study is that DMHCA inhibited the enzyme 3 β -hydroxysterol- Δ 24-reductase resulting in an accumulation of desmosterol in the plasma and in feces. Thus, the reduction of plasma cholesterol was due to a block in the final step of cholesterol biosynthesis. Taken together DMHCA is an interesting compound with properties distinct from other LXR ligands and might be used to study desmosterol-mediated effects in cells and tissues.

Keywords

Cholesterol absorption; cholesterol biosynthesis; desmosterol; DHCR24; DMHCA; liver X receptor

INTRODUCTION

The liver X receptors LXR α (NR1H3) and LXR β (NR1H2), members of the nuclear receptor family for which oxysterols have been identified as natural ligands [1], are key regulators of cholesterol homeostasis [2]. LXR α is mainly expressed in the liver, small intestine, and macrophages, whereas LXR β is ubiquitously expressed in many cell types. These sterol-responsive transcription factors control the expression of multiple genes

involved in cholesterol uptake, transport and efflux as well as fatty acid and glucose metabolism [3]. High plasma cholesterol levels represent a risk factor for atherosclerosis. Thus, a tightly regulated balance has to be maintained between intestinal cholesterol absorption, cholesterol excretion, and endogenous cholesterol biosynthesis.

Since several years, LXR activation has been considered as an attractive therapeutic approach for modulating cholesterol homeostasis and treating atherosclerosis [4]. LXR activation in animal models by the well-established synthetic LXR agonist T0901317 induces reverse cholesterol transport through induction of ATP-binding cassette (ABC) transporter A1 and ABCG1 [5-7], increases cholesterol excretion into the bile by upregulation of ABCG5 and ABCG8 and reduces intestinal cholesterol absorption [8,9]. Apart from these regulatory effects, T0901317 has also been shown to reduce Niemann-Pick C1 like 1 (NPC1L1) mRNA levels in duodenum, which underlines the role of LXR activation on the dietary cholesterol uptake [10]. Recent studies have demonstrated that synthetic LXR agonists increase HDL-cholesterol (HDL-C) concentrations [11] and decrease atherosclerosis [12-15]. Nevertheless, T0901317 treatment increases LDL-cholesterol (LDL-C) levels in mice [16,17]. Notably, LXR activation leads to reduced uptake of LDL through ubiquitination of the LDL receptor [18]. In addition, the concomitant induction of lipogenic genes via induction of sterol regulatory element binding protein 1c (SREBP1c) results in hypertriglyceridemia and liver steatosis, which are undesirable effects of non-steroidal synthetic LXR agonists such as T0901317 [19,20], GW3965 [21] and SB742881 [22]. Three synthetic steroidal LXR agonists, ATI-829 [3 α ,6 α ,24-trihydroxy-24,24-di(trifluoromethyl)-5 β -cholane], hypocholamide [3 α ,6 α -dihydroxy-5 β -cholanoic acid-N-methyl-N-methoxy-24-amide] and DMHCA [N,N-dimethyl-3 β -hydroxycholenamide] have been reported as potent LXR ligands *in vitro* and *in vivo* with only negligible effects on hepatic SREBP-1c activation [15,23-25]. We have previously shown that DMHCA significantly reduces lesion formation in apolipoprotein E (apoE)-deficient mice suggesting that this compound might be a promising candidate as therapeutic agent for the treatment or prevention of atherosclerosis [15].

In the present study, we investigated the impact of short term treatment of DMHCA on cholesterol metabolism in C57BL/6 mice. We provide evidence that DMHCA treatment inhibits endogenous cholesterol biosynthesis by blocking 3 β -hydroxysterol- Δ 24-reductase (DHCR24), which catalyzes the last step in cholesterol biosynthesis. As a consequence, DMHCA significantly lowers plasma cholesterol levels.

