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Curcumin induces changes in expression of genes involved in cholesterol homeostasis

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Abstract

Curcuminoids, the yellow pigments of curcuma, exhibit anticarcinogenic, antioxidative and hypocholesterolemic activities. To understand the molecular basis for the hypocholesterolemic effects, we examined the effects of curcumin on hepatic gene expression, using the human hepatoma cell line HepG2 as a model system. Curcumin treatment caused an up to sevenfold, concentration-dependent increase in LDL-receptor mRNA, whereas mRNAs of the genes encoding the sterol biosynthetic enzymes HMG CoA reductase and farnesyl diphosphate synthase were only slightly increased at high curcumin concentrations where cell viability was reduced. Expression of the regulatory SREBP genes was moderately increased, whereas mRNAs of the PPARα target genes CD36/fatty acid translocase and fatty acid binding protein 1 were down-regulated. LXRα expression and accumulation of mRNA of the LXRα target gene ABCg1 were increased at low curcumin concentrations. Although curcumin strongly inhibited alkaline phosphatase activity, an activation of a retinoic acid response element reporter employing secreted alkaline phosphatase was observed. These changes in gene expression are consistent with the proposed hypocholesterolemic effect of curcumin.

Keywords: Curcumin; Cholesterol; LDL receptor; SREBP; Liver X receptor; Retinoic acid response element; Alkaline phosphatase

1. Introduction

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1, 6-heptadien-3,5-dione] and its derivatives, the curcuminoids, are the predominant phenolic compounds in most curcuma species. Rhizomes of *Curcuma longa* are the major components of the spices turmeric and curry. These spices have been widely used in traditional medicine in Southeast Asia, and numerous biological effects have been associated with curcumin. Its capability to induce apoptosis and its antioxidative and anti-inflammatory effects make it a promising compound in the prevention and treatment of cancer, cardiovascular diseases and inflammatory processes [1]. Recently, it was also shown that curcumin is able to correct cystic fibrosis defects [2]. The molecular mechanisms underlying such effects have been analyzed to some detail, but have not been understood completely [1,3].

Several studies suggest that curcumin has hypocholesterolemic properties [4–6]. Zhang et al. [7] reported increased LDL-receptor amount in vascular smooth muscle cells of rats that were orally treated with turmeric extract, and Arafa [8] demonstrated an attenuation of hypercholesterolemia in rats by curcumin feeding. On the molecular level, Kapoor et al. [9] showed that curcumin interferes with Raf-1-mediated accumulation of LDL-receptor mRNA.

Lipid homeostasis is tightly controlled by the interaction of cellular and extracellular signals. Synthesis and cellular uptake are regulated by the sterol regulatory element binding proteins (SREBPs), which are considered to be the key elements in the control of cellular cholesterol homeostasis [10]. SREBPs are encoded by two genes, SREBP-1 and SREPB-2, where SREBP-2 predominantly regulates sterol metabolism and SREBP1 controls fatty acid biosynthesis. They are synthesized as inactive, membrane-bound precursors and are proteolytically activated if cellular sterol concentrations are low. These soluble, active SREBPs are then transported into the nucleus and bind to sterol regulatory elements (SREs), thus facilitating transcription of target genes such as the LDL-receptor or HMG-CoA

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reductase. Additionally, SREBPs may be activated by phosphorylation by MAP kinases [11].

Several nuclear receptors interact with the SREBP system to further control sterol and fatty acid metabolism. Liver X receptor (LXR) is a nuclear receptor that binds various oxidized cholesterol derivatives. It forms heterodimers with retinoic acid receptors (RXR) and activates several genes involved in cholesterol metabolism [12]. Cholesterol oxidation by cholesterol-7α-hydroxylase (*cyp7a*) is the rate-limiting step in bile acid synthesis and therefore cholesterol elimination. The *cyp7a* gene is repressed by a feedback activation of the farnesoyl X receptor (FXR) which acts via activation of the nuclear receptor "small heterodimeric binding partner" and also by activation of fibroblast growth factor 19 (*fgf-19*) and its receptor FGFR-4. *Klotho*, which encodes a membrane-bound, putative glycosidase, is also a repressor of *cyp7a* expression [13]. Additionally, cholesterol homeostasis is under the control of hormones such as the insulin system. Insulin increases LDL-R- and HMG CoA reductase expression via activation of the SREBPs [14] and down-regulates *cyp7a* expression via HNF-transcription factors [15].

