Gene expression profiling of rat liver reveals a mechanistic basis for ritonavir-induced hyperlipidemia

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Received 18 April 2007; accepted 19 June 2007
Available online 23 August 2007

Abstract

The molecular mechanisms of action of a HIV protease inhibitor, ritonavir, on hepatic function were explored on a genomic scale using microarrays comprising genes expressed in the liver of Sprague–Dawley rats (Rattus norvegicus). Analyses of hepatic transcriptional fingerprints led to the identification of several key cellular pathways affected by ritonavir treatment. These effects were compared to a compendium of gene expression responses for 52 unrelated compounds and to other protease inhibitors, including atazanavir and two experimental compounds. We identified genes involved in cholesterol and fatty acid biosynthesis, as well as genes involved in fatty acid and cholesterol breakdown, whose expressions were regulated in opposite manners by ritonavir and bezafibrate, a hypolipidemic agonist of the peroxisome proliferator-activated receptor α. Ritonavir also upregulated multiple proteasomal subunit transcripts as well as genes involved in ubiquitination, consistent with its known inhibitory effect on proteasomal activity. We also tested three other protease inhibitors in addition to ritonavir. Atazanavir did not impact ubiquitin or proteasomal gene expression, although the two other experimental protease inhibitors impacted both proteasomal gene expression and sterol regulatory element-binding protein-activated genes, similar to ritonavir. Identification of key metabolic pathways that are affected by ritonavir and other protease inhibitors will enable us to understand better the downstream effects of protease inhibitors, thus leading to better drug design and an effective method to mitigate the side effects of this important class of HIV therapeutics.

Keywords: Ritonavir; Atazanavir; Proteasome; Hyperlipidemia

Infection with HIV and the development of AIDS continue as a global epidemic. As summarized in the 2006 UNAIDS report (http://www.unaids.org/en/HIV_data/2006GlobalReport/default.asp), an estimated 38.6 million people are living with HIV worldwide. Though the epidemic continues, the management of HIV and AIDS has improved considerably in recent years, due in large part to the treatment advances in combating HIV with combination therapy consisting of HIV reverse transcriptase inhibitors (RTIs) and HIV protease inhibitors (PIs).

One of the first commercially available PIs for the treatment of HIV was ritonavir (RTV) [1], and there are now several additional PIs in the HIV treatment arsenal, including amprenavir, indinavir, nelfinavir, saquinavir, lopinavir, and atazanavir (ATZ). The HIV PIs, used together with RTIs in HAART (highly active antiretroviral therapy), have been very effective in lowering HIV viral loads [2–4]. However, because the effectiveness of antiretroviral therapy increases the successful management of HIV infection and improves longevity, drug side effects and toxicity have become of increasing concern [5].

Studies have shown that RTV, even in low-dose monotherapy, can affect serum lipid levels [6]. Patients treated with RTV or other PIs (often metabolically boosted with RTV) can develop hypercholesterolemia and hypertriglyceridemia, an

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anomaly known as hyperlipidemia [7–11]. Although patients on HAART that develop hyperlipidemia are put on cholesterol-lowering drugs such as statins or fibrates, the molecular mechanism(s) by which PIs contribute to hyperlipidemia is still being investigated. The mechanistic contribution of HIV PIs to hyperlipidemia is complicated by an association of this effect with HIV infection alone [12] and the potential influence of genetic predispositions [13], even though RTV does induce hyperlipidemia in healthy volunteers [6]. Due to the ever-increasing rate of infection and use of anti-HIV therapies, it is important to get as good an understanding as possible of the molecular mechanisms behind the hyperlipidemia effect, leading to improved screening methods for drugs with the fewest side effects for hyperlipidemia.

There is now considerable evidence that some of the PI effects on lipids are due to the inhibition of the 20S subunit of the proteasome [14,15]. Researchers have shown that this affects the levels of active sterol regulatory element binding proteins (SREBPs) in both the liver and adipocytes [16,17]. SREBPs are transcription factors that regulate gene expression of both cholesterol and fatty acid synthesis genes [18,19]. The impact of proteasome function would predictably affect the levels of many other proteins that are turned over in the proteasome. For example, in mice treated with RTV on a high-fat diet, ApoB levels were markedly increased [20]. ApoB protein is turned over in the proteasome via the proteasome–ubiquitin pathway [21,22]. Researchers have also shown that RTV impacts adipocyte differentiation by monitoring changes in gene expression of key adipogenic genes such as adiponectin [23,24].

