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Different mRNA expression profiling of nuclear retinoid, thyroid, estrogen and PPARgamma receptors, their coregulators and selected genes in rat liver and spleen in response to short-term *in vivo* administration of 13-*cis* retinoic acid

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ABSTRACT

Retinoic acids (RAs) and also their analogs (synthetic retinoids and rexinoids) have been regarded as major therapeutic and/or chemopreventive agents and can regulate a number of diverse processes—such as immune system, hormonal systems. In this work we describe different effects of short-term treatment of Wistar male rats with 13-*cis* retinoic acid on the regulation of retinoic acid receptors (RARs), retinoid-X receptors (RXRs), thyroid hormone receptors (TRs), ERs, 5′-DI, EGFR and erb-B2/neu genes in liver and/or spleen. Using RT-PCR analysis we have found that administration of 13-*cis* retinoic acid enhanced expression of RARβ and PPARγ mRNA, and decreased expression of RARα, RARγ, RXRβ and TRβ mRNA in liver. On the other hand, in spleen this treatment resulted in decreased expression of RARα, RARβ, RARγ, TRα and ERβ mRNA. Our findings indicate distinct modulation of various signal pathways by short-term administration of 13cRA, which also differ in spleen when compared to liver. We suggest that even a short-term treatment of rats with 13cRA may affect a reasonable number of steps in retinoid signaling pathways, a number of which might be very likely extended by long-term treatment of mammals by 13-*cis* retinoic acid.

1. Introduction

Retinoic acids (RAs) – all-*trans* (ATRA), 9-*cis* (9cRA) and 13-*cis* retinoic acid (13cRA) – and also their analogs (synthetic retinoids and rexinoids), thyroid hormones, steroids and vitamin D_3 can regulate a number of diverse processes as homeostasis, reproduction, development, differentiation, and oncogenesis (Morris-Kay, 1992). These actions are mediated through their cognate nuclear receptors. The steroid/thyroid/retinoid/vitamin D nuclear receptors belong to a superfamily of ligand-modulated transcription factors, which link extracellular signals directly to transcriptional responses. In fact, nuclear receptors represent one of the largest transcription factor families known (Beato, 1989; Evans, 1988). The receptors share a highly conserved cysteine-rich C-region, the DNA-binding domain (DBD) that forms two zinc finger structures that allow protein–DNA as well as protein–protein interaction (Luisi et al., 1991). A second, but less well conserved region is found in all receptors in the hydrophobic carboxy-terminal part and is usually referred to as ligand-binding domain (LBD). In addition to ligand recognition, this domain encodes either receptor dimerization and transactivation or repressor functions (Forman et al., 1989; Zhang et al., 1991a,b). The nuclear receptors mediate their action by binding to specific DNA sequences (response elements) of target genes as homo- or heterodimers (Beato, 1989; Pfahl et al., 1994) or by interacting with other factors, notably the transcription factor AP-1 (Pfahl, 1993).

RAs and their synthetic derivatives can induce the expression of the β-form of all-*trans* retinoic acid receptor (RARβ) and can also activate this receptor. The effects of ATRA on retinoic acid receptor (RAR) proteins stability were described in numerous cell lines (Andela and Rosier, 2004; Boudjelal et al., 2002; Gianni et al., 2002; Pratt et al., 2003; Tanaka et al., 2001). Retinoids have been regarded as major therapeutic and/or chemopreventive agents for skin disorders and many types of cancers, including human breast cancers (Hong and Sporn, 1997; Lotan, 1996; Miller, 1998; Singh and Lippman, 1998; Wu et al., 2002). Isotretinoin (13cRA) is widely used for oral treatment of severe recalcitrant nodular acne and is promising chemopreventive or chemotherapeutic agent for various types of cancers (Jaeckle et al., 2003; Reynolds and Lemons, 2001; Wieder et al., 2002). Long-term chronic administration of 13cRA often resulted in hypertriglyceridemia (Gerber and Erdman, 1982), dry skin and cheilitis (Farrell et al., 1980), ocular side effects

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(Egger et al., 1995) or hypocalcaemia (Villablanca et al., 1993). 13cRA, which has been demonstrated to retain the most of the pharmacological activities of retinoic acids, is better tolerated for *in vivo* administration (Goodman, 1984). It has been reported that 13cRA enhanced methionine catabolism by activation and induction of hepatic glycin *N*-methyltransferase (Rowling and Schalinske, 2001). Retinoid-mediated hypomethylation of DNA has significant implications due to link between DNA methylation and a number of important processes, such as gene expression and development (Hoffman, 1984, 1990; Wainfan and Poirier, 1992).

