

# Application of Pharmacogenomics in Drug Discovery and Development: Correlations Between Transcriptional Modulation and Preclinical Safety Observations

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**Abstract:** An integrated systems biology approach of measuring mRNA, protein and enzyme activity, was used to determine the molecular mechanisms responsible for reductions in thyroid hormone levels observed in rats given 1000 mg/kg/day of a nonsteroidal progesterone agonist (NSP). The effect of NSP on drug metabolizing enzyme (DME) expression was determined in livers from treated and vehicle control rats. In treated males, CYP1A1, CYP2B1, CYP2B2, CYP2C12, CYP3A1 and UGT1A mRNAs increased by 2.2, 31.0, 9.4, 13.0, 6.4 and 2.3 fold, while CYP2C11 and CYP3A2 levels decreased by 4.8 and 15.0 fold respectively. CYP1A, CYP2B and UGT1A enzyme activities increased by 2.9, 6.2 and 1.4 fold while CYP2C and CYP3A activities decreased by 2.2 and 1.8 fold respectively. CYP2B and CYP2C proteins increased by 2.1 and 1.3 fold but CYP2C11, the male-specific isozyme, and CYP3A protein decreased by 2.0 and 1.4 fold respectively. In treated females, CYP1A, CYP2B, CYP2C, CYP3A and UGT activities increased by 1.9, 12.0, 23.0, 13.0 and 2.2 fold respectively; with corresponding increases in mRNA ranging from 1.5 to 783 fold. CYP2B, CYP2C and CYP3A proteins increased by 3.6, 2.2 and 6.4 fold respectively, but CYP2C11 remained unchanged. These data suggest that NSP modulates the transcriptional regulation DME in rats and could account for the observed reductions in thyroid hormones, since UGT conjugation is the main pathway of thyroid hormone elimination in rats. These data also show gender and isozyme-specific regulation of some genes, thus demonstrating the value of an integrated approach in determining the contribution of individual genes in drug safety and metabolism observations.

**Key Words:** Non steroidal progestins, preclinical safety assessments, pharmacogenomics, systems biology approach, transcriptional regulation, thyroid hormone regulation.

## INTRODUCTION

Gene expression profiling is a tool that can be used to characterize chemically induced toxicity in cells and/or animal models, in order to provide plausible explanations for observed toxicity in preclinical testing [1-4]. Its applications in drug discovery and development to understand pathways affected by compounds in development and to identify potential biomarkers of toxicity and/or efficacy are gaining recognition [5-7]. However, one of the limitations of the technology, which has hampered its widespread application, is the reported poor correlation between changes in mRNA expression and protein expression [8,9]. This supposed lack of correlation can be attributed to multiple factors, including posttranscriptional and translational modifications, subcellular location and availability of co-factors, turnover rates and difficulties associated with measuring protein expression across wide dynamic ranges [10-15]. In addition, the lack of specific tools with which to measure protein and enzyme activities has often meant that protein and enzyme activity data for specific genes in a subfamily cannot be determined, while at the mRNA level, this specificity is achievable. Thus comparisons between mRNA and these data sets, which are generated using different parameters, often contribute to this poor correlation. Despite these observations, it is evident that most drug metabolizing genes are primarily regulated at the

level of transcription and minimally affected by posttranslational modifications [16-18]. The challenge therefore, for drug metabolism researchers is to generate mRNA, protein and enzyme activity data that can be used to delineate those genes regulated at the level of transcription, with direct correlations to phenotypic expression, and those with a disconnect between mRNA changes and enzyme activities. This will enable gene expression data to be used to determine the contribution of gene regulation to the metabolism and disposition of drug molecules and enhance our understanding of the underlying molecular mechanisms responsible for expected and unexpected findings during the drug development process, potentially leading to the identification of biomarkers of toxicity and/or efficacy.

In this paper, we report how this integrated approach was used to determine whether a non-steroidal progesterone agonist (NSP) has the potential to transcriptionally modulate the expression of drug-metabolizing enzymes in rat, and whether this regulation could account for changes in circulating thyroid hormone levels observed following repeated dosing with NSP. In a previous toxicology study, NSP had been administered orally *via* gavage, to male and female rats at dosages up to 1000 mg/kg/day for 14 days. Thyroid hypertrophy/hyperplasia were observed at  $\geq 100$  mg/kg/day. In a subsequent study to assess the tolerability and toxicokinetic parameters of NSP administered as a diet admixture for 14 days, liver samples from the highest dose group (1000 mg/kg/day dose), in which effects on the thyroid were expected, based on previous data, were collected and used to

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determine mRNA, protein and enzyme activity levels of cytochrome P450 and UGT genes. The hypothesis that at high doses, NSP causes changes in thyroid hormone levels and thyroid hypertrophy *via* regulation of drug metabolizing enzymes, was based on published reports showing that steroid hormones modulate the expression of microsomal enzymes in the rat, with one outcome being changes in circulating thyroid hormone levels and thyroid hypertrophy [19-24].

