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ORIGINAL CONTRIBUTION

Ralph Rühl
Ronny Sczech
Nico Landes
Paul Pfluger
Dirk Kluth
Florian J. Schweigert

Carotenoids and their metabolites are naturally occurring activators of gene expression via the pregnane X receptor

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Dr. R. Rühl (✉) · R. Sczech · F. J. Schweigert
Institute of Nutritional Science
University of Potsdam
Arthur-Scheunert-Allee 114–116
14558 Potsdam-Rehbrücke, Germany
Tel.: +49-172/317-0905
Fax: +49-33200/88-553
E-Mail: ralphruehl@web.de

N. Landes · P. Pfluger · D. Kluth
Dept. of Vitamins and Atherosclerosis
German Institute of Human Nutrition (DIfE)
Potsdam-Rehbrücke, Germany

R. Rühl
Present address: Dept. of Biochemistry & Molecular Biology
University of Debrecen
Debrecen, Hungary

Summary Carotenoids are important micronutrients in the human diet and are present in human serum at micromolar concentrations. In addition to their antioxidant potential, carotenoids obtain physiologically relevant properties such as influencing cellular signal pathways, gene expression or induction of detoxifying enzymes. In this study, we determined the transactivation of PXR by cotransfection with the full-length receptor and a PXR-responsive reporter gene. Carotenoids and retinol revealed a 5–6-fold reporter gene activity in HepG2 cells in comparison to a 7-fold induction by the well known PXR agonist rifampicin whereas apo-carotenals and lycopene exerted less or no activation potential. The inductive efficacy was hereby concentration-dependent. In addition, carotenoid or retinol mediated gene expression of PXR responsive genes like CYP3A4/CYP3A7, CYP3A5, MDR-1 and MRP-2 has been determined in HepG2 cells by RT-PCR with upregulative properties of β-carotene or retinol being comparable or even higher than that of rifampicin. In conclusion, PXR-mediated upregulation of CYP3A4/CYP3A7 and CYP3A5 as well as MDR1 and MRP2 by carotenoids points to a potential interference on the metabolism of xenobiotic and endogenous relevant compounds.

Key words β-carotene – retinol – PXR – nuclear receptor – carotenoid

Introduction

Carotenoids are a substance class which is widely distributed in flora and fauna. Plenty of plants and a few animals serve as human nutrients containing high concentrations of carotenoids. Several carotenoids are metabolized to retinoids in the mammalian organism either by central cleavage via the β,β-carotene-15,15'-oxygenase [1] or by an exocentric cleavage mediated by a lipoxygenase or free radicals, and hence represent a source for vitamin A [2]. Chylomicron remnants in plasma carry the carotenoids which may be converted to retinoids in the liver or are incorporated into very low density lipoproteins (VLDL), and thus be again transported to peripheral cells [3]. Carotenoids are capable of scavenging free radicals, inhibition of lipid peroxidation [4], modulation of immune functions [5] and activation of various cytochromes [6, 7].

In addition to the antioxidant properties of carotenoids, their metabolites and vitamin A derivates are able to serve as ligands for nuclear hormone receptors and thereby control gene expression [8]. All-*trans* and 9-*cis*-retinoic acid are known to activate the retinoic acid receptor (RAR) and the retinoid X receptor (RXR), which are transcription factors interacting with retinoic acid response elements (RARE) in the promotor region of retinoic acid-sensitive genes [3]. As structurally related ligands, phytol metabolites like phytanic acid or phytenic acid, are also able to stimulate the RXR at physiological relevant concentrations [9].

The RXR has the ability to heterodimerize with sev-

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eral other receptors of the nuclear hormone receptor superfamily including the pregnane X receptor (PXR), the constitutive androstane receptor (CAR), the peroxisome proliferator-activated receptors (PPARs) and the vitamin D receptor (VDR) [10, 11]. The PXR is a promiscuous nuclear receptor that has evolved to protect the organism from toxic substances. In vertebrate species, PXR is abundantly expressed in liver and, to a lesser extent, in colon, lung and intestine [12]. These are the same tissues in which CYP (cytochrome P450) 3A genes are mainly expressed. PXR mediates the effects of a wide range of structurally diverse xenobiotics as well as endogenous substances on CYP3A gene induction resulting in hydroxylation of various drugs and endogenous substances [13]. Frequently, these xenobiotics are also substrates for the inducible CYPs, and therefore enhance their own metabolism via autoregulation [14]. The diverse collection of xenobiotics activating the PXR includes also lipophilic compounds like bile acids and steroids [15]. PXR also regulates ABC transporters including MDR (multidrug resistance protein) 1 [16] and MRP (multidrug resistance-associated protein) 2 [17, 18] as well as glutathione S-transferase A2 [19] and sulfotransferases [20].

The aim of the study was to investigate whether β-carotene, lycopene, retinol and apo-carotenals obtain activating potential comparable to other nutritionally relevant substances like vitamin E [21] on the PXR using transiently transfected PXR reporter cell lines. In addition to the PXR receptor activating potential the mediation of several PXR responsive genes will be determined in HepG2 cells by PCR (polymerase chain reaction) techniques to clarify the involvement of carotenoids in cytochrome induction [6, 7] via PXR responsive pathways.