Retinoids exert their action on the properties of thyroid hormone receptors (TRs) at either a transcriptional or a post-translational level. It was shown that an inadequate level of dietary retinol results in a decreased level of TRs mRNA and subsequently in the decreased binding capacity of TRs (Higueret et al., 1989; Pailler-Rodde et al., 1991). The selenoenzyme iodothyronine 5′-deiodinase type I (5′-DI) deiodinates the prohormone thyroxine to the biologically active thyroid hormone 3,3′,5-triiodothyronine. Since gene for 5′-DI contains in promotor-specific sequence of nucleotides (RARE and TRE) for binding of heterodimers RAR/RXR and TR/RXR, respectively (Jakobs et al., 1997a) it has been shown that 9cRA, 13cRA and ATRA are capable of inducing 5′-DI activity (Schreck et al., 1994).

Recent findings indicate that cross-talk between RA, thyroid hormones and estrogens pathways plays important roles in regulation of many processes in various tissues and it has been suggested that cancer progression may be associated with alteration in metabolism and/or signalling pathways of these components (Garcia-Solis and Aceves, 2003).

The nonsteroid receptors (RARs and TRs) actively silence basal transcription by recruiting one or more corepressors, the silencing mediator for retinoid and thyroid receptors (SMRT) and nuclear receptor corepressor (N-CoR). Ligand activation involves a dissociation of corepressors followed by recruitment of transcriptional coactivators (e.g. steroid hormone receptor coactivator 1, SRC-1) (Horlein et al., 1995; Chen and Evans, 1995).

It has already been shown that RAs and their derivatives play an important role in regulation of cell growth and differentiation (Morris-Kay, 1992) by affecting epidermal growth factor receptor (EGFR) and its family member erb-B2/neu pathway encoding transmembrane tyrosine kinase sharing high homology with the EGFR.

The expression of many genes involved in xenobiotic/drug metabolism and transport is regulated also by nuclear receptors belonging to nuclear receptor superfamily, i.e., constitutive androstane receptor (CAR), and pregnane X receptor (PXR) that are capable to cross-talk with other transcription factors inducible by thyroid hormone, retinoids, rexinoids or by dihydroxyvitamin D_3 (Pascussi et al., 2008). The xenobiotic-mediated induction of three major human liver cytochrome P450 genes, CYP2B6, CYP2C9, and CYP3A4, is known to be regulated by the CAR and the PXR that are regulated, at least in part, by the glucocorticoid receptor (GR) (Dvorak et al., 2003). This cascade and the cross-talks with other nuclear receptors thus explain how physiopathological stimuli affect xenobiotic/drug disposition, and how xenobiotics/drugs may affect physiological functions and generate toxic responses (Pascussi et al., 2008).

Retinoic acid receptors and retinoid-X receptors (RXRs) are typical nuclear receptors that are involved in essential endogenous processes and they are often targets in human pharmacotherapy (Brtko and Thalhamer, 2003). Our recent data has shown that expression of RARα, RXRα and some of their coregulators in intact rat was significantly increased in postlactating mammary gland when compared to that of nonlactating mammary tissue. Postlactating mammary glands were found to express all RAR and RXR subtypes studied when compared to nonlactating mammary tissues that express exclusively RARα and RXRα subtypes (Macejova et al., 2005).