The Affymetrix™ microarray gene expression platform was used as a primary screening tool to determine which genes were affected by NSP. A custom drug metabolism chip designed in-house that contains genes involved in drug metabolism, nuclear receptors, drug transporters and signal transduction pathway genes, was used. This was then followed by Taqman RT-PCR to quantify and validate data generated by chip analysis. Where antibodies were available, western analysis was used to determine protein levels of genes showing changes at the mRNA level, followed by enzyme activity assays using prototypic probe substrates.

## MATERIALS AND METHODS

### Materials

NSP was synthesized in-house at Wyeth Research (Pearl River, NY), Testosterone and hydroxylated testosterone metabolites were purchased from Steraloids Inc. (Newport, RI). NADPH, UDP-glucuronic acid (UDPGA), p-nitrophenol, p-nitrophenol glucuronide, thyroxine, triiodothyronine, disodium hydrogen phosphate dihydrate, triton X-100, resorufin, 7-ethoxyresorufin and 7-pentoxeresorufin were obtained from Sigma Chemical Co. (St Louis, MO). Phenobarbital and  $\beta$ -naphthoflavone-induced rat liver microsomes were purchased from *In Vitro* Technology, Inc. (Baltimore, MD). TRIzol was purchased from Gibco BRL (Gaithersburg, MD), TaqMan™ probes and primers were ordered from BioSource International (Camarillo, CA) and the "One-Step RT-PCR Master Mix was from Applied Biosystems (Foster city, CA). Reagents for genechip analysis were purchased from various vendors, as recommended by Affymetrix (Santa Clara, CA). For Western blotting, Hybond-P membrane, Hyperfilm-ECL film, ECL-plus Western blotting detection kits were ordered from Amersham International (Arlington Heights, IL) and all buffers were purchased from Bio-Rad Laboratories (Hercules, CA). The primary antibodies were purchased from Gentest (Woburn, MA) and secondary antibodies from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). All other solutions/reagents were of analytical grade.

### Methods

#### Dosing of Rats

NSP was administered to male and female rats (4 rats/sex/group) as a diet admixture for 14 days. Dosages of 0, 100, 300 and 1000mg/kg/day were used. After the last dosage on day 14, surviving animals were fasted overnight and necropsy performed on day 15 for the standard battery of toxicity evaluations. For gene expression analysis, approximately 100–200 mg portions of liver from the highest dose and vehicle control groups were immediately frozen and stored at  $-80^{\circ}\text{C}$  until processed.

#### Preparation of Liver Microsomes

Prior to preparing microsomes, approximately 100 mg of tissue was set aside for total RNA isolation and microsomes prepared from the remaining tissues by differential ultracentrifugation as previously described [25,26]. Microsomal protein contents were determined by the Bradford method using IgG as a standard, and cytochrome P450 contents by determining P450 spectra, using a Beckman-650 spectrophotometer [27].

#### Enzyme Activity Assays

The activities of CYP1A and CYP2B in liver microsomes were measured using ethoxyresorufin and pentoxeresorufin as substrates for microsomal alkoxyresorufin O-dealkylase (EROD and PROD respectively), as previously described with minor modifications [28-30]. The production of resorufin was monitored using a Hitachi F-4500 Fluorescence Spectrometer (Hitachi Ltd, Tokyo, Japan). The standard curve of resorufin was generated by incubating known concentrations of resorufin in phosphate buffer without NADPH. Phenobarbital and  $\beta$ -naphthoflavone-treated male rat liver microsomes were used as positive controls. Enzyme activities are given as pmol of resorufin formed/mg protein/min.

The activities of CYP2C and CYP3A were measured using a testosterone hydroxylation assay as previously described [27]. Quadrupole Mass Spectrometer (Micromass, Inc., Beverly, MA), equipped with a positive electrospray ionization (ESI), interfaced to an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA), was used to quantify the formation of 6 $\beta$ -hydroxyl-testosterone (CYP3A) and 16 $\alpha$ -hydroxyl-testosterone (CYP2C) metabolites. Standard curves were generated by incubating hydroxylated metabolites (0.5 to 50  $\mu\text{M}$ ) in microsomes in the absence of NADPH. Phenobarbital-treated male rat liver microsomes were used as a positive control. Enzyme activity is given as nmol of metabolite formed/mg protein/min.