Materials and methods

Materials

Cell culture media and fetal calf serum were purchased from Biochrom AG (Berlin, Germany). All carotenoids were kindly provided by K. Krämer and H. Ernst (BASF, Ludwigshafen, Germany). CAT (chloramphenicol acetyltransferase) assay was purchased from Molecular Probes (Leiden, The Netherlands). Additional compounds were purchased from Merck KGaA (Darmstadt, Germany) if not indicated otherwise. PCR primers were purchased from Sigma-Ark GmbH (Darmstadt, Germany). PCR materials were purchased from Eppendorf AG (Hamburg, Germany).

Cell culture

HepG2 cells were maintained in RPMI 1640 medium supplemented with 10 % fetal calf serum. The cell line was cultured in 150 cm^2 tissue culture flasks at 37 °C in 5 % CO_2 atmosphere with a relative humidity of 95 %.

Expression and reporter plasmids

The plasmid pSG5-hPXR contains the human PXR cloned into the pSG5 expression vector (Stratagene, Amsterdam, The Netherlands). The reporter plasmid pCAT-DR3 containing the CAT was constructed by cloning two copies of the CYP3A1 PXR binding site followed by a tk promoter into the pBL-CAT2 basic vector. Both plasmids were kindly provided by S. Kliewer (Southwestern Medical-Center, University of Texas, Dallas, TX). The plasmid pCH110 (Amersham, Freiburg, Germany) expressing β-galactosidase was used as internal control for normalizing transfection efficiency.

Transient transfections and reporter gene assays

Cells were transiently transfected in 24 well plates by using 3 µl TfX-20 reagent (Promega, Hamburg, Germany) per µg DNA according to the manufacturer's instruction; in detail 0.5 µg/well PCH110, 0.17 µg/well pCAT-DR3 and 0.17 µg/well pSG5-hPXR were used. After 1 h of incubation at 37 °C, normal medium enriched with carotenoids, retinol or apo-carotenals (each 10 µM) was added up to 1 ml and incubation continued for 48 h. β-Carotene and lycopene were applicated as beadlets dissolved in water, apo-carotenals and retinol in DMSO solution. Control experiments using DMSO or beadlets as a vehicle without carotenoids or retinol exerted an equivalent base level induction indicated as control in the figures. The beadlets powder consists of the embedded carotenoid-nano-particles (~10 %) in a matrix of fish-gelatin, glucose sirup and d,l-alpha tocopherol, sodium ascorbate as antioxidants in an overall amount of 1.5 %, respectively, for the antioxidants. The amounts of d,l-alpha tocopherol was shown to be nonrelevant for PXR mediated activation [21].

Cells were harvested by using 100 µl reporter lysis buffer (Promega, Hamburg, Germany) per well. To 40 µl lysate 10 µl component A (1/10) and 10 µl acetyl coenzym A (7.8 mg/ml) were added according to the manufacturer's instructions (FAST CAT Green, Molecular Probes, Leiden, The Netherlands). After 3 h incubation at 37 °C the samples were extracted with ethyl acetate and evaporated to dryness. The residue was redissolved in the HPLC eluent containing 55 % acetonitrile and 45 % water. The amounts of fluorescence-labeled acetylated chloramphenicol were subsequently measured by a

HPLC system using a 1046A fluorescence detector (Hewlett Packard, Waldbronn, Germany) and a 250 × 4 mm RP18 column with a flow of 0.6 ml/min (SepServ, Berlin, Germany) as described [21].

For the β-galactosidase assay 40 µl lysate and 150 µl assay buffer (100 mM sodium phosphate buffer pH 7.5; 1 mM magnesium chloride; 50 mM 2-mercaptoethanol; 2.92 mM 2-nitrophenyl-β-D-galactopyranoside) were added to a translucent 96 well plate followed by 3 h incubation at 37 °C. The plate was subsequently analyzed in a BioRad 550 microplate reader measuring absorption at 405 nm (BioRad, Munich, Germany).

RT-PCR

HepG2 cells were seeded in 6 well plates and grown to 80 % confluency. Cell culture medium enriched with carotenoids or rifampicin (all 10 µM) was added and incubation continued for 48 h. Total RNA was isolated using the RNAGold solution from PeqLab (Erlangen, Germany). The first strand cDNA was synthesized in a 25 µl reaction from 3 µg RNA by reverse transcription with MMLV-RT (Promega, Hamburg, Germany) using 100 ng random hexamers. Subsequently, 2 µl of the cDNA was amplified using a Taq-Polymerase system (Promega, Hamburg, Germany). PCR samples were denaturized for 4 min at 94 °C, then cycled at 94 °C for 40 s, annealed at 58 °C or 60 °C for 1 min and elongationed at 72 °C for 2 min. The details are presented in Table 1. All mRNA amplifications were tested to be in the linear range. The degree of CYP3A4/CYP3A7, CYP3A5, MRP2 and MDR1 induction was calculated relative to β-actin mRNA expression of the respective sample measured by densitometry of ethidium bromide stained PCR products in 1.5 % agarose gel (MultiAnalyst, Biorad, Munich, Germany). Experiments with 0.1 % DMSO as a control sample were set as 100 % relative expression. Control experiments using DMSO or beadlets as a vehicle without carotenoids or retinol exerted an equivalent base level induction indicated as control in Fig. 5.

Statistics

Standard deviations have been calculated with MS-Excel using student's t-test, a value of $P < 0.05$ indicated statistical significance.