MATERIALS AND METHODS

Animals and Diet

All animal experiments were performed in accordance with the standards established by the Austrian Federal Ministry of Education, Science and Culture, Division of Genetic Engineering and Animal Experiments (Vienna, Austria). Male C57BL/6 mice (8-10 weeks old; Himberg, Austria) were used in all experiments. Mice received a standard laboratory chow diet (V1530, Ssniff, Soest, Germany). For treatment regimens, mice were fed chow diet supplemented with 80 mg/kg body weight/day of DMHCA for a period of 4 days. DMHCA was synthesized and the purity was verified by nuclear magnetic resonance (NMR) as previously described [15]. Unless otherwise specified, mice were killed and tissues were obtained at the end of the dark cycle, thus mice were in a fed-state at the time of the studies. In all experiments, duodenum (1 cm adjacent to pyloric sphincter), jejunum (10 cm from pyloric sphincter), and ileum (1 cm from cecum) were harvested. Food intake was measured and feces were collected daily for a continuous 4-day period.

Analysis of DMHCA by Liquid Chromatography and Tandem Mass Spectrometry (LC-MS/MS)

DMHCA was extracted from mouse tissues (200 mg) by liquid extraction with dichloromethane (1 ml). Triamcinolone acetonide (12.5 ng) was added to each sample as internal standard before vortexing. After phase separation by centrifugation at 10,000 g for 5 min, dichloromethane was collected and evaporated under nitrogen stream. The residue was dissolved in 250 μ l water, methanol (25,75 v/v) and subjected to LC-MS/MS analysis.

Determination of Lipid Concentration in Plasma and Tissue

Blood was collected by retroorbital bleeding and EDTA-plasma was prepared within 20 minutes. Plasma total cholesterol (TC) concentrations were measured enzymatically (Greiner Diagnostics AG, Langenthal, Switzerland). Plasma samples from 6 mice of each group were pooled and used for lipoprotein separation by fast protein liquid chromatography (FPLC) using the Pharmacia P-500 FPLC System and a Superose 6 column (Amersham Biosciences, Piscataway, NJ).

Plasma, liver, brain, small intestine and feces were saponified, total lipids were extracted with chloroform, methanol (2,1), and the neutral sterol concentrations were measured by gas chromatography (GC)-MS as described previously [26].

Measurement of Intestinal Cholesterol Absorption

Mice (n = 6/group) were fed chow diet or chow diet supplemented with 80 mg/kg body weight/day of DMHCA. After 4 days of feeding, mice fasted for 4 h were gavaged with 0.2 ml corn oil containing 2 μ Ci of [3 H]cholesterol (ARC Inc., St. Louis, MO). After 4 h and 8 h plasma was collected. The animals were killed, the livers and small intestines were excised and the radioactivity in plasma and tissues was determined by liquid scintillation counting.

Fractional cholesterol absorption was measured by using the fecal dual-isotope ratio method [27]. Briefly, mice were fed chow diet or chow diet supplemented with 80 mg/kg body weight/day of DMHCA for 4 days and fasted for 4 h before they were given a single intragastric dose of [3 H]sitostanol (2 μ Ci; ARC Inc., St. Louis, MO) and 1 μ Ci [14 C]cholesterol (ARC Inc., St. Louis, MO) in 0.2 ml corn oil. Feces were collected 24 h post-gavage.

Measurement of Cholesterol and Desmosterol Biosynthesis

HepG2 cells were obtained from American Type Culture Collection (Manassas, VA). Starved cells were incubated with different concentrations of DMHCA or U18666A (Sigma-Aldrich St. Louis, MO; dissolved in DMSO) plus [14 C]mevalonate (1 μ Ci/ml, ARC Inc., St. Louis, MO) for 24 h. After treatment, total lipids were extracted with hexane, isopropanol (3,2) and analyzed by thin-layer chromatography (TLC) (acetic acid, acetonitrile (1,1) 70% saturated with undecane) as previously described [28]. Cholesterol and desmosterol spots were marked, cut and the radioactivity was measured by liquid scintillation counting. Proteins were dissolved in 0.3 M NaOH at room temperature and the concentration was quantified using Bradford assay.