UGT activity was measured using 4-nitrophenol as a substrate, with the formation of 4-nitrophenol glucuronide measured by LC/MS. The incubation conditions were as published with minor modifications [31]. Acetonitrile (1 ml), containing 5  $\mu\text{M}$  of a proprietary internal standard, was added to terminate reactions and glucuronides quantified by Quattro Ultima, equipped with a negative ESI interface. A standard curve was generated by incubating 4-nitrophenol glucuronide (NPG) in the absence of UDPGA. 4-NPG formation was calculated from the standard curve and UGT activity recorded as nmol 4-NPG formed/mg protein/min.

#### Messenger RNA and Protein Determination

Total RNA was isolated using the TRIzol reagent from Gibco BRL (Gaithersburg, MD), following the manufacturer's protocol. Microarray analysis on the Affymetrix platform using a Wyeth Custom Drug Metabolism Chip was carried out as an initial screen and genes that showed regulation of 2-fold or more (up or down regulation) were further analyzed using Taqman RT-PCR. For microarray analysis, samples were pooled by treatment and gender (2 samples/array) and 10  $\mu\text{g}$  used to generate biotin-labeled cRNA,

following procedures recommended by Affymetrix (Santa Clara, CA) with minor modifications as follows: After fragmentation, 10 µg of the biotin-labeled cRNA was mixed with 11 Wyeth spike-in internal control transcripts, and hybridized in duplicates to a Wyeth Custom Drug Metabolism oligonucleotide array containing approximately 600 distinct rat drug metabolism genes. Microarray hybridization, staining, and visualization were performed following the manufacturer's protocol with modifications as previously described [32]. Absolute decision calls ("present," "absent," or "marginal") for each gene were determined using the Affymetrix Microarray Suit (MAS 4) and transcript levels, indicated as gene frequency and fold change, were quantified following the method described by Hill *et al.* [33]. Fold change values of 2 or more in either direction were considered significant. One-step Taqman RT-PCR was performed on the 7700 Sequence Detector from Applied Biosystems (Foster City, CA) using the "One-Step" RT-PCR Master Mix reagent following the manufacturer's protocol. Primers and probes (Table 1) were designed following criteria previously described [34] and the standard curve method was used for quantitation.

**Table 1. Taqman RT-PCR Primers. Primers and Probes were Designed Using the Primer Express Software (Applied Biosystems, Foster City, CA) Using the Selection Criteria Provided by the Vendor. Where Possible, Primers and Probes were Positioned Across Intron/Exon Boundaries to Prevent Cross-Hybridization to Genomic DNA. All Sequences were then Validated Against Plasmid cDNA to Determine Specificity to Intended Target**

| Gene    | Primer and probe sequences (5'→3')   |
|---------|--|
| CYP1A1  | F – AAAGATCCAGGAGGAGTTAGACACA<br>R – GAGGTCGTGAGAAAGCCGG<br>P – TGATTGGCAGGGATCGGCAGC                        |
| CYP2B1  | F- GGTTACACCGGCTACCAA<br>R – TGAATCTCATGGATAACTGCATCA<br>P – CCTTGATGACCGCAGTAAAATGCCATACA                   |
| CYP2B2  | F – GTGATTGGCTCTCACAGGCC<br>R – TGAATCTCGTGGATGACTGCAT<br>P – TCCCTTGATGATCGTACCAAAATGCCA                    |
| CYP2C6  | F – CAACCTAGTGATGTCATCATTAGGCTCCTCTTTG<br>R – GGACTTTAGCTGTGACCTCTGGAC<br>P – CTGGGACAGAGACAACAAGCACAACACTGA |
| CYP2C11 | F – TGGGATGCAATGGAAGGAGA<br>R – TCTTGCCATCCAAAAGTC<br>P – CCGGCGTTTCTCCATCATGACCC                            |
| CYP2C12 | F – ATTAATAATTGTGAGCACTCCTGCAT<br>R – TTAATAGCAGCAAAATGTTTTGAATG<br>P – ATCCCTATTCTTCTGGATTATTGTCCAGGAAATCA  |
| CYP3A1  | F – AATGCTCATAATGATTCTAAAGACAAAAGAA<br>R – GTGCTGCTGGTGGGTTTCATAT<br>P – TGGAGATCAGCCAGTCAATCATTTTTATTTTT    |
| CYP3A2  | F – GCTCATAATAATTCCAAAGACGAAGTG<br>R – GGTGAGTGGCCAGGAAATACAA<br>P – CTGCTGATGTTGAAATCATAGCCAGTCAGTTATCTTT   |