In this study, we used the human hepatoma cell line HepG2 as a model system to analyze whether the hypocholesterolemic effects of curcumin can be explained by alterations of the expression of genes that are involved in cholesterol/lipid homeostasis in liver cells. Furthermore, the expression of key regulators of cholesterol homeostasis was analyzed to gain insight into the molecular mechanisms underlying the curcumin effects.

2. Materials and methods

2.1. Cell culture and incubation conditions

HepG2 cells, which were obtained from ATCC (via Promochem, Wesel, Germany), were cultured in RPMI 1640 medium (Gibco, Karlsruhe, Germany) supplemented with 10% fetal bovine serum (Gibco) and gentamicin (50 mg/L) at 37°C and 5% CO_2 in 25-cm^2 flasks. Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione] was obtained from Merck (Darmstadt, Germany) and was dissolved directly into the growth medium (RPMI 1640, Gibco) to circumvent the effects of the commonly used solvents, ethanol or DMSO, on gene expression: 50 ml of medium with curcumin, corresponding to 500 μM, was shaken at 37°C for 24–48 h. After centrifugation, the resulting curcumin concentration was measured photometrically at 435 nm and compared to a curcumin standard, diluted from DMSO-dissolved curcumin (100 mM). For gene expression studies, 7×10^5 cells were plated per well of a 24-well plate. After 24 h, the medium was substituted by dilutions of the curcumin solution from 50 to 2 μM and medium for control. Cells were incubated for 24 h for RNA isolation and for 24–48 h for viability tests. Experiments were performed in six replicates. Cell viability was controlled by optical inspection and by using the MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide) viability test [16]. Absorption was measured using a microplate reader (Tecan Spectra Fluor Plus, Crailsheim, Germany) at 595 nm.

2.2. RNA isolation and cDNA synthesis

Culture medium was removed and 250 μl of Trizol Reagent (Invitrogen, Karlsruhe, Germany) was added per well for RNA extraction according to the manufacturer's protocol. For reverse transcription, 1.2 μg RNA was incubated with 0.125 pmol T_{18} primer (Operon, Cologne, Germany), 0.4 mM dNTPs (GeneCraft, Muenster, Germany) and 60 U Revert Aid reverse transcriptase (MBI Fermentas, St. Leon-Roth, Germany). Reverse transcription was performed at 42 °C for 1 h, followed by 10 min at 70 °C using a thermocycler.

2.3. Primer design

Primers for RT-PCR were designed using the sequence entries from GenBank (Table 1). To exclude amplification of contaminating genomic DNA, primers were usually designed to overlap intron spanning mRNA regions. Amplification products were in the range from 300 to 550 bp. For primer design, Primer Select software ver. 3.11 (DNASTAR, GATC Biotech, Konstanz, Germany) was

Table 1
Primer pairs used in RT-PCR experiments

Gene	Primer sequence	Amplicon	Annealing temperature
β-actin	forward: gagcgggaaatcgtgcgtgac reverse: gcctagaagcatttgcggtggac	518 bp	60 °C
ABCG1	forward: ccgtgcgctttgtgctgtttg reverse: caatgaggcggagggagatgaag	405 bp	60 °C
Fatty acid binding protein (FABP-1)	forward: agggagctctattgccaccat reverse: actttctcccctgtcattgtctc	255 bp	60 °C
Fatty acid translocase FAT/CD36	forward: gggctatagggatccatttttg reverse: cctttcagattaacgtcggattc	321 bp	55 °C
Farnesyl diphosphate synthase (FDPS)	forward: ggaattgatggcgagaaggagcac reverse: cgcgcaagccccagaaagac	386 bp	60 °C
Hydroxymethyl glutaryl CoA reductase (HMG-CoA reductase)	forward: taccatgtcaggggttacgtc reverse: caagcctagagacataatcatc	247 bp	55 °C
LDL-receptor	forward: ccccgcagatcaaaccccactc reverse: agacccccaggcaaaggaagacga	369 bp	60 °C
LXR-α	forward: tcagccgggaggaccagattg reverse: ccggaggctcaccagtttcattag	405 bp	65 °C
SREBP1	forward: gtggcggctgcattgagagtgaag reverse: aggtacccgagggcatccgagaat	362 bp	60 °C
SREBP2	forward: cgccacctgcccctctccttcc reverse: tgccctgccacctatcctctcacg	390 bp	65 °C

used. Primers were purchased from Operon (Cologne, Germany) or MWG (Ebersberg, Germany).