DHCR24 Activity Assay

DHCR24 activity was assayed as described previously [29]. Briefly, the synthesis of cholesterol from desmosterol (Sigma-Aldrich, St. Louis, MO) was measured by incubating 100 μ g of HepG2 cell homogenates in 250 μ l Buffer A for 4 h at 37°C. Final concentrations of Buffer A, 100 mM Tris/HCl (pH 7.23), 0.1 mM EDTA, 1 mM dithiothreitol, 30 mM nicotinamide, 3.5 mM nicotinamide adenine dinucleotide phosphate, 30 mM glucose-6-

phosphate, 2 U glucose-6-phosphate dehydrogenase/ml, 0.5 mg bovine serum albumin/ml, and 168 μ M desmosterol (prepared as 420 μ M stock in 1.25% methyl- β -cyclodextrin (Sigma-Aldrich, St. Louis, MO), in Tris/HCl, pH 7.23). DMHCA and U18666A (10 nM, 100 nM, 1 μ M and 10 μ M) were added to Buffer A. After incubation for 24 h, cholesterol and desmosterol levels were analyzed by GC-MS as described previously [26], and the ratio of cholesterol to desmosterol was calculated.

RNA Isolation and Quantitative Real-Time PCR

Total RNA from mouse tissues was isolated using the TriFast reagent according to the manufacturer's protocol (Pierce, Erlangen, Germany). Total RNA (1 μ g) was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA). Quantitative real-time PCR was performed with a Roche LightCycler 480 (Roche Diagnostics, Mannheim, Germany) using the QuantiFastTM SYBR[®] Green PCR Kit (Qiagen, Hilden, Germany). Samples from two independent experiments were analyzed in triplicate and normalized to cyclophilin A as an internal control. The expression levels and associated statistical parameters were determined using the public domain program Relative Expression Software Tool - REST (<http://www.gene-quantification.com/download.html>). Real-time PCR primers were designed with primer designer version 2.0, NPC1L1-Fwd, 5'-GCAAGGTGATCAGGAGGTTGA-3', NPC1L1-Rev, 5'-ATCCTCATCCTGGGCTTTGC-3', SR-BI-Fwd, 5'-GCGCTCGGCGTTGTCATGAT-3', SR-BI-Rev, 5'-TGGCCGTTGAGGACC TCGTT-3', CD36-Fwd, 5'-GCAGGTCTATCTACGCTGTG-3', CD36-Rev, 5'-GGTGTCT GGATTCTGGAGG-3', ABCA1-Fwd, 5'-CTCTTCATGACTCTAGCCTGGA-3', ABCA1-Rev, 5'-ACACAGACAGGAAGACGAACAC-3', ABCG5-Fwd, 5'-CAAGACTGCTTCTCCT ACGTCCTGC-3', ABCG5-Rev, 5'-CCAGCTCAGCCAAGAGAAGACAAT-3', ABCG8-Fwd, 5'-AGTGGTCAGTCCAACACTCTG-3', ABCG8-Rev, 5'-GAGACCTCCAGGGTATC TTGAA-3', CyclophilinA-Fwd, 5'-TTCCAGGATTCATGTGCCAG-3', CyclophilinA-Rev, 5'-CCATCCAGCCATTCAGTCTT-3'.

Cell Viability Assay

Cell viability was measured using Cytotoxicity Detection Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's protocol.

Statistical Analysis

Statistical differences between groups were analyzed using unpaired Student's t-test (GraphPad Prism 5.0, San Diego, CA). Data are reported as mean $\pm$ SEM for the specified number of animals. * p < 0.05, ** p < 0.01, *** p < 0.001 for DMHCA vs. control.

RESULTS AND DISCUSSION

DMHCA Reduces Plasma Cholesterol Concentrations

First, we examined the impact of orally administered DMHCA on plasma cholesterol and lipoprotein profiles in C57Bl/6 mice. DMHCA significantly reduced plasma TC levels by 24% (68.2 ± 9.5 mg/dl vs. 90.2 ± 11.4 mg/dl), which was mainly due to decreased HDL-C and LDL-C levels (Fig. (1)). From these results we concluded that a 4-day treatment might be sufficient to study the effects of this compound on whole-body cholesterol homeostasis in mice.