|        |   |
|--------|---|
| UGT1A1 | F – AACAAAGTCAAAAAGGACTCCAGT<br>R – TTCCAGAGAGGCCATAAACTCG<br>P – CTGCTGTCTGGCTGCTCCACCTTC                  |
| UGT1A5 | F – GAAACATCCGAGATTTGAAAAACATA<br>R – TGTCAAAGGAGCTGGAATTCAGA<br>P – CGACAGTTCTCTTAAGGTCTGTATGAATCTATTGCACA |
| UGT1A6 | F – TCTCTGGAGCACATGCTTGGT<br>R – GTTGGCCAGCCGTTGG<br>P – AAGCCCCGTATCTATGTGTTCCAGATTCTACA                   |
| UGT1A7 | F – TGTAGGAGTTTGTGTTAAGGACAAGAAGTTA<br>R – GGCAACAGTTAAGCCACACACAT<br>P – TTCGTTTGATGCTGTGTTTCTGGATCCTTT    |

CYP2B, CYP2C and CYP3A subfamily proteins were quantified by western immunoblotting following standard protocols. The amount of microsomal protein loaded on the gel for each treatment group was based on enzyme activity and mRNA data. Anti-rat CYP2B, CYP2C and CYP3A primary antibodies, and horseradish peroxidase conjugated secondary antibodies were used.

## RESULTS

There were no differences in the total protein content of microsomes from both treated male groups, compared to vehicle controls. The P450 contents of treated female microsomes increased by approximately 2-fold compared to the vehicle group (data not shown).

### Enzyme Activities

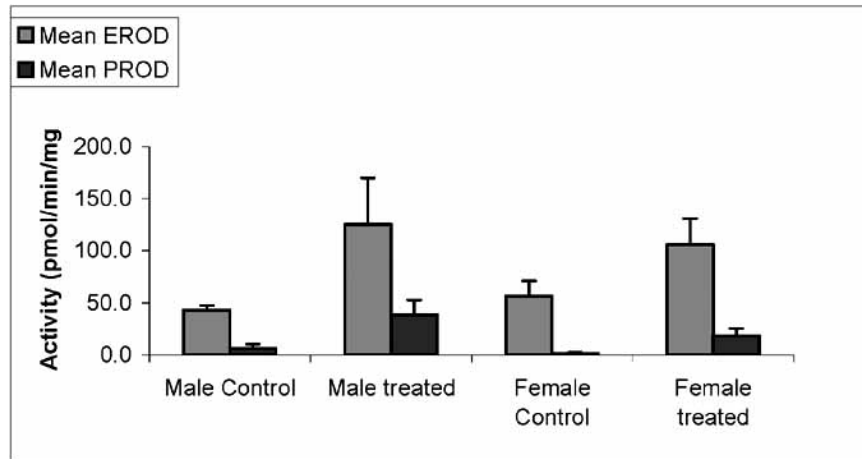
CYP1A (EROD) and CYP2B (PROD) activities increased significantly in treated males (2.9 and 1.9 fold respectively) and treated females (6.2 and 12.0 fold respectively). See Fig. (1). The activities of CYP3A and CYP2C decreased by 1.8 and 2.2 fold respectively in treated males, but increased by approximately 13 and 23 fold respectively, in treated females. See Fig. (2). The baseline activities of CYP2B, CYP3A and CYP2C showed gender related differences, with female controls showing significantly lower baseline levels than the male controls. There were a moderate increases in UGT activity towards p-nitrophenol in both treated males (1.5 fold) and treated females (2 fold). See Fig. (3).