2.4. Quantitative real-time PCR

Real-time PCR was performed with the Brilliant SYBR Green QPCR Master Mix (Stratagene, Amsterdam, The Netherlands) according to the manufacturer's instructions in a final volume of 15 μl. Amplification was performed on a DNA Engine OPTICON real-time thermocycler (MJ-Research, Biozym-Diagnostik, Hessisch Oldendorf, Germany). Melting curves and agarose gel electrophoresis were used to control the specificity of the PCR. The amplification efficiency was determined using a dilution series of the PCR products.

mRNA amount was calculated in relation to the housekeeping gene β-actin, and relative expression values of the control experiments were set to 100%. Data of all experiments were pooled and used for calculation of expression values relative to the control experiment and standard error. Kruskal–Wallis one-way analysis of variance (ANOVA) by ranks and multiple comparisons according to Dunn's method were performed using the SigmaStat software (ver. 2.0) to determine statistical significance.

2.5. Reporter gene expression studies

Analysis of the activity of the LDL-receptor promoter was performed by reporter gene assays using the dual luciferase reporter gene system. For transfection experiments, cells were plated at 1.3×10^5 cells/well in a 96-well non-crosstalk plate (Greiner, Frickenhausen Germany). pHLDLR1 and pHLDLR4 [14] and phRL-CMV (Promega, Heidelberg, Germany) were cotransfected in a 10:1 ratio using the FuGENE 6 transfection reagent (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. pAP1-Luc and pRARE-TA-SEAP were obtained from Clontech (Heidelberg, Germany) and used as described above for pHLDLR vectors.

Dual luciferase assays were either performed using the PROMEGA Dual-Luciferase Reporter Assay System following the manufacturer's instructions or as described in Ref. [17] in a Mithras multilabel reader (Berthold Technologies, Bad Wildbad, Germany). Alkaline phosphatase assays were performed with the chemiluminescent substrate 3-(4-methoxyspiro(1,2-dioxetane-3,2′(5′-chloro)tricyclo [3,3.1.13.7]decan)-4-yl) phenyl phosphate (CSPD) (Calbiochem, Schwalbach, Germany). One hundred microliters of the reagent (Tris/Cl 50 mM, pH 9.8, $MgCl_2$ 5 mM, NaCl 150 mM, CSPD 0.125 mM) was injected into 20 μl of culture medium and the luminescence determined in a Mithras reader (Berthold) for 10 s several times within 15 minutes.

2.6. Western blot analysis

For short-term (5 min to 2 h) experiments, HepG2 cells were grown to confluence in 24-well cell culture plates and then serum starved for 2 days in 1 ml of fresh RPMI medium. Curcumin was dissolved in the medium and added to final concentrations ranging from 2 to 50 μM. In control wells, the equivalent amount of medium was added. Fetal calf serum (10%) and human insulin (Sigma, Deisenhofen, Germany) were added as positive controls. To investigate the curcumin effects on stimulated cells, curcumin was added at 20 μM concentration 60 min before the cells were stimulated with serum (10%) or insulin for further 30 min. For long-term experiments (24 h), medium was replaced with fresh medium containing fetal calf serum (10%) and curcumin at concentrations ranging from 1 to 50 μM, as described for the gene expression experiments.

Cells were lysed by shaking in Tris/Cl (50 mM, pH 6.8) containing sodium dodecyl sulfate (SDS) (2%), sodium vanadate (1 mM) and ocadaic acid (0.1 μM) and proteinase inhibitor mix (1×) (Sigma). Sodium dodecyl sulfate sample buffer was added and protein samples (20 μl) subjected to denaturing SDS-polyacrylamide gel electrophoresis (10%). Proteins were transferred to nitrocellulose (BA85, Schleicher and Schuell, Dassel, Germany) by semidry blotting in a Trans-Blot SD cell (BioRad, Munich, Germany) in a blotting buffer (CAPS 50 mM, 3-mercaptopropionic acid 1 mM, methanol 10%, pH 10).

After staining with poinceau red and blocking with Tris buffered saline (Tris/Cl 50 mM, NaCl 150 mM, pH 7.5), containing bovine serum albumin (2%), sodium azide (0.03 mM) and NP40 (0.2%), the blots were incubated with primary antisera overnight at 4°C. Phospho-specific, rabbit monoclonal antibodies for p42/44-MAPK (ERK), p38-MAPK, and Akt-kinase were obtained from Cell Signalling Technology (Beverly, MA, USA), and αSREBP2 SREBP2 (H-164) from Santa Cruz Biotechnology (Heidelberg, Germany). After washing, detection was performed using a secondary antibody coupled to peroxidase (Dianova, Hamburg, Germany). Chemiluminescence was detected with hydrogen peroxide and luminol (both 2.5 mM) as substrate and 40 μM p-coumaric acid as enhancer in Tris–HCl (50 mM, pH 8.5), using a Fuji LAS 3000 imaging system (Raytest, Straubenhardt, Germany) and quantified using the