DMHCA Decreases Intestinal Cholesterol Absorption

The hypocholesterolemic plasma phenotype of DMHCA-treated mice differs from that commonly induced by nonsteroidal synthetic LXR agonists, but is consistent with that of the oxysterol ligand hypocholenamide [25]; this suggests that synthetic steroidal LXR ligands trigger a mechanism distinct from that of non-steroidal ligands. To explore possible mechanisms of the reduction of plasma cholesterol levels in DMHCA-treated mice, we measured its effects on intestinal cholesterol absorption by the dual fecal isotope ratio method. Fractional cholesterol absorption was decreased by 41% in DMHCA-treated compared to control mice (Fig. (2A)). To examine the effect of DMHCA on intestinal and plasma cholesterol levels after an acute cholesterol load, mice were gavaged with [^{14}C]cholesterol followed by monitoring [^{14}C]cholesterol concentrations in the small intestine and plasma. As shown in Fig. (2B), DMHCA treatment reduced the intracellular concentration of [^{14}C]cholesterol in the duodenum and jejunum by 39% and 33%, respectively, whereas [^{14}C]cholesterol levels in the ileum were comparable to control mice. DMHCA decreased [^{14}C]cholesterol concentrations in the plasma by more than 70% (Fig. (2C)). Thus, DMHCA significantly decreases intestinal cholesterol absorption in mice. These data might reflect the efficacy of DMHCA to regulate gene expression in the small intestine, i) downregulation of NPC1L1 expression (Fig. (3A)) might result in lower intestinal cholesterol uptake and ii) higher transcriptional activation of intestinal ABCA1 and ABCG5/G8 (Fig. (3B)) might cause increased cholesterol efflux into the lymph, plasma and the intestinal lumen, respectively. DMHCA treatment, however, failed to alter SR-BI or CD36 mRNA expression in the small intestine, genes known to mediate cholesterol absorption (Fig. (3A)) [30].

DMHCA Increases Fecal Desmosterol Loss

Next, we measured the amount of neutral sterols in feces within 4 days. DMHCA markedly increased fecal loss of neutral sterols after 2, 3 and 4 days of treatment (Fig. (4A)). Analysis of individual neutral sterols in the feces determined by GC-MS revealed that DMHCA treatment did not affect fecal cholesterol excretion (Fig. (4B)) but strongly increased the concentration of fecal desmosterol compared to untreated mice (Fig. (4C)). These findings differed from all previous published work on LXR agonist and suggest that DMHCA lowered plasma cholesterol concentrations by mechanisms distinct from previously described effects of LXR ligands.

DMHCA Decreases Endogenous Sterol Biosynthesis by Blocking DHCR24

The increase in fecal desmosterol concentration indicated that DMHCA inhibits endogenous cholesterol biosynthesis by blocking DHCR24, the enzyme responsible for the conversion of desmosterol into cholesterol [29]. Therefore, we compared the impact of DMHCA on endogenous sterol biosynthesis and DHCR24 activity with U18666A, a specific inhibitor of DHCR24 [31]. First, we measured cholesterol and desmosterol biosynthesis in HepG2 cells in the presence of DMHCA or U18666A using [^{14}C]mevalonate as a tracer. As shown in (Fig. (5A)), DMHCA decreased cellular cholesterol levels in a concentration-dependent manner comparable to U18666A. This decrease was accompanied by a marked increase in desmosterol concentration (Fig. (5B)).

In addition, both DMHCA and U18666A inhibited the activity of DHCR24 (Fig. (5C)), however, the effect of DMHCA on DHCR24 activity lacked statistical significance. These data suggest that DMHCA inhibits DHCR24 only partially, and this inhibition is not consistent with competitive inhibition. Treatment of cells with the doses of DMHCA or U18666A applied in our studies resulted in unchanged lactate dehydrogenase (LDH) release suggesting unchanged cell viability (data not shown).

To confirm the relevance of these findings *in vivo*, endogenous levels of cholesterol and desmosterol were examined in plasma, liver, brain, and small intestine of control and DMHCA-treated mice. In control mice, the relative content of desmosterol of total sterols in plasma and in the investigated tissue ranged from 1 to 5%, respectively (Fig. (6A)). In contrast, in DMHCA-treated mice the relative desmosterol content was 30-40% increased in plasma, the liver, and the small intestine (Fig. (6B)). Notably, cholesterol and desmosterol concentrations in the brain were not affected by DMHCA-treatment (Fig. (6B)). This latter result is in accordance with our finding that DMHCA was detected in the small intestine, but also in the lung, kidney and heart, whereas no DMHCA was found in the brain (Fig. (6C)).