### Messenger RNA Expression

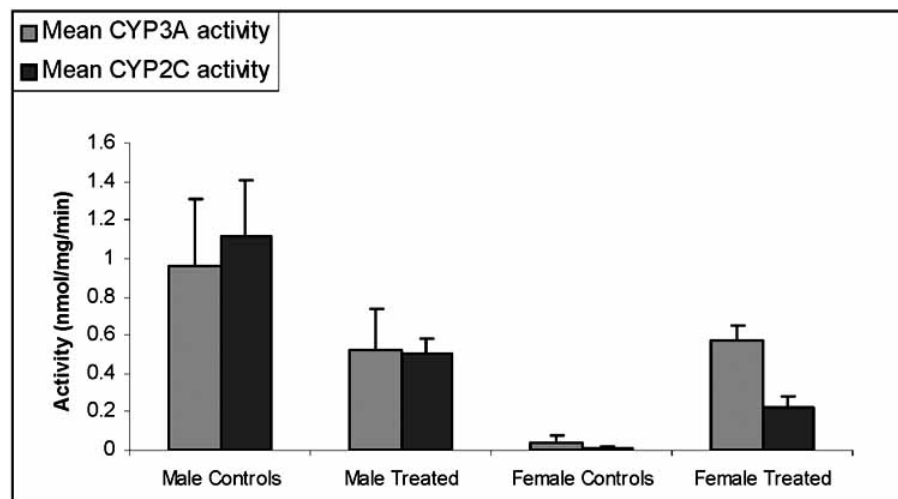
Table 2 shows gene expression data generated by genechip analysis. In treated males, CYP2A1 (2.2 fold), CYP2C12 (3.5 fold) and UGT1A (4.9 fold) were upregulated while CYP2C11 and CYP3A2 were downregulated (2.4 and 4.3 fold respectively). In treated females CYP3A2 and UGT1A genes were induced by approximately 2.8 fold each. Fig. (4) shows data generated by Taqman analysis for the individual genes. In addition to the gene changes detected by genechip analysis, Taqman analysis also detected significant induction of CYP2B1 and CYP2B2 mRNA in both treated groups.

### Protein Contents

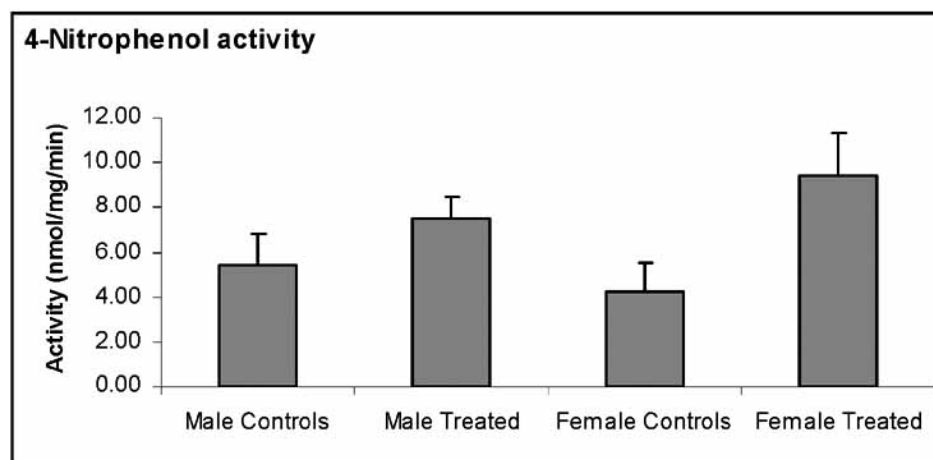
CYP2B microsomal protein increased by 2.1 and 3.6 fold in treated males and females respectively. In treated males, using a CYP2C11-specific primary antibody, a decrease of



**Fig. (1).** CYP1A and CYP2B activities in liver microsomes from treated and untreated male and female rats. Ethoxyresorufin and pentoxyresorufin were used as substrates for microsomal alkoxyresorufin O-dealkylase, EROD (CYP1A) and PROD (CYP2B) activities respectively. Data is presented as rate of formation of resorufin (pmol resorufin formed/mg protein/min).



**Fig. (2).** CYP2C and CYP3A activities in liver microsomes from treated and untreated male and female rats, using testosterone as the substrate. Rate of formation of 6 $\beta$ -hydroxyl-testosterone (CYP3A) and 16 $\alpha$ -hydroxyl-testosterone (CYP2C) metabolites were measured and data presented as pmol of metabolite formed/mg protein/min.



**Fig. (3).** UGT activity in liver microsomes from treated and untreated male and female rats, using 4-nitrophenol as the substrate. Data is presented as nmol/mg protein/min of 4-nitrophenol glucuronide.