In addition to effects on serum lipids, RTV is known to produce effects on the clinically important cytochrome P450 enzymes, notably its impact on CYP3A4 activity. RTV treatment also gives rise to Pregnane X Receptor-mediated induction of other cytochrome P450 genes responsible for the metabolism of many drugs in humans and rats [25–27]. Since RTV both induces and inhibits CYP3A4 [28], in addition to being a substrate, preclinical and clinical pharmacokinetic behaviors are complicated.

To date, most studies describe monitoring only sets of predetermined genes. Here we describe an unbiased approach, in which the effects of RTV on thousands of genes are monitored simultaneously using microarrays in a common preclinical model, the rat. We also compared RTV to a compendium of 52 other unrelated compounds with known toxicities [29]. Together with 52 other compounds, we monitored the overall transcriptional activity of RTV over three different doses and treatment duration of 3 or 7 days. We showed that RTV elicited a robust signature with several groups of genes showing regulation in a dose-dependent manner (Fig. 1A). These included genes encoding proteasomal subunits, genes involved in β-oxidation, and cholesterol metabolism genes. In subsequent sections, we also describe the characteristics of the RTV gene signature relative to the other compounds in the compendium.

Impact on PXR by RTV

Since RTV induces, inhibits, and is metabolized by the hallmark PXR-responsive cytochrome P450 isoform, CYP3A4 in humans, and is a potent inhibitor of this enzyme, we investigated the impact of RTV on rat homologues of cytochrome P450 genes that may be regulated by the xenobiotic receptor PXR. Consistent with PXR-mediated induction, Fig. 1B shows a PXR-responsive rat orthologue Cyp3a1 [30,31] (NM_013105, Entrez 25642, also known as Cyp3a4 and as Cyp3a23) that was substantially upregulated in a dose-dependent fashion. The upregulation of Cyp3a was confirmed by Western blot using antibodies that recognize the family of Cyp3a (Fig. 1D). RTV also upregulated other cytochrome P450 genes such as the phenobarbital-inducible Cyp2b and cytochrome P450 genes involved in cholesterol synthesis and metabolism, consistent with observations of cross talk between the nuclear receptors CAR, PXR, and other nuclear receptors [32,33]. Lanosterol 14α-demethylase (CYP51) was upregulated in RTV-treated rats. Cytochrome P450s Cyp7a1 and Cyp8b1, both involved in bile acid metabolism, were downregulated. In addition, Cyp1a1 and Cyp1a2, both AhR-responsive genes that are induced by 3-
methylcholanthrene, responded differently to RTV. Only Cyp1a2 was downregulated in RTV-treated rats, whereas the expression of the AhR bellwether gene, Cyp1a1, was not affected (Fig. 1D). It is interesting to note that fibrate-inducible Cyp4b1 and Cyp4a3 were downregulated in response to RTV treatment. The significance of this finding will be discussed later.

**RTV affects proteasomal and ubiquitination pathway gene expression**

Genes encoding various proteasomal subunits contributed to the uniqueness of the RTV signature, as determined unbiasedly by principal component analysis (PCA) of the RTV signature within the context of a 52-compound compendium of gene expression.

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**Fig. 1.** (A) Heat map of expression changes in one-dimensional clustering of 2410 signature genes in RTV-treated rats with three different dosing regimens, together with vehicle controls. Signature genes were defined as those with threefold or more change in expression ratio and p value less than 0.001 in at least three experiments. Genes that are upregulated are shown in red and downregulated in green. Black denotes genes with no significant expression change. (B) Logarithmic expression ratios for a set of cytochrome P450 genes in all RTV-treated rats together with vehicle controls. (C) Similar to (B) for a set of proteasomal and ubiquitin-related genes with upregulation. Color scales are shown universally for (A), (B), and (C). Dynamic range of color scale is between log –0.3 and log 0.3. (D) Western blot using antibodies that recognize the family of CYP3A confirms the upregulation of CYP3A.
expression profiles. PCA established that the two high doses of RTV administered to rats for 3 days elicited transcriptional profiles that were unique to RTV’s profile (see Supplemental Fig. S1). A total of 790 sequences contributed to RTV’s distinct signature. Among the genes that contributed significantly to the PCA were transcripts encoding proteasomal subunits or that were annotated as having homology to proteasomal genes. The transcriptional profiles of all proteasomal-related sequences that contributed to the loading of the principal component analysis are shown in Fig. 1C. Transcripts that were impacted include those with homology to the human 26S proteasome-associated pad1 homologue, proteasome subunit, α type 1 and β type 3, proteasome 26S subunit, ATPase 2, and others (see supplemental data for complete list). The upregulation of the various proteasomal subunits was dose dependent. Since RTV is now known to also inhibit eukaryotic proteasomal function [34], it is likely that the transcriptional effects on proteasomal components is a feedback response to this inhibition [35]. The expression levels of some sequences related to the ubiquitination pathway were also upregulated.