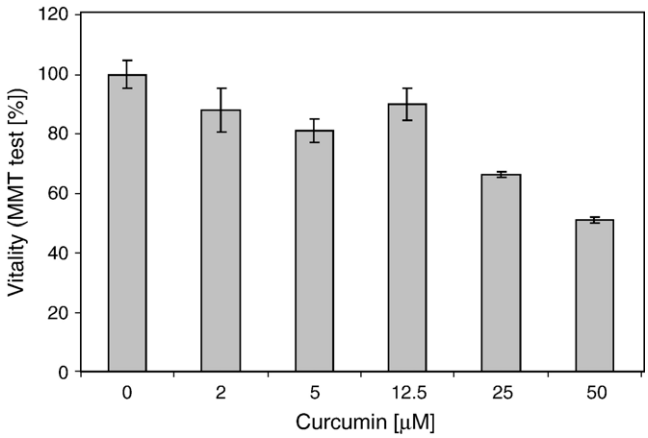

Fig. 1. Cell viability assay using the MTT test as described in Material and Methods. Data are given as mean±S.D. of three experiments with six replicates for each concentration.

Table 2
mRNA accumulation determined by real-time RT-PCR

	Control	2 μM	11.25 μM	25 μM	50 μM
LDL-R	1.00±0.28	0.85±0.16	1.56±0.35*	3.24±1.80*	7.38±2.37*
HMG-CoA reductase	1.00±0.41	1.15±0.46	0.97±0.29	0.70±0.26	2.57±0.86*
FDPS	1.00±0.28	1.71±0.45	1.74±0.80	1.87±0.77	2.36±1.07*
SREBP1	1.00±0.31	1.09±0.31	1.46±0.66	1.15±0.44	1.26±0.38
SREBP2	1.00±0.23	0.99±0.24	1.50±0.64	1.91±1.03	1.87±0.59*
CD36	1.00±0.42	0.66±0.21	1.07±0.55	0.51±0.28	0.34±0.21*
FABP1	1.00±0.28	1.10±0.23	1.23±0.43	0.72±0.35	0.18±0.05*
LXRα	1.00±0.30	1.50±0.91	2.62±1.43*	2.16±0.96	1.79±0.61
ABCG1	1.00±0.40	2.60±0.88*	2.43±1.45*	1.31±0.81	0.96±0.74

Relative mRNA amount to actin in the controls was set to 1.

* Statistically significant at $P < .05$ (ANOVA) in pooled data of all experiments performed. Standard error of the mean is given from three experiments with six replicates each.

AIDA software package (ver. 3.5, Raytest). Blots were normalized in the same way, using anti-GAPDH antiserum (Upstate, Biomol, Hamburg, Germany).

3. Results

In the first set of experiments, we determined the effects of curcumin on the vitality of HepG2 cells. Based on the MTT cell viability assay [16], cells tolerated curcumin up to 12.5 μM for 24 h. Significantly decreased viability became evident at 25 and 50 μM of curcumin (Fig. 1).

To understand the molecular basis of the hypocholesterolemic effect of curcumin, we analyzed the mRNA accumulation of selected genes of cellular lipid homeostasis by RT-PCR at curcumin concentrations ranging from 2 to 50 μM (Table 2). The LDL-receptor mRNA accumulated significantly and concentration dependently up to sevenfold above the expression level in control cells. The transcripts of HMG-CoA reductase and farnesyl pyrophosphate synthase (FDPS) enzymes, which are directly involved in cholesterol biosynthesis, accumulated only twofold above the values observed in untreated cells and this was only significant at 50 μM curcumin. An even lower accumulation was observed for the transcripts of the two SREBP genes, which regulate the expression of the LDL-receptor as well as of HMG-CoA reductase and FDPS. LXRα-mRNA, which encodes a regulator of several ABC transporters, was significantly induced at intermediate curcumin concentrations. Transcript levels of fatty acid binding protein 1 (FABP1) and the fatty acid translocase CD36, which both encode proteins involved in cellular lipid transport and are targets of PPARs, were strongly reduced at toxic curcumin concentrations but remained unchanged at lower curcumin concentrations.