**Table 2. Microarray Data Generated Using the Wyeth Custom Drug Metabolism Array. 10 µg of Total RNA was Used as Starting Material for Microarray Experiments. Absolute Decision Calls ("Present," "Absent," or "Marginal") for each Gene were Determined by the Affymetrix Microarray Suit (MAS 4). Transcript Levels, Indicated as Gene Frequency in ppm (Parts per Million), were Generated from Standard Curves of Spiked-in cDNAs of Known Concentrations. Fold Change Values Between Treated and Vehicle Controls were Calculated. Only Fold Changes of 2 or More in Either Direction are Considered Significant**

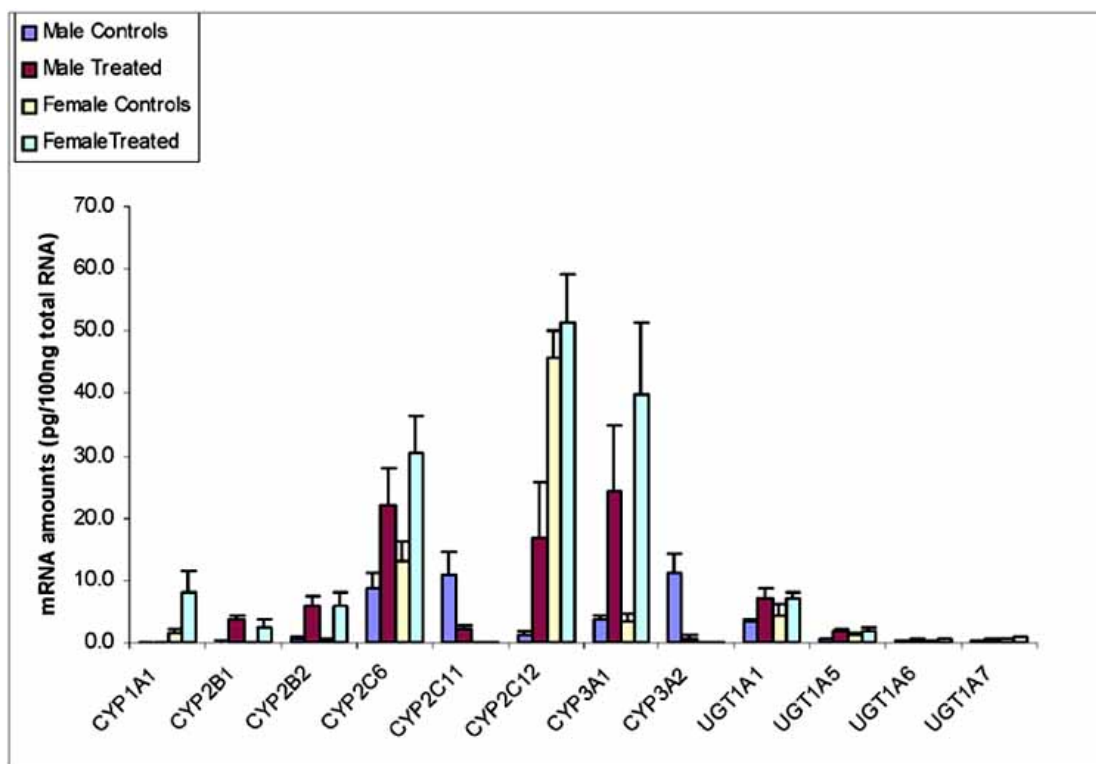
| Gene    | Controls (ppm) | TREATED (PPM) | Fold Change |
|---------|----------------|---------------|-------------|
| CYP2A1  | 60.0           | 134.0         | 2.2         |
| CYP2C11 | 360.0          | 154.0         | 2.4↓        |
| CYP2C12 | 91.0           | 320.0         | 3.5         |
| CYP3A2  | 285.0          | 66.0          | 4.3↓        |
| UGT1A   | 32.5           | 158           | 4.9         |

2.0 fold in protein was observed but when a CYP2C6 primary antibody that cross hybridized to other CYP2C proteins was used, total CYP2C protein increased by 1.3 fold. In females, the protein levels remained unchanged when the CYP2C11 antibody was used, but increased by 2.2 fold using nonspecific CYP2C antibodies. CYP3A protein decreased by 1.4 fold in treated males but increased by 6.4 fold in treated females (Figs. 5 & 6).