The aim of this work was to compare the effect of short-term (five doses in 10 days) administration of 13cRA (0.8 mg/kg) on expression of RARs (α, β, γ), RXRs (α, β), TRs (α1, β), estrogen receptors (ERα, ERβ), γ-form of the peroxisome proliferator activated receptor (PPARγ), their coregulators N-CoR, SRC-1, SMRT and genes for 5′-DI, EGFR and erb-B2/neu in liver and/or spleen of male Wistar rats.

2. Materials and methods

2.1. Animals and treatment with 13-cis retinoic acid

Male Wistar rats (Dobra Voda, Slovakia) were used in this experiment. Prior to the experiments, animals were housed for 1 week four per cage in a controlled environment (22 ± 2 °C, 12 h light/dark cycle, light on at 6:00 a.m.). Food and water were available *ad libitum*. The Ethic Committee of the Institute of Experimental Endocrinology, Slovak Academy of Sciences, approved all presented experiments.

Animals were treated with 13-*cis* retinoic acid (Roaccutane®) by gavage. Each animal got five doses of retinoic acid (each dose 0.8 mg/kg in tylose), during a period of 10 days. Control rats were treated in exactly same way with tylose only. Rats were decapitated 24 h after last treatment. Liver and spleen were removed, frozen separately in liquid nitrogen and stored at −70 °C.

2.2. Reverse transcription-PCR analyses

Total RNA was isolated using Trizol® reagent according to the manufacturer's instructions. Concentration of RNA was quantified by spectrometry at 260 nm and purity was assessed from the ratio of absorbances A_{260nm}/A_{280nm}. Reverse transcription (RT) was performed with 2 μg of total RNA and the Ready-to-Go You-Prime First-Strand Beads (Amersham Pharmacia Biotech, Inc., USA) according to the manufacturer's protocol. PCR was performed in a 25 μl total volume consisting of 2 μl RT mixture, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 25 pmol of each specific gene primer set and 0.6 U of DyNAzyme II DNA polymerase (Finnzymes OY, Finland) in buffer provided by the manufacturer. After treatment of samples at 94 °C for 3 min to inactivate reverse transcriptase, PCR consisted of 35 cycles of denaturing (95 °C, 45 s), annealing (30 s), extension (72 °C, 90 s), and a final extension at 72 °C for 10 min. These conditions were proven to be in the log phase for each amplified sequence by us or were already described elsewhere (Davis and Snyderwine, 1995; Hou et al., 2002; Ohata et al., 2000). Oligonucleotide sequences of primers used in PCR as well as annealing temperatures and sizes of expected PCR products are summarized elsewhere (Macejova et al., 2005). The oligonucleotide sequences of additional primers used in PCR are PPARγ: sense 5′-CATTTCTGCTCCACACTATGAA-3′, antisense 5′-CGGGAAGGACTTTATGTATGAG-3′ (51 °C, 550 bp) and GAPDH: sense 5′-TGAACGGGAAGCTCACTGG-3′, antisense 5′-TCCACCACCCTGTTGCTGTA-3′ (60 °C, 307 bp) (Kimura et al., 2002). The PCR products were separated on 2% agarose gel and stained with ethidium bromide. The band intensities were measured using the STS 6220I Documentation System (Ultralum, USA) and normalized to the band intensity of PCR product corresponding to the housekeeper gene GAPDH.

2.3. Statistical analysis

Data are expressed as mean ± S.D. Statistical significance was assessed using an unpaired Student's *t*-test.

3. Results

In liver and spleen of male Wistar rats treated with 13-*cis* retinoic acid (13cRA, 0.8 mg/kg, under experimental conditions described in the Materials and Methods section), we compared expression of genes for all known subtypes of RARs, two subtypes of RXRs, both subtypes of TRs, estrogen receptors (ERs) and PPARγ as well as expression of EGFR, proto-oncogene erb-B2/neu, type I iodothyronine 5′-deiodinase (5′-DI) and nuclear coregulators: N-CoR, steroid hormone receptor coactivator 1 (SRC-1) and SMRT.

3.1. Liver

In liver, administration of 13cRA selectively modulated the expression of RAR genes (Fig. 1a); it down-regulated RARα and RARγ mRNAs, whereas it up-regulated RARβ mRNA when