RTV affects expression of β-oxidation and fatty acid and cholesterol biosynthesis genes

Bezafibrate is a member of the fibrate class of hypolipidemic drugs that signal through the peroxisome proliferator-activated receptor α (PPARα) to upregulate fatty acid β-oxidation. Since RTV is known to produce the opposite lipid effect in patients, it was of interest to compare the gene expression profiles of RTV and bezafibrate to determine potential differences in effects on cellular pathways. Clinical chemistry data from rats treated with RTV showed that serum cholesterol concentrations were mildly elevated in all rats dosed at 500 mg/kg/day for 3 days. At 7 days, rats fed with 50 and 175 mg/kg/day also showed elevated cholesterol levels. Earlier studies showed that rats showed increased cholesterol levels even at 25 mg/kg/day when they were administered RTV over longer periods of time (data not shown). Mildly increased serum triglyceride levels were also observed in some of the rats given higher doses of RTV. When the transcriptional response of RTV was compared with that of bezafibrate, we observed that many genes involved in fatty acid and cholesterol metabolism were oppositely regulated by the two compounds (Fig. 2). Using a simple t test to identify genes that are significantly oppositely regulated in RTV- and bezafibrate-dosed animals, we found that the fibrate hallmark genes, such as genes encoding enzymes involved in β-oxidation, such as acetyl-CoA acyltransferase, peroxisomal enoyl-CoA: hydroxase-3-hydroxyacyl-CoA bifunctional enzyme, carnitine octanoyltransferase, and acyl CoA oxidase, were among the most significantly affected. Transporters of the peroxisomal membrane, such as the ATP-binding cassette half-transporters and carnitine octanoyltransferase, were also upregulated after bezafibrate but downregulated after RTV administration. Fatty acid synthase was also oppositely regulated by the two compounds. Genes encoding proteins involved in cholesterol biosynthesis and breakdown, such as Cyp51 and NADH-cytochrome b5 reductase, and ESTs with homology to lanosterol synthase, 7-dehydrocholesterol reductase, and acetyl-CoA synthetase also showed significant opposite regulation between RTV and bezafibrate. Another set of oppositely regulated PPARα-inducible genes are Cyp4b1 and Cyp4a3. In addition, RXR, which forms an obligate heterodimer with PPARα, was also significantly downregulated at a level of threefold with a p value <0.01 in RTV-treated rats. Interestingly, ESTs with homology to mouse Angptl4 (PPARγ angiopeptin-related protein) and Adfp (adipose differentiation related protein), both known PPARγ-inducible proteins, were also oppositely regulated between RTV and bezafibrate, consistent with previous findings of RTV’s effect on adipogenesis. Many of the above-mentioned genes are also SREBP-regulated genes, such as the cholesterol biosynthetic genes and FASN (fatty acid synthase) (see supplemental data for complete list).

Do differences in gene expression signatures caused by other PIs help explain lipid-related side effects?

To follow up on the results from the first study, we conducted another set of experiments in rats to compare hepatic gene expression profiles of two experimental HIV PIs (Merck A and Merck B), RTV, and ATZ. We wanted to see if the gene expression signatures brought on by these different PIs could further help dissect the pathways that impact lipid levels. ATZ is generally considered to have minimal hyperlipidemia-related side effects; hence it was included in the study [36,37]. Detailed pharmacokinetic analyses for this study were performed to help us better interpret the gene expression signatures (Table 1).

We found that Cmax for all compounds and doses tested were between 4 and 20 μM, indicating that compound exposure was achieved (Table 1). However, for both doses of Merck A and Merck B and for the ATZ low dose, marked differences in AU0–∞ were observed in the replicated (N=2) pharmacokinetic satellite animals. This may indicate that individual animals in the N=5 expression profiling study cohort have variable drug exposure. After normalization of AUC to a uniform 100 mg/kg/day dose, we found that RTV exposure was 5- to 10-fold higher than that of the other three compounds. RTV is both an inducer and an inhibitor of the cytochrome P450 enzymes responsible for its metabolism [28], so the difference in AUC across compounds may be related to low absorption and/or metabolism. Accordingly, average exposures showed low-dose RTV to be most comparable to the high dose of the other three compounds.