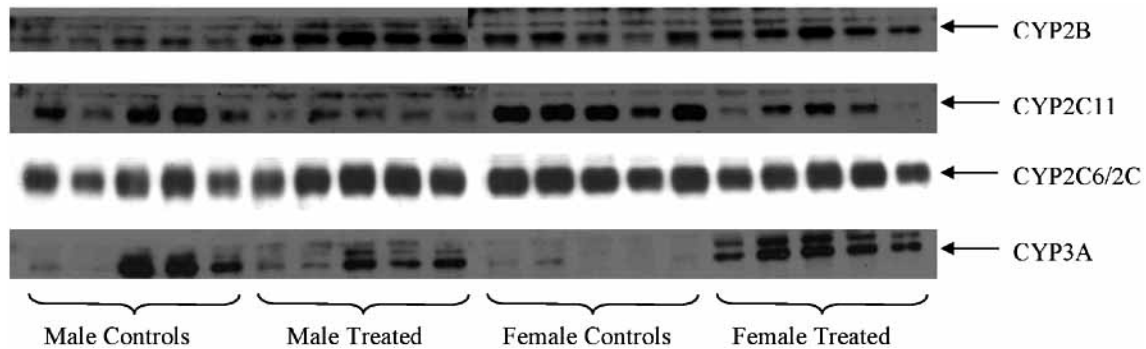
## DISCUSSION

An integrated approach of measuring mRNA and protein levels, and enzyme activities was used to study the potential

for a nonsteroidal progestin agonist (NSP) to modulate the expression of drug metabolizing enzymes in rats. The results show that NSP transcriptionally regulates the expression of CYP and UGT enzymes in both male and female rats a dose of 1000 mg/kg/day, in a gender and isozyme-specific manner (Table 3). Genes in the CYP2C and CYP3A families were differentially regulated, such that while the male-specific CYP2C11 and CYP3A2 were downregulated in males, CYP2C6, CYP2C12 and CYP3A1 were upregulated, resulting in an overall reduction in these isozymes at the level of enzyme activity. In females, on the contrary, all genes were upregulated in female rats, resulting in a synergistic effect at



**Fig. (4).** Real-time quantitative PCR of P450 and UGT mRNA in treated and untreated rat livers. Total RNA was isolated using TRIzol and One step RT-PCR carried out on the Taqman, with 100ng of total RNA per reaction. For quantitation, serially diluted plasmid cDNA for each gene were used to generate a standard curve, from which amounts of mRNA were extrapolated. Data is expressed as pg/100ng of total RNA.

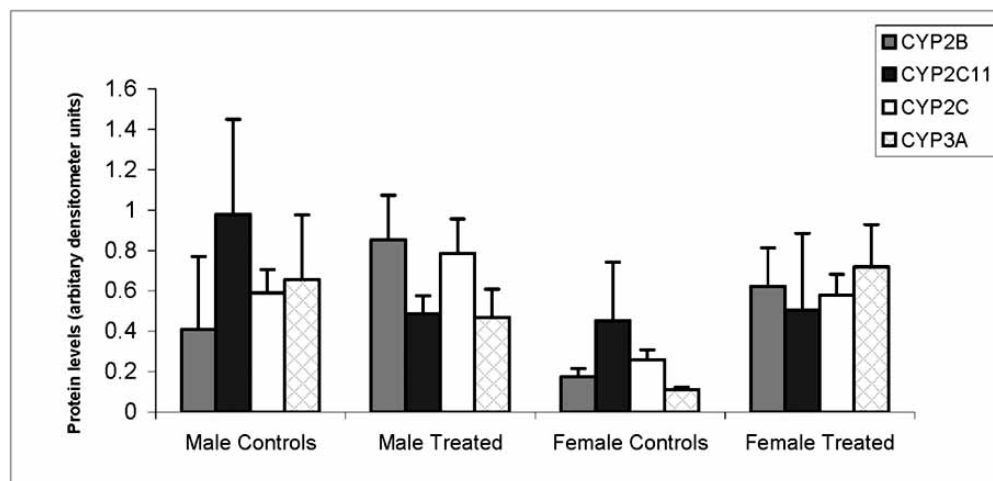


**Fig. (5).** Autoradiograph of CYP2B, CYP2C11, CYP2C and CYP3A proteins in liver microsomes from treated and untreated rat livers. For male samples and treated females, 1  $\mu\text{g}$  of microsomal protein was loaded while 3  $\mu\text{g}$  of protein was used for the female control samples. After electrophoresis, bands were transferred to nitrocellulose membranes, stained and exposed to x-ray film. A Densitometer was used to determine arbitrary intensity bands. Data was normalized against amount of protein loaded (1  $\mu\text{g}/\text{ml}$ ) and the difference in expression between treated and vehicle controls presented as fold change of treated over vehicle controls with loaded amount as the common.

the level of activity. Gender and isozyme-specific regulation of CYP2C and CYP3A genes in the rat has previously been reported [35,36] and may account for some of the discordance that has been reported between CYP mRNA levels and enzyme activity [37]. Due to lack of specific probe substrates, most enzyme activity assays determine overall CYP activity in a particular subfamily, while mRNA measurements are isozyme-specific. Although it is generally accepted and indeed echoed by the FDA in its guidance on *in vitro* induction methods, that mRNA expression for a specific CYP enzyme is not necessarily informative of enzyme activities, nevertheless measurement of mRNA levels is helpful when both enzyme inhibition and induction are operative. Thus our data provides another rationale for measuring mRNA levels when determining if differential transcriptional regulation is responsible for unexpected observations in preclinical testing.