We used LDL-receptor promoter-luciferase constructs [14] to determine curcumin effects on the LDL-receptor promoter. In a control experiment, the effect of curcumin on the dual luciferase reporter assay by transfection of SV40 promoter-driven firefly luciferase and SV40 promoter-driven *Renilla* luciferase was determined. Two different incubation periods were analyzed: 5 h after treatment, no effect of curcumin on the promoter fragment was evident; 24 h after addition, however, the activity of both reporter genes was strongly reduced with higher curcumin concentrations and the ratio of firefly to renilla luciferase decreased for all firefly

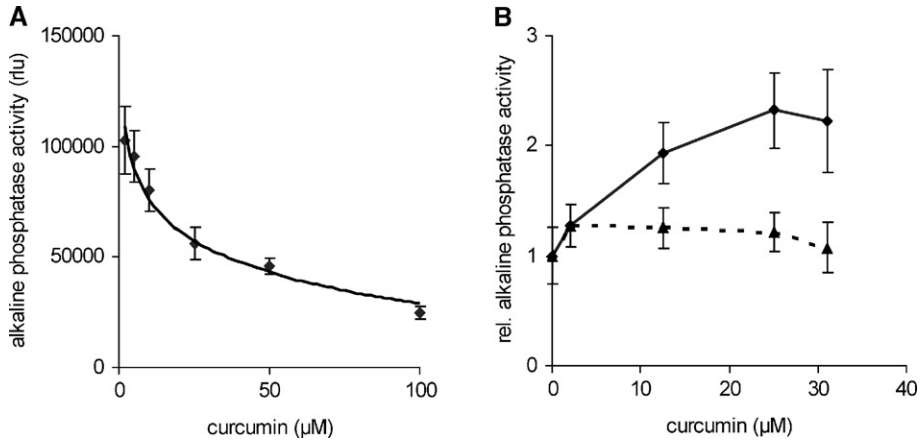

Fig. 2. (A) Inhibition of alkaline phosphatase by curcumin. Cell culture medium containing fetal calf serum (10%) was used as source for alkaline phosphatase. Activity was determined in triplicate using CSPD as substrate as described in Material and Methods and expressed as relative light units (rlu). (B) Alkaline phosphatase activity in the medium of HepG2 cells transfected with the RARE-SEAP reporter vector. Alkaline phosphatase was determined using CSPD as substrate, and the light emission (dashed line) was corrected for inhibition by curcumin (solid line) using the data from (A). The result of a representative experiment is shown. Data are given as mean of six replicates±S.D.

luciferase reporter vectors tested (data not shown). Thus, the dual luciferase assay was disturbed by the curcumin treatment and could not be used to determine the effects of curcumin on promoter activity.

In the case of experiments using the RARE-SEAP reporter gene, we observed an inhibition of serum alkaline phosphatase by curcumin with an IC_{50} of 30 μM (Fig. 2A) in control experiments. On the basis of the inhibitor curves of alkaline phosphatase by curcumin, we corrected the inhibitory effect of curcumin in transfection experiments. These data showed an activation of RARE-SEAP activity with increasing amounts of curcumin added to the medium (Fig. 2B).

4. Discussion

Several studies have been conducted to determine the effects of curcumin on gene expression, mainly focusing on its antiproliferative potential in colon cancer cells [24,25]. In this study, we intended to evaluate the molecular basis of the described hypocholesterolemic effects [5,8] of dietary curcumin in the human hepatoma cell line HepG2 on the level of gene expression. This cell line has been used as a model system in numerous studies on cholesterol and lipoprotein metabolism in human hepatocytes [11,26,27]. However, these hepatoma cells, although maintaining many aspects of hepatocyte physiology, exhibit certain differences compared to hepatocytes [28], which might cause different responsiveness to exogenous compounds. Nevertheless, unlimited availability and reproducibility has made HepG2 cells an established model for cholesterol and lipoprotein metabolism.

We analyzed the response curcumin at micromolar concentrations reaching from 2 to 50 μM. Such concentrations are widely used, although systemic bioavailability of curcumin is low [18–21]. Most authors could detect curcumin effects at concentrations between 2 and 10 μM [22–25] depending on the effect and cell type analyzed. Garcea et al. [20] observed curcumin concentrations in plasma of up to 2 μM after oral intake of very high amounts of curcumin. It was also found that bioavailability depended on the formulation and other compounds present in the actual meal [29], thus higher concentrations might be possible, depending on the actual composition of food. Nevertheless, most pharmacokinetic studies in humans pointed out that only low micromolar levels of curcumin can be found in blood.