Principal component analysis of a 505-gene signature set obtained from one-way ANOVA showed that RTV is still differentiated from the other PIs via its hepatic gene expression fingerprint in this second study (see Supplemental Fig. S2). We then performed a more focused pathway analysis around the genes that we found to be impacted by RTV in the first study. We found that all the compounds tested showed very similar patterns with respect to SREBP-related genes, PXR-responsive genes, and the mainstream Absorption, Distribution, Metabolism, and Excretion-related genes (Fig. 3). In contrast, ATZ is the only protease inhibitor among those tested that did not induce the expression of genes encoding the proteasomal subunits (Fig. 3). Genes involved in the ubiquitin degradation pathway were also...
not impacted by ATZ though they were upregulated by the other PIs. Because ATZ has little effect on plasma lipid levels in human, this particular proteasomal molecular fingerprint may represent the differences between ATZ and the other PIs in this regard.

**Discussion**

*A working model for RTV-induced hyperlipidemia in rats*

As depicted in the cellular response model in Fig. 4, we propose that RTV exerts its hyperlipidemia effects by a combination of several pathways, interfering with the degradation of key proteins via proteasomal function and antagonism of the PPARα signaling pathway. A variety of genes in SREBP and lipid pathways showed gene expression changes that are consistent with this model, and PXR-related changes in the cytochrome P450 class (such as Cyp7a1, 8b, and 51) may be compensating for lipid dysregulation or perturbations of bile acid homeostasis [38]. A comparison of the gene expression profiles of RTV to the hypolipidemic drug bezafibrate shows RTV to be a functional antagonist of PPARα in rats. Based on our results, one of the affected pathways was PPARα-induced fatty acid β-oxidation. Genes involved in fatty acid oxidation were downregulated in RTV-treated rats. The gene expression pattern of RTV was almost a mirror image of the expression pattern of bezafibrate. Moreover, the expression of RXR, which forms an obligate heterodimer with PPARα, was also significantly downregulated at a level of threefold and p value <0.01 in RTV-treated rats. Thus, one possibility is that RTV is an antagonist to the PPARα signaling pathway, producing an
inhibitory effect on triglyceride metabolism and stimulating triglyceride synthesis. Patients on HAART that experience hyperlipidemic side effects are treated with fibrates, including bezafibrate [39] to control triglycerides and statins to control cholesterol. Our results, derived from gene expression studies in rat liver, provide a mechanism for the effectiveness of this

![Fig. 3. Heat map of the four groups of genes for atazanavir, RTV, Merck A, and Merck B compounds. SREBP, SREBP-responsive genes; PXR, PXR-responsive genes; ADME, ADME genes; Proteasomal, genes encoding proteasomal subunits and ubiquitin pathway. Dynamic range of color scale is between log –0.3 and log 0.3.](image-url)

Table 1
Pharmacokinetic analysis summary for rats in study 2

<table>
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<th>Compound</th>
<th>Dose (mg/kg/day)</th>
<th>Animal</th>
<th>AUC (0–∞) (μM·h)</th>
<th>AUC average (0–∞) (μM·h)</th>
<th>C_{max} (μM)</th>
<th>AUC normalized to 100 mg/kg/day (μM·h)</th>
<th>Average normalized AUC (μM·h)</th>
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Drug concentrations in female Sprague–Dawley rat plasma were determined by LC–MS/MS following the last of three once-daily oral doses (N=2/dose/compound). Noncompartmental analysis was used to derive area under the concentration–time curve (AUC) and the maximum plasma concentration (C_{max}) and average results were calculated. AUC was normalized to a dose of 100 mg/kg/day to compare relative exposure across compounds.
Design of new PIs with limited hyperlipidemia liabilities

Monitoring the changes in expression of tens of thousands of DNA sequences in rat produced by different PIs has provided us with some distinct gene signatures to investigate further. In addition, our compendium of gene expression profiles showed how hypolipidemic drugs, such as bezafibrate, may ameliorate these side effects. Further, we tested this by conducting a pharmacokinetically guided comparison of RTV with three other PIs and evaluated effects on expression of PXR-related genes, SREBP-related genes, proteasomal subunit genes, and genes involved in fatty acid metabolism.

