Short Communication

The effect of all-*trans* retinoic acid and/or colchicine on expression of rexinoid and thyroid hormone nuclear receptors and their coregulators in primary rat hepatocytes

D. Macejová[1], Z. Dvořák[2], R. Vrzal[2], J. Ulrichová[2], S. Ondková[1] and J. Brtko[1]

[1] *Laboratory of Molecular Endocrinology, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárska 3, 833 06 Bratislava, Slovakia*
[2] *Institute of Medical Chemistry and Biochemistry, Faculty of Medicine, Palacký University Olomouc, Hněvotínská 3, 775 15 Olomouc, Czech Republic*

Abstract. In the present work, the effects of colchicine (COL) and/or all-*trans* retinoic acid (ATRA) on expression of rexinoid receptors (RXRs) (α, β, γ), thyroid hormone receptor α and coregulators N-CoR, SMRT and SRC-1 mRNA in primary rat hepatocytes as a model of no-proliferating cells were investigated. Treatment with these components, either alone or in combination, induced differences of the expression profiles between distinct treatment groups.

Key words: Nuclear receptors — Transcription factors — Colchicine — ATRA

Retinoic acids – all-*trans* (ATRA), 9-*cis* (9cRA) and 13-*cis* (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; Macejová et al. 2005; Ondková et al. 2006). These actions are mediated through their cognate nuclear receptors. The steroid/thyroid/retinoid/vitamin D receptors, and also other nuclear hormone receptors, represent a superfamily of ligand-modulated transcription factors which link extracellular signals directly to transcriptional responses. Moreover, rexinoid receptors (RXRs) (α, β and γ) play a crucial role as heterodimerizing partners for a number of nuclear receptors (retinoic acid receptors (RARs), thyroid hormone receptors (TRs), vitamin D_3 receptor, peroxisome proliferator activated receptors (PPARs) and orphans receptors) (Germain et al. 2006). Retinoids also exert their action on the properties of TRs at either a transcriptional or a post-translational level. Also nonsteroid receptors (RARs, RXRs and TRs) actively silence basal transcription by recruiting one or more corepressors, the silencing mediator for retinoid and thyroid (SMRT) receptors and nuclear receptor corepressor (N-CoR). Ligand activation involves a dissociation of corepressors followed by recruitment of transcriptional coactivators (e.g. SRC-1) (Chen and Evans 1995; Hörlein et al. 1995).

Retinoids have been regarded as major therapeutic and/or chemopreventive agents for skin disorders and many types of cancers, including human breast cancers (Lotan 1996; Hong and Sporn 1997; Miller 1998). Microtubule interacting agents (colchicine, nocodazol, taxol) and retinoids are used in human pharmacotherapy and possible drug interactions may occur. For instance, synergistic cytotoxicity exhibited by combination treatment with selective retinoid ligands and taxol has been reported (Vivat-Hannah et al. 2001).

In the present work we investigated the effect of colchicine (COL) and/or ATRA on expression levels of RXRs (α, β, γ), TRα and coregulators N-CoR, SMRT and SRC-1 in primary rat hepatocytes as model non-proliferating cells.

Primary rat hepatocytes were isolated by two-step collagenase perfusion according to a published protocol (Moldeus et al. 1978). Following isolation, the cells were plated on collagen-coated culture dishes using cell density 2×10^5 cells/cm^2. Williams´ medium E supplemented with 2 mmol/l L-glutamine, 10 μmol/l streptomycin, 100 U/ml penicillin, 350 nmol/l insulin, and 1 μmol/l dexamethasone, was used for culture maintenance. The medium was enriched for plating with 5% foetal calf serum (FCS) (v/v). After 4 h, culture medium was replaced by a serum-free one and the

Correspondence to: Dana Macejová, Laboratory of Molecular Endocrinology, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárska 3, 833 06 Bratislava, Slovakia
E-mail: ueenmace@savba.sk

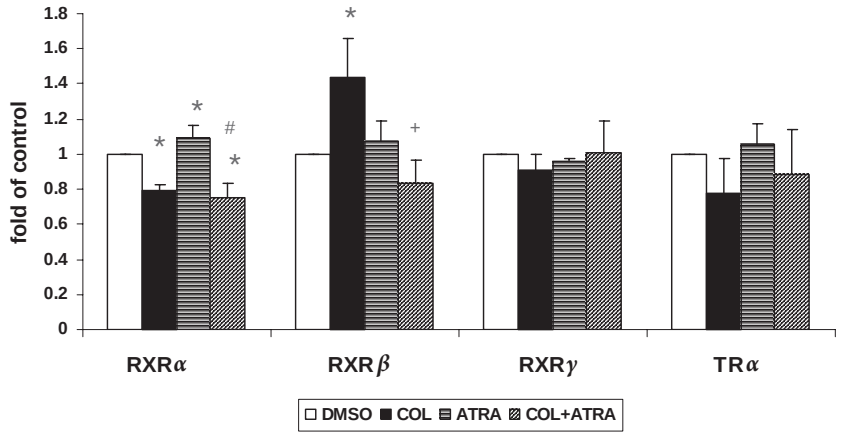

Figure 1. Effects of colchicine (COL) and/or all-*trans* retinoic acid (ATRA) on the expression of rexinoid receptors and TRα receptor mRNAs. Primary rat hepatocytes were treated 24 h with COL (1 μmol/l) and/or ATRA (1 μmol/l) and/or with DMSO as vehicle for control. Total RNA was isolated and the levels of rexinoid receptors RXRα, RXRβ, RXRγ, TRα and GAPDH mRNAs were determined by RT-PCR. The data were normalized *per* GAPDH content in the respective samples. Bar graphs represent means ± SD of three independent experiments. * the value significantly different from the control value (DMSO) at $p < 0.05$; $^{+}$ the value significantly different from COL at $p < 0.05$; $^{\#}$ the value significantly different from ATRA at $p < 0.05$.