Curcumin could act in several ways to lower plasma LDL-bound cholesterol. First, uptake of cholesterol in the gastrointestinal tract could be inhibited; second, LDL-cholesterol could be eliminated from the blood via LDL-receptor; and, finally, the activity of cholesterol-degrading enzymes, namely, cholesterol-7-hydroxylase, could be increased. In this study, we focused on the effect of curcumin on hepatoma cells, following the hypothesis that curcumin effects on plasma cholesterol are due to changes in hepatic cholesterol homeostasis.

Indeed, curcumin caused changes in gene expression in HepG2 cells that are consistent with the hypocholesterolemic effects of dietary curcumin, assuming that these changes are associated with altered protein levels and activity. The observed strong increase in LDL-R mRNA and the negligible increase in FDPS- and HMG-CoA reductase mRNA levels should result in a higher net uptake of LDL-cholesterol from plasma. It remains to be shown whether the observed changes in mRNA accumulation indeed correlate with changes in protein expression and activity. In the case of mouse macrophages, a correlation between curcumin-induced accumulation of LDL-receptor mRNA and protein content was recently described [30,31].

To further analyze the molecular basis of this effect, we determined the expression of regulator genes of lipid metabolism and selected target genes. As SREBP genes themselves contain SREs, an increase in SREBP expression is a marker for increased amounts of active SREBP protein in the nucleus. Only the mRNA of SREBP-2 was significantly increased in curcumin-treated cells and only at toxic concentrations. The strong increase in LDL-receptor mRNA, especially at lower curcumin concentrations, can hardly be explained by this moderately enhanced SREBP-2 expression. Additionally, we could not detect changes in active SREBP-1 and SREBP-2 after 25 μM curcumin treatment by Western blotting (data not shown).

Targets of PPARα (CD36 and FABP1) were significantly down-regulated at high curcumin concentrations. This observation is in accordance with the described correlation of elevated SREBP activity and decreased PPARα activity [35].

In contrast, at low micromolar concentrations, which might be reached under physiological conditions, a significant accumulation of LXRα mRNA and also a significant increase in expression of the ATP-binding cassette transporter ABCG1 [32], which is a direct target of LXRα, were observed. ABCG1 as well as ABCA1 is ubiquitously expressed in cholesterol and phospholipid efflux carrier, and high levels of expression are found in macrophages, liver and intestinal cells. Their function is crucial for cellular lipid homeostasis in these cell types [33]. ABCG1 was selected for our study because this gene responds much stronger to LXR activation in HEPG2 cells than the ABCA1 gene [34], which is frequently used as a marker for LXR activation in macrophage cell lines. We assumed that ABCG1 mRNA levels should be a more sensitive indicator for LXRα activation than ABCA1 expression in HepG2 cells. Higher expression of ABCA1 and ABCG1 is expected to cause increased HDL-dependent lipid efflux, thus increasing plasma HDL-cholesterol concentrations, which was indeed observed by Arafa [8] in rats that were fed with a curcumin-containing diet.

As LXR obligatorily heterodimerizes with the retinoid acid receptor RXR [12] and RXR ligands are potent inducers of many sterol-metabolizing pathways, we tested for activation of the retinoic acid response element (RARE)

by curcumin. Indeed, a reporter gene construct consisting of the RARE element and secreted alkaline phosphatase was activated by curcumin already at concentrations of 2–10 μM. Unexpectedly, we found that curcumin was an effective inhibitor of alkaline phosphatases in plasma, assayed by using the luminescent substrate CSPD. If curcumin can also inhibit specific intracellular protein phosphatases it would interfere with protein phosphorylation. It has previously been shown that curcumin interferes with protein phosphorylation events [36–38] and an inhibition of protein phosphatases might contribute to these effects. Interestingly, berberine, a novel cholesterol-lowering compound, activates LDL-receptor expression by increasing the phosphorylated form of ERK [39]. However, we were not able to detect an influence of curcumin on the amount of phosphorylated MAP-kinases ERK and p38 and on protein kinase b (Akt) (data not shown). Under serum-starved conditions, curcumin did not alter phosphorylation status of the kinases, and also after stimulation with serum or insulin no reduction or further stimulation was observed. Therefore, we consider it unlikely that curcumin acts via activation of MAP-kinase pathways.