Results

Transcriptional activation of PXR by carotenoids in HepG2 cells

We investigated the transactivational effect of several carotenoids (Fig. 1) on the PXR response element reporter construct pCAT-DR3, co-transfected with plasmids expressing the human PXR and the β-galactosidase as internal control in HepG2 cells. Incubation for 48 h with 10 µM carotenoid, retinoid or rifampicin as positive control revealed a significant ($P < 0.05$) stimulation of CAT activity except for lycopene in the following ranking: rifampicin > β-carotene > retinol > apo-8-carotenal > apo-12-carotenal ≈ apo-4-carotenal > lycopene (Fig. 2). β-Carotene and retinol revealed the strongest effects with a 5–6.5-fold induction, respectively. In contrast apocarotenals formed from β-carotene exerted a lower induction on PXR (2.5- to 3.5-fold), whereas lycopene showed no significant induction at all. Omitting PXR in the transfected system decreased the total induction level of transcription but did not alter the relative inductions because of the sufficient amount of endogenous PXR in HepG2 cells (Fig. 3). Overexpression was not necessary but increased the sensitivity of the assay. Dose response experiments elucidated a slower decline of induction for rifampicin compared to β-carotene and retinol, which lost their induction at 0.5 µM (Fig. 4).

mRNA expression in HepG2 cells in response to carotenoids

A most reliable indicator of PXR activation is the induction of CYP3A-type enzymes. The induction of the human relevant subtypes CYP3A4/CYP3A7 and CYP3A5

Table 1

Gene	Cycles	Tm	Sequence	Position	GenBank	Ref.
β-Actin	23	60°C	caagagatggccacggctgct tccttctgcatcctgtcggca	714–734 968–988	X00351	[50]
MDR1	25	58°C	aaaaagatcaactcgtaggagtg gcacaaaatacaccaacaa	2108–2131 2250–2269	AF016535	[51]
MRP2	30	58°C	cttcggaaatccaagatcctgg tagaattttgtgctgttcacattct	4391–4412 4650–4674	NM_000392	[52]
CYP3A4/CYP3A7	32	60°C	gggaagcagagacaggcaag gagcgtttcattcaccacca	553–572 1141–1160	M14096	[53]
CYP3A5	28	60°C	gaagaaaagtcgcctcaac aagaagtccttgcgtgtcta	855–873 1533–1552	J04813	[54]

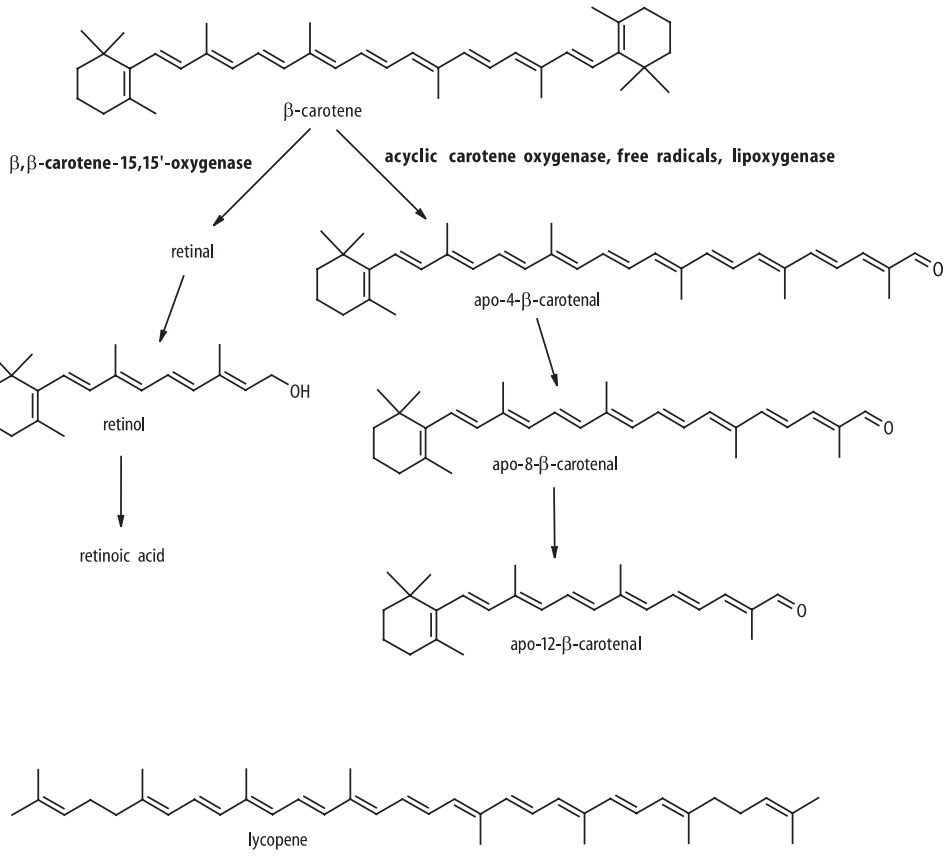

Fig.1 Metabolic pathway of β-carotene to retinol via central cleavage by a 15,15′-β,β-carotene oxygenase or to apo-carotenals via asymmetric cleavage by a different carotene oxygenase, a lipoxygenase or free radicals and the carotenoid lycopene