cells were ready for the treatments. Cultures were maintained at 37°C in 5% CO_2 (air : CO_2, 95 : 5) humidified incubator. Rat hepatocytes were plated on Petri dishes (100 mm I.D.) in a density of 1×10^7 cells/well using culture media enriched with foetal calf serum (5% v/v). After 4 h, culture medium was replaced by a serum-free one. Thereafter, the cells were treated 24 h with ATRA (1 μmol/l), COL (1 μmol/l) and/or with DMSO as vehicle for control.

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 4 μl RT mixture, 1 × PCR buffer, 1.5 mmol/l $MgCl_2$, 0.2 mmol/l dNTP, 25 pmol of each specific gene primer set and 0.3 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 RT, 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. Oligonucleotide sequences of primers used in PCR as well as annealing temperatures and sizes of expected PCR products are summarized in our previous published paper (Macejová et al. 2005). 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 house keeper gene GAPDH.

We have recently shown that treatment of primary rat hepatocytes with ATRA (1 μmol/l) down-regulated RARα and RARγ mRNAs whereas it up-regulated RARβ mRNA (Dvořák et al. 2007). COL diminished the expression of RARs in dose-dependent manner (Dvořák et al. 2007). As shown in Fig. 1, treatment of primary rat hepatocytes with ATRA resulted in slight up-regulation of RXRα and down-regulation of RXRγ mRNA. The expression of RXRβ as well as TRα mRNA remained unaffected. COL alone significantly inhibited expression of RXRα and slightly TRα mRNA but on the other hand significantly enhanced expression of RXRβ mRNA. Treatment with combination COL+ATRA resulted in significant inhibition of expression RXRα mRNA when compared to DMSO-treated control. In addition, this expression was significantly down-regulated also in comparison with expression of RXRα up-regulated by ATRA itself. Similarly, expression of RXRβ mRNA was significantly reduced after treatment with COL+ATRA when compared to significantly enhanced expression of this receptor by COL alone. Treatment with ATRA and COL or in combination did not affect expression of corepressors N-CoR and SMRT, and coactivator SRC-1 except for decreased expression of SRC-1 after treatment with combination COL+ATRA (Fig. 2).

Recently, we have shown that expression, protein stability and transcriptional activity of RARs may be affected by both microtubules interfering agents and ATRA (Dvořák et al. 2007). The data lead us to conclusion that microtubules may play the role in regulation of RARs expression. This subse-

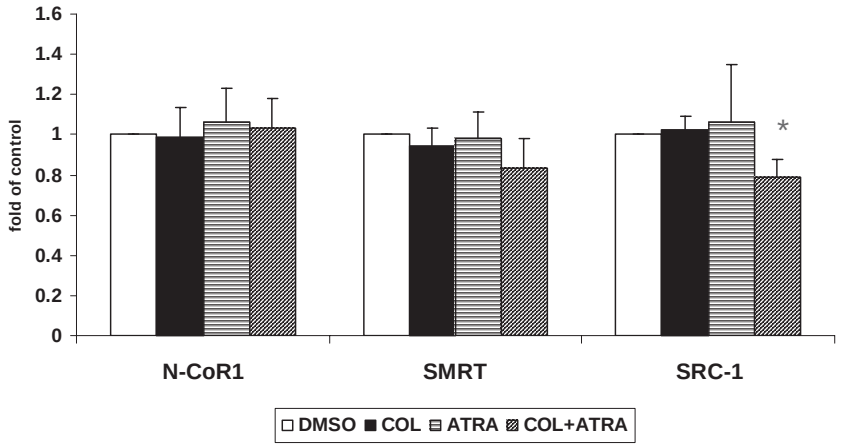

Figure 2. Effects of colchicine (COL) and/or all-*trans* retinoic acid (ATRA) on the expression of coregulators mRNAs. Primary rat hepatocytes were treated 24 h with COL (1 μmol/l) and/or ATRA (1 μmol/l) and/or with DMSO as vehicle for control. Total RNA was isolated and the levels of coregulators N-CoR1, SMRT, SRC-1 and GAPDH mRNAs were determined by RT-PCR. The data were normalized *per* GAPDH content in the respective samples. Bar graphs represent means ± SD of three independent experiments. * the value significantly different from the control value (DMSO) at $p < 0.05$.

quently has raised our attention on further investigation of the role of COL and/or ATRA on RXRs heterodimerizing partners RXRs as well as selected coregulators of RARs and TRs. The data obtained have confirmed our expectation that microtubule interfering agent, COL, differentially affects expression of RXRs. This might strengthen and extend previous conclusion on the possible role of microtubules interfering agents in pathways regulated by RARs but also very likely by RXRs. On the other hand, COL did not exert any significant effect on corepressor (N-CoR, SMRT) expression in primary rat hepatocytes.

Summing up, in this work we describe for the first time differential effects of COL and/or ATRA on the regulation of RXRs, TRα and coregulators genes in primary rat hepatocytes. The further work on role of microtubules in nuclear receptor-mediated signaling is warranted.

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