Among the other three PIs tested, gene expression patterns were generally similar to that of RTV except for ATZ, which differed from the rest of the PIs by the lack of upregulation of genes encoding the proteasomal subunits. Paradoxically, ATZ resembled the other PIs in gene responses related to SREBP response. While this is puzzling, it is plausible that ATZ has sufficient, albeit weak, proteasomal inhibitory activity to affect immediate SREBP degradation (with subclinical effects on cholesterol biosynthesis), but is not sufficient to trigger a compensatory increase in proteasomal gene expression or clinically significant change in lipid levels. But the impact on the proteasomal function, weak as it may be, may have already affected the gene expression of SREBP-activated genes because of the more direct effect of SREBP on gene expression. From Figs. 1C and 2, we observe that the SREBP-responsive genes were already upregulated at 3 days (50 mg/kg), while the proteasomal genes were not significantly upregulated until day 7 (50 mg/kg). The alternative explanation for this is that the proteasomal fingerprint and the SREBP fingerprint can be empirically derived cotherapy for PI-related hyperlipidemia. However, even though we now have a better handle on the overall impact of ritonavir on pathways as schematically depicted in the working hypothesis, it is still unclear exactly how these genes respond to RTV treatment. Further studies will be needed to dissect direct effects of RTV on transcription versus indirect effects via the proteasomes or PXR interplay. There is also cross talk between transcription factors such as NF-κB and AP-1 with PPARα; hence it is likely that several pathways that could impact fatty acid and cholesterol biosyntheses and RTV could impact one or several of them at different levels [40]. Future experiments will be needed to dissect these various possibilities at a deeper level. In addition, though RTV induces hyperlipidemia even in healthy volunteers, some of the mechanistic contribution of HIV PIs to hyperlipidemia could be complicated by an association of this effect with HIV infection alone [12] and the potential influence of genetic predispositions [13], as mentioned in the introduction. HIV rodent models [41,42] might help to investigate this aspect further.
independent and can each affect lipid levels to different degrees. Recently, the ubiquitin-proteasome system has been implicated in the stability of diabetic atherosclerotic plaques [43,44]. In mice treated with RTV on a high-fat diet, ApoB levels were also markedly increased [20], most likely through impeded turnover via the proteasome–ubiquitin pathway [21,22]. All this suggests that the ubiquitin–proteasome system could impact the lipid profile independently, playing a larger than expected role in hyperlipidemia and other related symptoms in a SREBP-independent manner. One of the ways to address this in the future is by testing various proteasomal inhibitors across a range of concentrations and monitoring the dynamics of the proteasomal genes with respect to the lipid biosynthesis genes.

We have uncovered gene expression patterns across multiple protease inhibitors and have begun to test more directly some of the specific hypotheses. In addition, based on the comparison of gene expression patterns alone for the PIs that we tested, we do not see the two experimental PIs as having an advantage over RTV with respect to the impact they have on both SREBP and proteasomal signatures. ATZ, a PI with limited lipid liability in humans, on the other hand, lacked the proteasomal signature. Practically speaking, a method of screening one could employ now in the preclinical setting, even without understanding the complete molecular mechanism of action, would be to utilize the more robust proteasomal signature as a filter to exclude compounds that might bring on lipid effects. This can be translated to a TaqMan assay in which key proteasomal and ubiquitination pathway genes are monitored in rats to help guide selection of future PIs.

Materials and methods

Materials

All solvents were reagent grade and obtained from routine commercial sources.

Animal studies

Two studies were conducted. In study 1, gene expression profiling and comparison to a compendium of profiles were done as described in Waring et al. [29]. Briefly, an oral dose regimen of 50, 175, or 500 mg/kg/day of RTV was given to male Sprague–Dawley rats over a period of 3 or 7 days. Tissues were collected 18 h after the last dose.

Study 2 compared gene expression profiles of four PIs. The study was based on current International Conference on Harmonisation Harmonised Tripartite Guidelines [46]. Animal care was in compliance with the USDA Animal Welfare Act (9 CFR Parts 1, 2, and 3) and ILAR guidelines [47]. Briefly, female Sprague–Dawley rats (age 9–10 weeks, CD (Crl: CD (SD)IGS BR)), seven animals/compound) were treated once daily for 3 days with two Merck PI candidate compounds and two marketed PIs, RTV and ATZ, at the following dose levels: RTV, 50 and 170 mg/kg/day; ATZ, 300 and 600 mg/kg/day; Merck A, 160 and 800 mg/kg/day; Merck B, 100 and 500 mg/kg/day. At 24 h after the last dose, livers were collected from five animals and RNA samples were prepared from the liver. In the other two animals, blood was collected by orbital sinus bleed at 0, 25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h after the last dose. Plasma was isolated and LC–MS/MS determination of plasma concentrations and pharmacokinetic analysis was done. Control rats (N = 5) for the vehicle (10 ml/kg of 5% ethanol:95% propylene glycol containing 2 eq of p-toluene sulfonic acid) were treated identically. This study was carried out in female rats as opposed to male rats as in the first study to look for common effects that are sex independent.