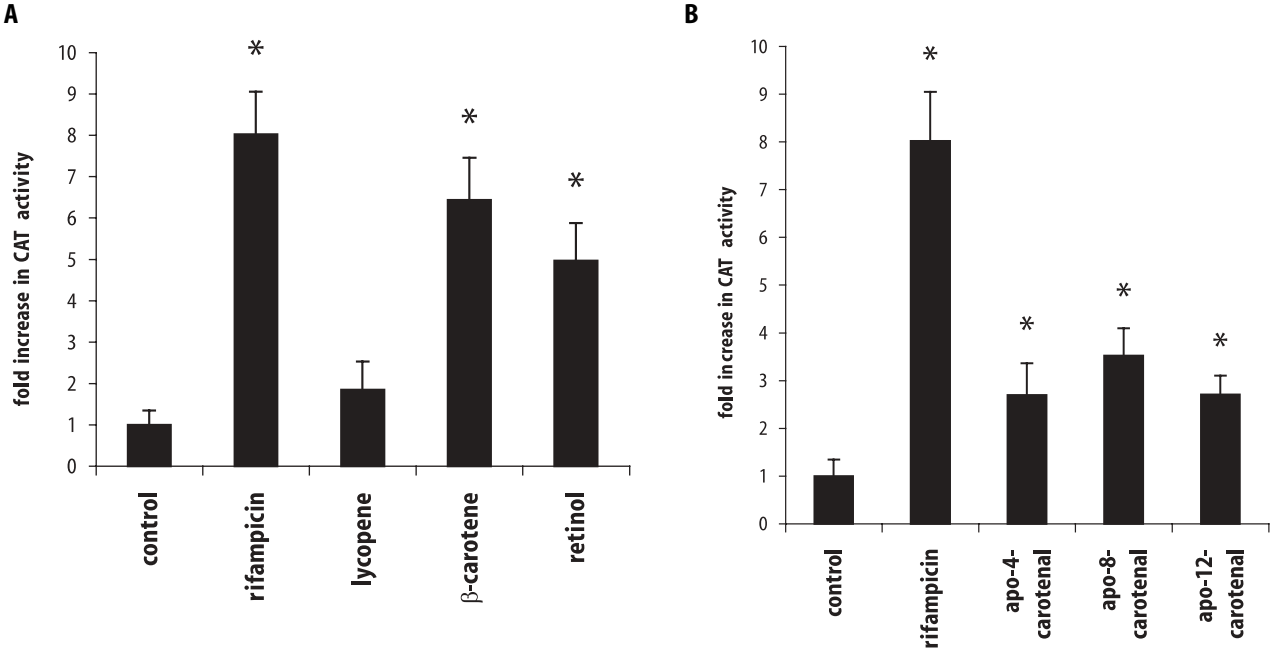

Fig. 2 Transactivation of PXR by several carotenoids. HepG2 cells were transiently transfected with plasmids expressing the human PXR (pSG5hPXR), the $(CYP3A1)_2$-tk-CAT reporter and β-galactosidase (pCH110) as transfection control. Cells were treated with 10 μM of different carotenoids and rifampicin. Data are means ± SD from 4 different experiments each performed in triplicate (* indicates $P < 0.05$ versus control). **A** Most inducing carotenoids β-carotene and retinol in comparison with rifampicin and control without carotenoid. **B** Rifampicin and apo-carotenals relative to control without carotenoid

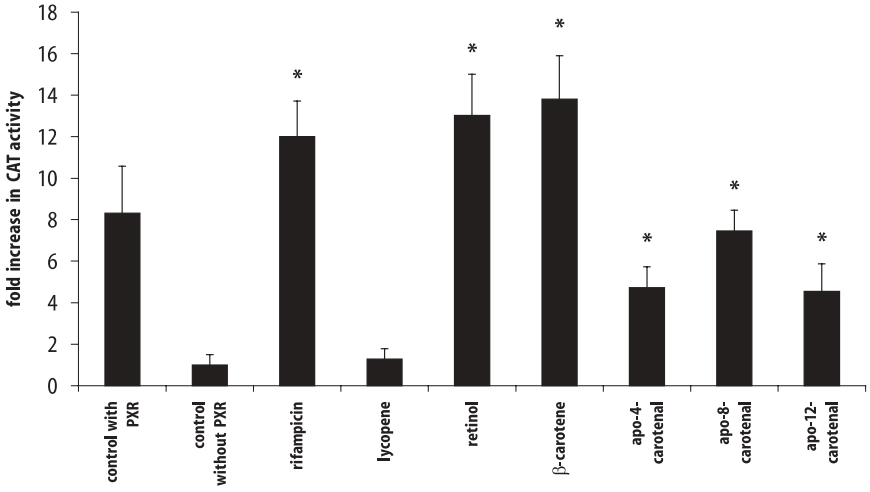

Fig. 3 Transactivation of the $(CYP3A1)_2$-tk-CAT reporter construct without the PXR expressing plasmid in comparison to Fig. 2. To compare the sensitivity of the assays the control from Fig. 2 was added to this figure (control with PXR). β-galactosidase expressing plasmid (pCH110) was used as transfection control (* indicates $P < 0.05$ versus control without PXR)

by β-carotene, retinol and lycopene was tested in comparison to rifampicin. Primers chosen for CYP3A4/7 mRNA additionally recognize CYP3A7 mRNA. After 48 h exposure to several chemicals the expression of the CYP3A genes was analyzed using standard RT-PCR. The expression of CYP3A4 mRNA increased significantly ($P < 0.05$) in HepG2 cells exposed to β-carotene and rifampicin relative to the endogenous levels of expression (control). β-Carotene, rifampicin and retinol had significant ($P < 0.05$) effects on the expression of CYP3A5 mRNA (Fig. 5).

Additionally, the mRNA induction of multi-drug resistance protein 1 (MDR1) as well as the multidrug resistance-associated protein 2 (MRP2) was examined by RT-PCR. Treatment with rifampicin and β-carotene enhanced significantly ($P < 0.05$) the expression of MDR1 mRNA in HepG2 cells. In contrast, the expression of MRP2 mRNA was elevated significantly ($P < 0.05$) by lycopene as well as rifampicin overtopped by β-carotene.