This study was performed to evaluate the basis of the hypocholesterolemic effect of curcumin in a liver model on the level of gene expression. The major effect of curcumin was a concentration-dependent increase in LDL-receptor mRNA, which became significant at 10 μM curcumin, which was nontoxic to the HepG2 cells. At this concentration, no increased accumulation of the mRNA amount of cholesterol biosynthetic enzymes was observed, but LXRα and ABCG1 expression and RXR activity were also elevated. At 50 μM curcumin, where a significant decrease of vitality occurred, SREBP-2 mRNA was increased and the expression of PPAR-target genes was lowered. This observation is consistent with the proposed activation of the SREBP system by activated caspases, which occurs when cells undergo apoptosis [40]. Nevertheless, these effects appear nonphysiological for liver cells due to the low bioavailability of curcumin. Also, we could not find evidence for a contribution of MAP-kinase pathways to the curcumin effects. Nevertheless, the observed activation of LXR and RXR that occurred at concentrations that can be reached by oral consumption of curcumin holds for a contribution of nuclear receptors to the hypocholesterolemic effect of curcumin.

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References

[1] Joe B, Vijaykumar M, Lokesh BR. Biological properties of curcumin-cellular and molecular mechanisms of action. Crit Rev Food Sci Nutr 2004;44:97–111.
[2] Egan ME, Pearson M, Weiner SA, Rajendran V, Rubin D, Gloeckner-Pagel J, et al. Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects. Science 2004;304:600–2.
[3] Lin JK. Suppression of protein kinase C and nuclear oncogene expression as possible action mechanisms of cancer chemoprevention by curcumin. Arch Pharm Res 2004;27:683–92.
[4] Ramirez-Tortosa MC, Mesa MD, Aguilera MC, Quiles JL. Oral administration of a turmeric inhibits LDL oxidation and has hypocholesterolemic effects in rabbits with experimental atherosclerosis. Atherosclerosis 1999;147:371–8.
[5] Asai A, Miyazawa T. Dietary curcuminoids prevent high-fat diet-induced lipid accumulation in rat liver and epididymal adipose tissue. J Nutr 2001;131:2932–5.
[6] Lodha R, Bagga A. Traditional Indian systems of medicine. Ann Acad Med Singapore 2000;29:37–41.
[7] Zhang W, Liu D, Wo X, Zhang Y, Jin M, Ding Z. Effects of *Curcuma longa* on proliferation of cultured bovine smooth muscle cells and on expression of low density lipoprotein receptor in cells. Chin Med J (Engl) 1999;112:308–11.
[8] Arafa HMM. Curcumin attenuates diet-induced hypercholesterolemia in rats. Med Sci Monit 2005;11:BR228–34.
[9] Kapoor GS, Atkins BA, Mehta KD. Activation of Raf-1/MEK-1/2/p42/44(MAPK) cascade alone is sufficient to uncouple LDL receptor expression from cell growth. Mol Cell Biochem 2002;236:13–22.
[10] Osborne TF. Sterol regulatory element-binding proteins (SREBPs): key regulators of nutritional homeostasis and insulin action. J Biol Chem 2000;275:32379–82.
[11] Kotzka J, Lehr S, Roth G, Avci H, Knebel B, Muller-Wieland D. Insulin-activated Erk-mitogen-activated protein kinases phosphorylate sterol regulatory element-binding protein-2 at serine residues 432 and 455 in vivo. J Biol Chem 2004;279:22404–11.
[12] Repa JJ, Mangelsdorf DJ. The role of orphan nuclear receptors in the regulation of cholesterol homeostasis. Annu Rev Cell Dev Biol 2000;16:459–81.
[13] Ito S, Fujimori T, Furuya A, Satoh J, Nabeshima Y, Nabeshima Y. Impaired negative feedback suppression of bile acid synthesis in mice lacking βKlotho. J Clin Invest 2005;115:2202–8.
[14] Streicher R, Kotzka J, Muller-Wieland D, Siemeister G, Munck M, Avci H, et al. SREBP-1 mediates activation of the low density lipoprotein receptor promoter by insulin and insulin-like growth factor-I. J Biol Chem 1996;271:7128–33.
[15] de Fabiani E, Crestani M, Marrapodi M, Pinelli A, Golfieri V, Galli G. Identification and characterization of cis-acting elements conferring insulin responsiveness on hamster cholesterol 7a-hydroxylase gene promoter. Biochem J 2000;347:147–54.
[16] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55–63.
[17] Dyer BW, Ferrer FA, Klinedinst DK, Rodriguez R. A noncommercial dual luciferase enzyme assay system for reporter gene analysis. Anal Biochem 2000;282:158–61.
[18] van Erk MJ, Teuling E, Staal YC, Huybers S, van Bladeren PJ, Aarts JM, et al. Time- and dose-dependent effects of curcumin on gene expression in human colon cancer cells. J Carcinog 2004;3:1–17.
[19] Chen HW, Yu SL, Chen JJW, Yang PC. Anti-invasive gene expression profile of curcumin in lung adenocarcinoma based on a high throughput microarray analysis. Mol Pharmacol 2004;65:99–110.
[20] Garcea G, Jones DJL, Singh R, Dennison AR, Farmer PB, Sharma RA, et al. Detection of curcumin and its metabolites in hepatic tissue and portal blood of patients following oral administration. Br J Cancer 2004;90:1011–5.

