ALTERED GENE EXPRESSION INDUCED BY MAMMALIAN PXR LIGANDS DOES NOT INDICATE THESE COMPOUNDS BIND THE PXR IN WINTER FLOUNDER (*PSEUDOPLEURONECTES AMERICANUS*)

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St. John's wort (SJW) and 4-nonylphenol (4-NP) have been shown to bind the Pregnane X-receptor (PXR) in mammalian species. SJW, a natural anti-depressant, has shown consistent agonism of PXR-mediated transcription and 4-NP has shown both agonism and antagonism, with 4-NP acting as an agonist in *in vitro* systems and as in antagonist in *in vivo* systems (Masuyama et al., Mol Endocrinol. 14:421-428, 2000; Laurenzana et al., Food Chem. Toxicol. 40:53-63, 2002). The objective of this research was to determine if SJW and 4-NP alter the expression of genes in a manner consistent with PXR agonism in winter flounder.

Winter flounder were injected ip with 500mg/kg/day SJW, 100mg/kg/day 4-NP, or both SJW and 4-NP every 24 hours for 48 hours. Following treatment, livers were excised, cut in two and snap frozen at –80°C. RNA was extracted and microsomes prepared from the livers. Testosterone hydroxylase assays were performed to determine whether 6β-hydroxylase activity had been induced by the PXR ligands. The PXR regulates the induction of CYP3A, which is primarily responsible for 6β-OH-testosterone production. The mRNA was used to determine changes in gene expression by subtractive hybridization. mRNA from the different groups were pooled, and both control vs. SJW, and control vs. 4-NP treated flounder livers were subtracted.

Production of 6β-OH testosterone was not altered by either 4-NP, SJW nor 4-NP + SJW in the winter flounder. This suggests that these compounds are not binding and activating the PXR in flounder. However, Laurenzana et al. (Food Chem. Toxicol. 40:53-63, 2002) did not show an increase in 6β-hydroxylation after 4-NP treatment in gestationally treated rats. Research with FVB/NJ mice in our laboratory has demonstrated that a 7-day exposure to 4-NP orally also does not induce CYP 3A or increase 6β-OH, but instead increases 16β-OH of testosterone and induces CYP 2B6 and 2B9 protein levels (unpublished). 16β-OH activity is very low in winter flounder. SJW also did not alter 6β-OH activity following ip injections. Recent work by Bray et al. (Toxicol Sci. 66:27-33, 2002) indicates that induction of CYP3A by St. John's wort takes up to three weeks of gavage treatment and that may also explain why we did not see induction of 6β-OH activity. Therefore, it is possible that longer treatments of winter flounder with SJW may increase 6β-OH activity.

St. John's wort seemed to cause the flounder to be slightly listless based on subjective observations while handling, but this did not translate into changes in gene expression. Subtractive hybridization indicated that few genes were altered by SJW. However, 4-NP, which binds both the pregnane X-receptor (PXR) and the estrogen receptor, altered the expression of several genes. The association of these genes with induction by PXR and estrogen receptor ligands is unknown. Since both males and females were injected and liver samples were pooled for subtractive hybridization, the estrogen receptor regulated genes may not have been sufficiently increased for detection, and nonylphenol may not bind the PXR in flounder. Several

genes altered by 4-NP were related to inflammatory responses and include antithrombin III, alpha 1-antitrypsin, complement components C3, C4b, C8b, C9 and cathepsin L. Many of these genes altered are acute phase proteins involved in the acute phase reaction inflammatory response mediated by the liver, and it has been hypothesized that the immune system is sensitive to environmental estrogen exposure (Yamashita et al., J UOEH 24:1-10, 2002). Other genes may be involved in cell proliferation or steroid activity and include adenylate kinase 2, 11-cis retinol dehydrogenase, RPS6 and IGF-1, which may suggest increased hepatic growth caused by estrogenic activity. Increased liver growth and mitotic activity have been observed following 4-NP treatment (Zumbado et al., Toxicol. 175:49-62, 2002). Table 1 lists the genes that were altered following exposure to 4-NP based on the subtractive arrays. None of these genes have been submitted to GenBank at this time.

Real-time-PCR is currently being conducted to confirm differential expression. Preliminary results indicate that none of the genes altered by 4-NP were altered by SJW, suggesting that 4-NP and SJW do not have similar acute effects on the liver. Some of the genes altered by 4-NP were also altered by 4-NP + SJW, and in a few cases the SJW may have enhanced the effect. This could be due to several possibilities, including activity on the same receptor or competition for metabolic enzymes that increased 4-NP's half-life in the flounder. Originally, this group was treated to determine if 4-NP might act as a PXR antagonist *in vivo*.

Table 1: Genes differentially expressed in flounder following treatment with 4-NP. Clone numbers beginning with a 1 are potentially down-regulated and those starting with a 2 are up-regulated.

Clone number	Gene name	Translated Blast E-value
1-2	C-type lectin domain	7e-12
1-4	complement component C4B	6e-32
1-7	fibronectin 1	2e-61
1-15	complement component C8 β chain	1e-90
1-23	ceruloplasmin	6e-59
1-27	complement component C9	2e-96
1-29	antithrombin III	3e-50
1-36	alpha-1-antitrypsin	1e-36
2-8	ribosomal protein S3A	9e-68
2-12	cytochrome c oxidase subunit II	2e-57
2-13	glucose transporter type 2	9e-23
2-15	IGF-1 precursor	2e-51
2-17	11-cis retinol dehydrogenase	3e-43
2-18	ribosomal protein S27A	e-140
2-23	rho guanine nucleotide exchange factor (GEF)	5e-09
2-29	cathepsin L	5e-34
2-52	ribosomal protein S6	9e-98
2-62	adenylate kinase 2	9e-60

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