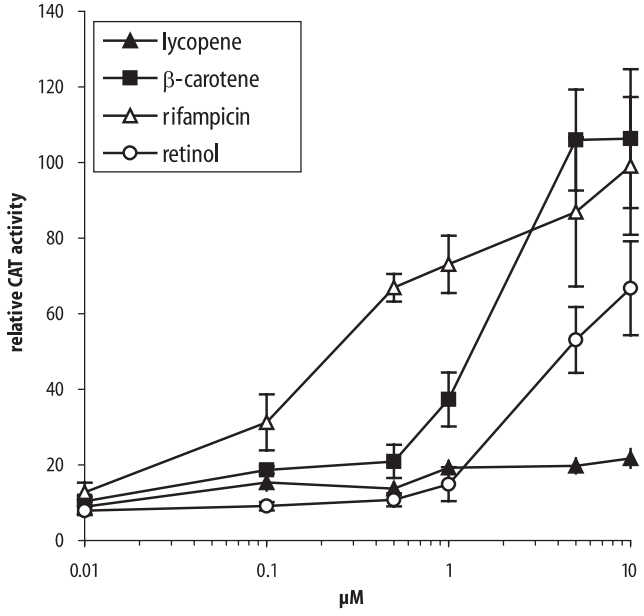

Fig. 4 Concentration-dependent activation of PXR with lycopene (black rhombs), β-carotene (black squares), rifampicin (open triangles) and retinol (open circles). HepG2 cells were treated as described in Fig. 2. Data represent means ± SD from 3 different experiments, each performed in triplicate

Discussion

PXR represents an important component in the body's defense mechanism against hydrophobic xenobiotics and endogenous substances such as bile acids [22]. Metabolizing enzymes like CYP3A4 and CYP3A5 are well known regulated targets of PXR activation. In addition target genes like MDR1 [16], MRP2 [18], glutathione S-transferase A2 [19] and sulfotransferase A2 [20] are regulated by PXR. Our data have shown the first time that β-carotene or retinol are able to activate PXR comparable to the well known PXR-inducer rifampicin. PXR can be activated also by various chemicals including pregnenolone 16α-carbonitrile (PCN), phenobarbital [23] as well as vitamin E [21]. PXR and other nuclear receptors like CAR (constitutive androstane receptor) cross talk and serve as xenobiotic sensors to form a safety net against toxic effects of harmful substances [24].

Concentrations in human serum are between 0.34–0.54 μM for β-carotene [25] and between 1.37–2.18 μM for retinol [26]. An average liver concentration of 4.4 μM (range from 0–19.4 μM) for β-carotene [27] and between 1.57–4.84 μM for retinol [28] has been observed. From these data it could be postulated that endogenous and nutritionally relevant concentrations of β-carotene and retinol in the organism have the ability to induce PXR (Figs. 2 and 3) dependent transcription of PXR-regulated genes. Due to a weak knowledge of human β-carotene levels after β-carotene supplementation