[21] Sharma RA, McLelland HR, Hill KA, Ireson CR, Euden SA, Manson MM, et al. Pharmacodynamic and pharmacokinetic study of oral *Curcuma* extract in patients with colorectal cancer. Clin Cancer Res 2001;7:1894–900.

[22] Gertsch J, Guttinger M, Heilmann J, Sticher O. Curcumin differentially modulates mRNA profiles in jurkat T and human peripheral blood mononuclear cells. Bioorg Med Chem 2003;11: 1057–63.

[23] Hong J, Bose M, Ju J, Ryu J-H, Chen X, Sang S, et al. Modulation of arachidonic acid metabolism by curcumin and related b-diketone derivatives: effects on cytosolic phospholipase A2, cyclooxygenases and 5-lipoxygenase. Carcinogenesis 2004;25: 1671–9.

[24] Jana NR, Dikshit P, Goswami A, Nukina N. Inhibition of proteasomal function by curcumin induces apoptosis through mitochondrial pathway. J Biol Chem 2004;279:11680–5.

[25] Jeong W-S, Kim I-W, Hu R, Kong A-NT. Modulation of AP-1 by natural chemopreventive compounds in human colon ht-29 cancer cell line. Pharm Res 200421649–60.

[26] König B, Eder K. Differential action of 13-HPODE on PPARα downstream genes in rat Fao and human HepG2 hepatoma cell lines. J Nutr Biochem 2006 [in press].

[27] Sirtori CR, Lovati MR, Manzoni C, Castiglioni S, Duranti M, Magni C, et al. Proteins of white lupin seed, a naturally isoflavone-poor legume, reduce cholesterolemia in rats and increase LDL receptor activity in HepG2 cells. J Nutr 2004;134:18–23.

[28] Wilkening S, Stahl F, Bader A. Comparison of primary human hepatocytes and hepatoma cell line HEPG2 with regard to their biotransformation properties. Drug Metab Dispos 2003;31: 1035–42.

[29] Sharma RA, Gescher AJ, Steward WP. Curcumin: the story so far. Eur J Cancer 2005;41:1955–68.

[30] Fan CL, Qian Y, Wo XD, Yan J, Gao LP. Effect of curcumin on the gene expression of low density lipoprotein receptors. Chin J Integr Med 2005;11:201–4.

[31] Fan C, Wo X, Qian Y, Yin J, Gao L. Effect of curcumin on the expression of LDL receptor in mouse macrophages. J Ethnopharmacol 2006;105:251–4.

[32] Kennedy MA, Venkateswaran A, Tarr PT, Xenarios I, Kudoh J, Shimizu N, et al. Characterization of the human ABCG1 gene. J Biol Chem 2001;276:39438–47.

[33] Schmitz G, Langmann T. Transcriptional regulatory networks in lipid metabolism control ABCA1 expression. Biochim Biophys Acta 2005; 1735:1–19.

[34] Albers M, Blume B, Schlueter T, Wright MB, Kober I, Kremoser C, et al. A novel principle for partial agonism of liver X receptor ligands. Competitive recruitment of activators and repressors. J Biol Chem 2006;281:4920–30.

[35] Kersten S, Desvergne B, Wahli W. Roles of PPARs in health and disease. Nature 2000;405:421–4.

[36] Leu TH, Maa MC. The molecular mechanisms for the antitumorigenic effect of curcumin. Curr Med Chem Anti-Canc Agents 2002;2: 357–70.

[37] Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res 2003;23:363–98.

[38] Lin JK. Suppression of protein kinase C and nuclear oncogene expression as possible action mechanisms of cancer chemoprevention by curcumin. Arch Pharm Res 2004;27:683–92.

[39] Kong W, Wei J, Abidi P, Lin M, Inaba S, Li C, et al. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nat Med 2004;10:1344–51.

[40] Higgins ME, Ioannou YA. Apoptosis-induced release of mature sterol regulatory element-binding proteins activates sterol-responsive genes. J Lipid Res 2001;42:1939–46.