Preparation of labeled cRNA and microarray hybridization

In study 1, DNase-treated rat liver total RNAs were isolated in Trizol and processed essentially as described in [45] on Agilent 25 k rat chips v1.0 (GEO Platform Series GPL4772). In study 2, after sample quality control (QC) procedures, RNA samples were amplified, labeled, and hybridized with Fluor reversal on Agilent 50k rat chips v2.1 (GEO Platform Series GPL3631) at the Rosetta Gene Expression Laboratory according to general methods developed in Hughes et al. [45]. All compound-treated samples (N = 5/group) were profiled against a common reference pool made of all control samples.

Pharmacokinetic analysis

HIV protease inhibitor analytes were isolated from EDTA-treated rat plasma by protein precipitation and quantified by LC–MS/MS in the positive ionization mode. Analysis was done on a Sciex API 3000 mass spectrometer interfaced via the Sciex heated nebulizer to a Micro-Aria LX4 LC system consisting of a HTS Twin PAL CTC autosampler and Rheos pumps. Chromatography was on a Waters Atlantis-C18, 2.1 × 50 mm (5 μm) column with a mobile-phase gradient at flow rate 0.5 ml/min: A, acetonitrile containing 0.1% formic acid (w/v); B, water containing 0.1% formic acid (w/v); gradient (time (min))/% B, 0.0/100; 0.5/100; 2.5/5; 3/0.5; 3.1/100; 5/100. LC–MS conditions were nebulization gas, nitrogen at setting 8; auxiliary gas, nitrogen at 1 L/min; heater, 450 °C; discharge current, 3; curtain gas, nitrogen at setting 12; collision gas, nitrogen at setting 4. LC–MS analysis parameters were for Merck B, standard curve range 20–10,000 ng/ml, LOQ 20 ng/ml, precursor → product 781.5 → 217.1; Merck A, standard curve range 5–10,000, LOQ (ng/ml) 5, precursor → product 756.4 → 192.0; RTV, standard curve range 10–10,000, LOQ (ng/ml) 10, precursor → product 721.6 → 268.1; ATZ, standard curve range 5–10,000, LOQ (ng/ml) 5, precursor → product 705.4 → 168.0; Crixivan (internal std), precursor → product 614.5 → 421.2, run acceptance criteria, 2/3 of the QC values within 20% CV and 25% of theoretical value.

Data analysis

After standard raw data processing, measured values of log(ratio) for each gene from each treated animal were reported together with log(ratio) error, p value for the significance of regulation relative to the control, and other quality control metrics. All profiles passed spike-in based data QC and other QC metrics. In addition to expression data, overview such as one-dimensional clustering and two-dimensional clustering and one-way ANOVA were used to identify a set of genes that discriminates among nine ANOVA groups (two doses for each of four compounds and one control group). Prior to the ANOVA, a preselection criterion required that a gene was significantly regulated based on a p value <0.01, was more than twofold changed in at least two compound-treated animals, and was regulated at p <0.01 in fewer than two vehicle-treated animals. With a cutoff ANOVA p value of 0.01, we identified 505 genes from ~25,000 sequences. This set of 505 genes was analyzed using PCA, and prespecified genes of interest, such as SREBP genes, fatty acid metabolism genes, and PXR genes, were extracted and clustered. PCA, a method that allows one to determine the key variables that discriminate the differences in the observations in a multidimensional data set, was used to determine if RTV can be differentiated from the rest of the reference compounds. PCA involves a set of linear transformations that converts a number of correlated variables in gene expression profiles into a set of new variables called principal components (PCs). PCs, orthogonal to each other, are taken together to explain all the variance contained in the original data. The transformations are defined in such a way that the projection of profiles onto key PCs allows one to capture features of profiles. The linear combination of genes that defines each PC is called PC loading. The projection of each profile onto PCs is called PC score. The microarray data were also analyzed using the Rosetta Resolver (Biosoft, Seattle, WA, USA) analysis program as well as in-house custom bioinformatics tools.

Acknowledgments

We thank Jim Yerger for critical discussions and the Rosetta Gene Expression Laboratory for microarray hybridizations.
Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ygeno.2007.06.004.

References