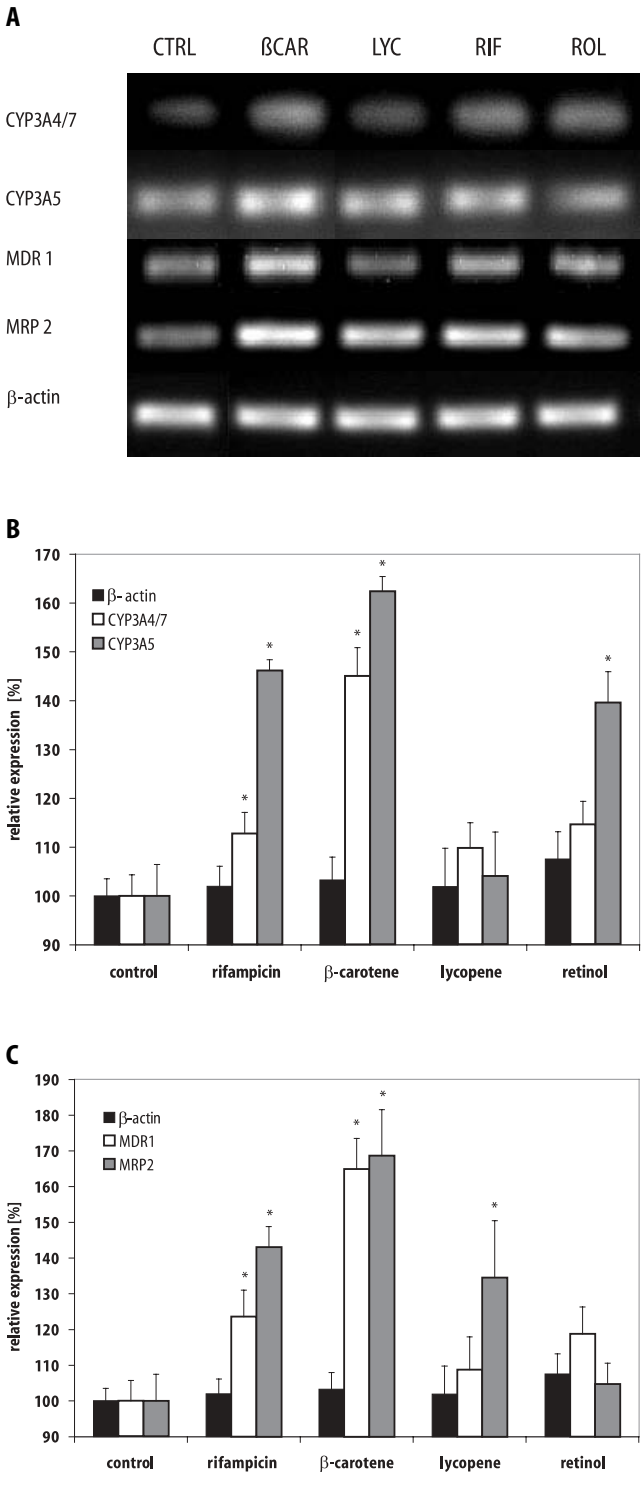

Fig. 5 Expression of mRNA in HepG2 cells treated with several carotenoids; **A** representative agarose gel of the result of RT-PCR stained with ethidium bromide (*CTRL* control; *βCAR* β-carotene; *LYC* lycopene; *RIF* rifampicin; *ROL* retinol); **B**, **C** densiometric analysis from different RT-PCR experiments (n = 4) representing mean ± SD (* indicates $P < 0.05$ versus control)

the human in vivo relevance of β-carotene mediated alteration of PXR responsive genes remains elusive.

β-Carotene could be converted mainly by two different types of β-carotene oxygenases, a cyclic [1, 29, 30] and an acyclic [31] cleavage enzyme. β-Carotene metabolites found in the organism or in cell culture are mainly retinal, retinoic acid or retinol (Fig. 1) or acyclic cleavage products like apo-carotenals [32]. Due to the low or undetectable concentrations of apo-carotenals and low induction properties of retinol possible activation of PXR in vivo may mainly be mediated directly or indirectly by β-carotene.

Xenobiotic metabolism plays a crucial role for human health. Cytochromes are the major enzymes in the activation or deactivation of xenobiotica or endogenous substances. The data presented in this paper demonstrate that expression of CYP3A4/7 and CYP3A5 in HepG2 cells is markedly induced by β-carotene and retinol indicating possible positive health effects of β-carotene or retinol on the activation/deactivation of a wide array of xenobiotica [33]. Additional regulated target genes of the PXR are the ABC transporters MDR1 and MRP2 which are involved in cellular drug excretion [16, 18]. ABC transporters are involved in the transport of various xenobiotics, various glutathione, glucuronate and sulfate conjugates and peptides [34–38].

Carotenoids have not yet been shown to be metabolized by CYP3A enzymes, but carotenoid metabolites like all-trans-retinoic acid are a target for this class of enzymes [39–41]. Retinoic acid metabolism is enhanced by coadministration of the PXR-inducer rifampicin, indicating a potential mechanism via PXR activated pathways [39]. In addition supplementation of β-carotene to ferrets enhances in vitro all-trans-retinoic acid catabolism via induction of CYPs [42]. In contrast CYP3A4 has also been associated with an increased generation of all-trans-retinoic acid by retinol [43].

In contrast to the beneficial effects also prooxidative effects of carotenoids could be explained. In the ATBC [44] and CARET study [45] tobacco smokers with additional β-carotene supplementation showed higher incidence of lung cancer. In contrast moderate and high dose supplementations with lycopene substantially inhibited smoke-induced squamous metaplasia [46]. PXR expression in the lung [47] and possible upregulation of CYPs [48] after β-carotene supplementation could be related with high concentrations of CYP induced stable or semi-stable substrate radicals [49]. An alternative pathway of adverse effects of CYP3A activation by carotenoids in humans would be the induction of high levels of CYPs that predispose an individual to cancer risk by metabolizing more tobacco-smoke xenobiotics to harmful carcinogens [7]. Lycopene has shown no effect on PXR mediated increase of CYP3A expression and additionally shown no increased risk for tobacco smoke induced squamous metaplasia [46]. In

summary, beneficial or adverse effects of β-carotene via PXR mediated mechanisms on human health are possibly relevant and should be clarified in detail in *in vivo* experiments.

In summary, β-carotene and retinol activate PXR responsive pathways such as the xenobiotica and endogenous substance metabolising cytochromes CYP3A4/CYP3A7 and CYP3A5 and the ABC transporters (MDR1/MRP2). Retinol and β-carotene have been determined as possibly important nutritionally relevant PXR activators interfering with the metabolic system of the organism.