While the regulation CYP and UGT enzymes by NSP did not appear to have any obvious consequences on the overall biochemistry and/or physiology of these rats, UGT enzymes

are known to be involved in the metabolism and elimination of both endogenous and exogenous substrates. In addition, there is data showing that naturally occurring progestins modulate the expression of microsomal enzymes in rodents, resulting in alterations in the circulating levels of endogenous substrates such as thyroid hormones, which are predominantly eliminated *via* UGT-mediated conjugation [22, 23]. Based on these data, it appears NSP modulates UGTs in a similar manner as naturally occurring steroid, thus offering a plausible explanation for the thyroid hypertrophy and slight decreases (<30%) in total thyroxin ( $T_4$ ) and free thyroxin observed in the safety studies. We propose, based on these data and the known mechanisms of thyroid hormone elimination, that UGT induction resulted in an increase in the conjugation and elimination of thyroid hormones, resulting in reductions in circulating levels. This decrease is thought to trigger a feedback mechanism involving the hypothalamus and pituitary glands that results in a sustained increase in thyroid stimulating hormone (TSH), and eventually, thyroid hypertrophy/hyperplasia [20,38].



**Fig. (6).** Relative amounts of CYP2B, CYP2C11, CYP2C and CYP3A protein in each treatment group determined by western analysis. After electrophoresis, bands were transferred to nitrocellulose membranes, stained and exposed to x-ray film. A Densitometer was used to determine arbitrary intensity bands from which fold changes were calculated. Samples from female controls were corrected for loading amounts.

**Table 3. Gene Expression Changes Determined at mRNA, Protein and Enzyme Activity Levels. Data is Presented as Fold Change of Treated Over Vehicle Controls. \*Data Obtained Using a CYP2C11-Specific Antibody and Generic CYP2C Antibody Respectively. ND = Not Determined. Gender and Isozyme Related Regulation is Observed for CYP2C and CYP3A Sub-families**

| Isozyme | mRNA<br>Fold Changes |         | Protein<br>Fold Changes |         | Enzyme Activity<br>Fold Changes |         |
|---------|----------------------|---------|-------------------------|---------|---------------------------------|---------|
|         | Males                | Females | Males                   | Females | Males                           | Females |
| CYP1A1  | 2.2                  | 5.0     | ND                      | ND      | 2.9                             | 1.9     |
| CYP1A2  | ND                   | ND      |                         |         |                                 |         |
| CYP2B1  | 30.6                 | 783.3   | 2.1                     | 3.6     | 6.2                             | 12.1    |
| CYP2B2  | 9.4                  | 20.3    |                         |         |                                 |         |
| CYP2C6  | 2.5                  | 2.3     | 0.5 / 1.3*              | 1.1     | 0.5                             | 22.6    |
| CYP2C11 | 0.2                  | 3.0     |                         |         |                                 |         |
| CYP2C12 | 13.2                 | 1.1     |                         |         |                                 |         |
| CYP3A1  | 6.4                  | 11.9    | 0.7                     | 6.4     | 0.6                             | 13.3    |
| CYP3A2  | 0.1                  | 6.7     |                         |         |                                 |         |
| UGT1A1  | 2.2                  | 1.6     | ND                      | ND      | 1.4                             | 2.2     |
| UGT1A5  | 3.0                  | 1.7     |                         |         |                                 |         |
| UGT1A6  | 1.7                  | 2.5     |                         |         |                                 |         |
| UGT1A7  | 2.4                  | 1.5     |                         |         |                                 |         |

In conclusion, we have used a systems biology approach to demonstrate that a nonsteroidal progestin receptor agonist can modulate the expression of drug metabolizing enzymes, resulting in perturbations of the thyroid and thyroid hormone homeostasis. We have shown that gene-specific transcriptional data can provide insights into how genes in the same subfamily can be independently regulated in both a gender and isozyme-specific manner and that such specific data can be directly correlated with toxicological findings. This integrated approach will enhance our understanding of how drug metabolism genes are regulated and how this knowledge can be better applied to provide value-added data to in the drug discovery and development to determine process.

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