Desmosterol itself activates LXR and in turn increases the expression of ABCA1 *in vitro* [32]. In mice with a genetic inactivation of Dhcr24, in which almost all cholesterol is replaced by desmosterol, LXR-regulated hepatic genes are significantly activated by desmosterol *in vivo* [33]. Thus, the effects of DMHCA on cholesterol homeostasis might be attributable to desmosterol. DMHCA, however, is a potent oxysterol-type LXR activator with similar binding potencies for LXR α and β isoforms [24,34]. Therefore, it is likely that the accumulation of desmosterol in the small intestine and liver enhances the effect of DMHCA on LXR activation. Notably, Dhcr24-deficient mice generated in 2003 by Quark Biotech Inc. were viable [35], but infertile due to abnormalities in male gonads and oocyte maturation. Moreover, the mice exhibited decreased subcutaneous and mesenteric fat, decreased myelination of the central nervous system, and hypotonia [35]. This phenotype is consistent with that observed in two patients with desmosterolosis who carry mutations in the Dhcr24 gene [29,36]. Their pathology is characterized by the accumulation of desmosterol in plasma and tissues, multiple congenital anomalies, and severe mental retardation. The viability of Dhcr24-deficient mice, in which desmosterol concentrations exceeded cholesterol on a molar basis by 80-fold in the plasma and by 15-fold in the brain, however, indicated that desmosterol can substitute for cholesterol in adult mice.

In that respect, it is important to mention that DMHCA (8 mg/kg/d) fed to apoE-deficient mice for 11 weeks did not result in any morphologic defects, but reduced atherogenesis in both male and female apoE-deficient mice [15]. Furthermore, female C57Bl/6 mice fed DMHCA (8 mg/kg/day) for 8 weeks were fertile and lacked cataracts (data not shown), as reported for other inhibitors of DHCR24, U18666A and triparanol [37,38]. These findings suggest that even long-term treatment with DMHCA does not affect oocyte development or induce obvious toxicologic effects. Due to its limited bioavailability, DMHCA was absent in the liver. However, the observed increase in hepatic desmosterol might result from desmosterol of intestinal origin that was transported to the liver. The lack of desmosterol accumulation in the brains of DMHCA-treated mice reflects the inability of DMHCA to traverse the blood-brain barrier. However, cholesterol still accounts for approximately 60% of total sterols in the liver and small intestine of DMHCA-treated mice. Therefore, DMHCA-treated mice are not comparable with either Dhcr24-deficient mice or patients suffering from desmosterolosis.

In summary, our findings reveal that DMHCA has dual effects on cholesterol homeostasis resulting in decreased plasma cholesterol concentrations. In addition to its known LXR-agonizing effects, DMHCA acts as an inhibitor of DHCR24, which leads to a reduction in the conversion of Δ 24-dehydrocholesterol to cholesterol and in turn to an accumulation of desmosterol in plasma and several organs but not in the brain. These properties of DMHCA, which differ from those of other synthetic LXR agonists, make DMHCA an interesting substance to study desmosterol-mediated effects in cells and tissues.

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LIST OF ABBREVIATIONS

ABC	ATP binding cassette
DHCR24	3 β -hydroxysterol- Δ 24-reductase
DMHCA	N,N-dimethyl-3 β -hydroxycholelamide
FPLC	Fast protein liquid chromatography
HMGCR	3-hydroxy-3-methyl-glutaryl-CoA reductase
LXR	Liver X receptor
NPC1L1	Niemann-Pick C1 like 1
RCT	Reverse cholesterol transport
SREBP	Sterol regulatory element-binding protein
TC	Total cholesterol
TLC	Thin layer chromatography
T0901317	<i>N</i> -(2,2,2-trifluoroethyl)- <i>N</i> -[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)-ethyl]phenyl]-benzenesulfonamide

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Fig. (1).