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References

1. Redmond TM, Gentleman S, Duncan T, Yu S, Wiggert B, Gantt E, Cunningham FX Jr (2001) Identification, expression, and substrate specificity of a mammalian beta-carotene 15.15'-dioxygenase. J Biol Chem 276:6560–6565
2. Wolf G (2001) The enzymatic cleavage of beta-carotene: end of a controversy. Nutr Rev 59:116–118
3. Silveira ER, Moreno FS (1998) Natural retinoids and β-carotene: end of a controversy. J Nutr Biochem 9:446–456
4. Stahl W (2000) Lipid oxidation and antioxidants. Curr Opin Clin Nutr Metab Care 3:121–126
5. Chew BP (1995) Antioxidant vitamins affect food animal immunity and health. J Nutr 125:1804S-1808S
6. Astorg P, Gradelet S, Leclerc J, Siess MH (1997) Effects of provitamin A or non-provitamin A carotenoids on liver xenobiotic-metabolizing enzymes in mice. Nutr Cancer 27:245–249
7. Paolini M, Antelli A, Pozzetti L, Spetlova D, Perocco P, Valgimigli L, Pedulli GF, Cantelli-Forti G (2001) Induction of cytochrome P450 enzymes and overgeneration of oxygen radicals in beta-carotene supplemented rats. Carcinogenesis 22:1483–1495
8. Kliewer SA, Lehmann JM, Willson TM (1999) Orphan nuclear receptors: shifting endocrinology into reverse. Science 284:757–760
9. Kitareewan S, Burka LT, Tomer KB, Parker CE, Deterding LJ, Stevens RD, Forman BM, Mais DE, Heyman RA, McMorris T, Weinberger C (1996) Phytol metabolites are circulating dietary factors that activate the nuclear receptor RXR. Mol Biol Cell 7:1153–1166
10. Aranda A, Pascual A (2001) Nuclear hormone receptors and gene expression. Physiol Rev 81:1269–1304
11. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P et al. (1995) The nuclear receptor superfamily: the second decade. Cell 83: 835–839
12. Bertilsson G, Heidrich J, Svensson K, Asman M, Jendeberg L, Sydow-Backman M, Ohlsson R, Postlind H, Blomquist P, Berkenstam A (1998) Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. Proc Natl Acad Sci USA 95:12208–12213
13. Waxman DJ (1999) P450 gene induction by structurally diverse xenochemicals: central role of nuclear receptors CAR, PXR, and PPAR. Arch Biochem Biophys 369:11–23
14. Goodwin B, Redinbo MR, Kliewer SA (2002) Regulation of cyp3a gene transcription by the pregnane x receptor. Annu Rev Pharmacol Toxicol 42:1–23
15. Kliewer SA, Willson TM (2002) Regulation of xenobiotic and bile acid metabolism by the nuclear pregnane X receptor. J Lipid Res 43:359–364
16. Geick A, Eichelbaum M, Burk O (2001) Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. J Biol Chem 276: 14581–14587
17. Payen L, Sparfel L, Courtois A, Vernhet L, Guillouzo A, Fardel O (2002) The drug efflux pump MRP2: regulation of expression in physiopathological situations and by endogenous and exogenous compounds. Cell Biol Toxicol 18: 221–233
18. Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM, Tontonoz P, Kliewer S, Willson TM, Edwards PA (2002) Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. J Biol Chem 277:2908–2915
19. Falkner KC, Pinaire JA, Xiao GH, Geoghegan TE, Prough RA (2001) Regulation of the rat glutathione S-transferase A2 gene by glucocorticoids: involvement of both the glucocorticoid and pregnane X receptors. Mol Pharmacol 60:611–619
20. Sonoda J, Xie W, Rosenfeld JM, Barwick JL, Guzelian PS, Evans RM (2002) Regulation of a xenobiotic sulfonation cascade by nuclear pregnane X receptor (PXR). Proc Natl Acad Sci USA 99: 13801–13806
21. Landes N, Pfluger P, Kluth D, Birringer M, Ruhl R, Bol GF, Glatt H, Brigelius-Flohe R (2003) Vitamin E activates gene expression via the pregnane X receptor. Biochem Pharmacol 65:269–273
22. Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, Liu Y, Klaassen CD, Brown KK, Reinhard J, Willson TM, Koller BH, Kliewer SA (2001) The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. Proc Natl Acad Sci USA 98:3369–3374
23. Xie W, Barwick JL, Downes M, Blumberg B, Simon CM, Nelson MC, Neuschwander-Tetri BA, Brunt EM, Guzelian PS, Evans RM (2000) Humanized xenobiotic response in mice expressing nuclear receptor SXR. Nature 406:435–439
24. Cai Y, Konishi T, Han G, Campwala KH, French SW, Wan YJ (2002) The role of hepatocyte RXR alpha in xenobiotic-sensing nuclear receptor-mediated pathways. Eur J Pharm Sci 15:89–96
25. Brown ED, Micozzi MS, Craft NE, Bieri JG, Beecher G, Edwards BK, Rose A, Taylor PR, Smith JC Jr (1989) Plasma carotenoids in normal men after a single ingestion of vegetables or purified beta-carotene. Am J Clin Nutr 49: 1258–1265
26. Gueguen S, Herbeth B, Siest G, Leroy P (2002) An isocratic liquid chromatographic method with diode-array detection for the simultaneous determination of alpha-tocopherol, retinol, and five carotenoids in human serum. J Chromatogr Sci 40:69–76
27. Schmitz HH, Poor CL, Gugger ET, Erdman JW Jr (1993) Analysis of carotenoids in human and animal tissues. Methods Enzymol 214:102–116
28. Schmidt CK, Brouwer A, Nau H (2003) Chromatographic analysis of endogenous retinoids in tissues and serum. Anal Biochem 315:36–48