Fast protein liquid chromatography (FPLC) separation of plasma lipoproteins of DMHCA-treated and control mice. C57BL/6 mice were fed a standard chow diet supplemented with or without DMHCA (80 mg/kg weight/day) for 4 days ($n = 6$ per group). Plasma from all individual mice per group was pooled and subjected to FPLC using a Superose 6 column. Cholesterol content in each fraction was measured enzymatically.

Fig. (2).

Cholesterol absorption and acute cellular cholesterol uptake in DMHCA-treated and control mice. **A)** Cholesterol absorption was measured using the fecal dual-isotope ratio method as previously described [27]. After 4 days of feeding a standard chow diet supplemented with or without DMHCA (80 mg/kg body weight/day), mice (n = 8 per group) were gavaged with [³H]sitostanol and [¹⁴C]cholesterol in 0.2 ml corn oil. Feces were collected 24 h post-gavage. **B)** Acute cellular cholesterol uptake was determined 8 h after gavaging of mice with [¹⁴C]cholesterol. After 4 days of feeding a standard chow diet supplemented with or without DMHCA (80 mg/kg body weight/day), mice (n = 8 per group) were gavaged with

[¹⁴C]cholesterol in 0.2 ml corn oil. 8 h later, animals were killed, the livers and small intestines were excised and the radioactivity was measured by liquid scintillation counting. C) Accumulation of [¹⁴C]cholesterol was determined in plasma 4 h and 8 h after gavaging mice with [¹⁴C]cholesterol. Data are expressed as mean ± SEM. **p < 0.01, ***p < 0.001 DMHCA vs. control.

Fig. (3).

Effects of DMHCA on the expression of genes involved in cholesterol metabolism in the small intestine. C57BL/6 mice were fed a standard chow diet supplemented with or without DMHCA (80 mg/kg weight/day) for 4 days. Gene expression of **(A)** cholesterol transporters NPC1L1, SR-BI, and CD36, and **(B)** ABC transporters was analyzed by real-time PCR. RNA amounts were normalized to cyclophilin A and controls were arbitrarily set to 1. Data are expressed as mean $\pm$ SEM (n = 8 per group). *p < 0.05; **p < 0.01; ***p < 0.001 DMHCA vs. control.

Fig. (4).

Fecal loss of neutral sterols in DMHCA-treated and control mice. C57BL/6 mice (n = 8 per group) were fed a standard chow diet supplemented with or without DMHCA (80 mg/kg body weight/day) for 4 days. Feces were collected, lipids were extracted and concentrations of (A) total neutral sterols, (B) cholesterol and (C) desmosterol were determined by GC-MS. Data are expressed as mean $\pm$ SEM. *p < 0.05, **p < 0.01, ***p < 0.001 DMHCA vs. control.

Fig. (5).

Effects of DMHCA and U18666A on cholesterol and desmosterol biosynthesis in HepG2 cells. HepG2 cells were incubated with DMHCA or U18666A at the indicated concentrations for 4 h, followed by 24 h incubation with the tracer [3 H]mevalonolactone. Subsequently, lipids were extracted and separated by TLC. (A) Cholesterol and (B) desmosterol spots were marked, cut and the radioactivity was measured by liquid scintillation counting. (C) DHCR24 activity in HepG2 cell homogenates was assayed using desmosterol as a substrate. After incubation with DMHCA or U18666 for 24 h, total cholesterol and desmosterol levels were analyzed by GC-MS, and the ratio of cholesterol to desmosterol was calculated. Data are expressed as mean values $\pm$ SEM. For each analysis

two independent experiments were performed in triplicate. * $p < 0.05$ DMHCA vs. U18666A.

Fig. (6).

Concentration of desmosterol in plasma and organs of control and DMHCA-treated mice. C57BL/6 mice were fed a standard chow diet supplemented without or with DMHCA (80 mg/kg weight/day) for 4 days. Plasma, liver, small intestine and brain were saponified, total lipids were extracted with chloroform:methanol (3:2), and the concentrations of cholesterol and desmosterol in (A) control and (B) DMHCA-treated mice were determined by GC-MS. The sum of cholesterol and desmosterol was set to 100% (n = 4 per group). (C) Tissue distribution of DMHCA. The concentration of DMHCA is expressed in µg per gram of tissue, feces or urine.