29. von Lintig J, Wyss A (2001) Molecular analysis of vitamin A formation: cloning and characterization of beta-carotene 15,15'-dioxygenases. Arch Biochem Biophys 385:47–52
30. Wyss A, Wirtz GM, Woggon WD, Brugger R, Wyss M, Friedlein A, Riss G, Bachmann H, Hunziker W (2001) Expression pattern and localization of beta,beta-carotene 15,15'-dioxygenase in different tissues. Biochem J 354:521–529
31. Kiefer C, Hessel S, Lampert JM, Vogt K, Lederer MO, Breithaupt DE, von Lintig J (2001) Identification and characterization of a mammalian enzyme catalyzing the asymmetric oxidative cleavage of provitamin A. J Biol Chem 276: 14110–14116
32. Yeum KJ, Lee-Kim YC, Yoon S, Lee KY, Park IS, Lee KS, Kim BS, Tang G, Russell RM, Krinsky NI (1995) Similar metabolites formed from beta-carotene by human gastric mucosal homogenates, lipoxygenase, or linoleic acid hydroperoxide. Arch Biochem Biophys 321: 167–174
33. Moore JT, Kliewer SA (2000) Use of the nuclear receptor PXR to predict drug interactions. Toxicology 153:1–10
34. Loo TW, Clarke DM (1999) Merck Frosst Award Lecture 1998. Molecular dissection of the human multidrug resistance P-glycoprotein. Biochem Cell Biol 77:11–23
35. Paulusma CC, Kothe MJ, Bakker CT, Bosma PJ, van Bokhoven I, van Marle J, Bolder U, Tytgat GN, Oude Elferink RP (2000) Zonal down-regulation and redistribution of the multidrug resistance protein 2 during bile duct ligation in rat liver. Hepatology 31:684–693
36. Buchler M, Konig J, Brom M, Kartenbeck J, Spring H, Horie T, Keppler D (1996) cDNA cloning of the hepatocyte canalicular isoform of the multidrug resistance protein, cMrp, reveals a novel conjugate export pump deficient in hyperbilirubinemic mutant rats. J Biol Chem 271:15091–15098
37. Schrenk D, Baus PR, Ermel N, Klein C, Vorderstemann B, Kauffmann HM (2001) Up-regulation of transporters of the MRP family by drugs and toxins. Toxicol Lett 120:51–57
38. Evers R, Kool M, van Deemter L, Janssen H, Calafat J, Oomen LC, Paulusma CC, Oude Elferink RP, Baas F, Schinkel AH, Borst P (1998) Drug export activity of the human canalicular multispecific organic anion transporter in polarized kidney MDCK cells expressing cMOAT (MRP2) cDNA. J Clin Invest 101: 1310–1319
39. Lampen A, Meyer S, Arnhold T, Nau H (2000) Metabolism of vitamin A and its active metabolite all-trans-retinoic acid in small intestinal enterocytes. J Pharmacol Exp Ther 295:979–985
40. Howell SR, Shirley MA, Ulm EH (1998) Effects of retinoid treatment of rats on hepatic microsomal metabolism and cytochromes P450. Correlation between retinoic acid receptor/retinoid x receptor selectivity and effects on metabolic enzymes. Drug Metab Dispos 26:234–239
41. Marill J, Cresteil T, Lanotte M, Chabot GG (2000) Identification of human cytochrome P450s involved in the formation of all-trans-retinoic acid principal metabolites. Mol Pharmacol 58: 1341–1348
42. Liu C, Russell RM, Wang XD (2003) Exposing ferrets to cigarette smoke and a pharmacological dose of beta-carotene supplementation enhance in vitro retinoic acid catabolism in lungs via induction of cytochrome P450 enzymes. J Nutr 133:173–179
43. Chen H, Howald WN, Juchau MR (2000) Biosynthesis of all-trans-retinoic acid from all-trans-retinol: catalysis of all-trans-retinol oxidation by human P-450 cytochromes. Drug Metab Dispos 28: 315–322
44. (1994) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. N Engl J Med 330:1029–1035
45. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL Jr, Valanis B, Williams JH Jr, Barnhart S, Cherniack MG, Brodkin CA, Hammar S (1996) Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. J Natl Cancer Inst 88:1550–1559
46. Liu C, Lian F, Smith DE, Russell RM, Wang XD (2003) Lycopene supplementation inhibits lung squamous metaplasia and induces apoptosis via up-regulating insulin-like growth factor-binding protein 3 in cigarette smoke-exposed ferrets. Cancer Res 63:3138–3144
47. Zhang H, LeCulyse E, Liu L, Hu M, Matoney L, Zhu W, Yan B (1999) Rat pregnane X receptor: molecular cloning, tissue distribution, and xenobiotic regulation. Arch Biochem Biophys 368: 14–22
48. Liu C, Wang XD, Bronson RT, Smith DE, Krinsky NI, Russell RM (2000) Effects of physiological versus pharmacological beta-carotene supplementation on cell proliferation and histopathological changes in the lungs of cigarette smoke-exposed ferrets. Carcinogenesis 21: 2245–2253
49. Wolf G (2002) The effect of beta-carotene on lung and skin carcinogenesis. Carcinogenesis 23:1263–1265
50. Sumida A, Fukuen S, Yamamoto I, Matsuda H, Naohara M, Azuma J (2000) Quantitative analysis of constitutive and inducible CYPs mRNA expression in the HepG2 cell line using reverse transcription-competitive PCR. Biochem Biophys Res Commun 267: 756–760
51. Renes, JW (2000) (Patho)physiological function of the multidrug resistance protein MRP1. Reiksuniversiteit Groningen, Groningen, The Netherlands
52. Nies AT, Konig J, Pfannschmidt M, Klar E, Hofmann WJ, Keppler D (2001) Expression of the multidrug resistance proteins MRP2 and MRP3 in human hepatocellular carcinoma. Int J Cancer 94: 492–499
53. Bowen WP, Carey JE, Miah A, McMurray HF, Munday PW, James RS, Coleman RA, Brown AM (2000) Measurement of cytochrome P450 gene induction in human hepatocytes using quantitative real-time reverse transcriptase-polymerase chain reaction. Drug Metab Dispos 28:781–788
54. Jover R, Bort R, Gomez-Lechon MJ, Castell JV (2001) Cytochrome P450 regulation by hepatocyte nuclear factor 4 in human hepatocytes: a study using adenovirus-mediated antisense targeting. Hepatology 33:668–675