BDE 47 AND PCB 153 INCREASE THYROXINE CATABOLISM IN PRIMARY RAT AND HUMAN HEPATOCYTES: THE UTILITY OF HEPATOCYTES AS SCREENING TOOLS FOR POTENTIAL THYROID HORMONE DISRUPTORS

Richardson VM[1,2], Sey YM[1], DeVito MJ[3]

[1]US EPA, ORD, NHEERL, ISTD, PB, Research Triangle Park, North Carolina, USA. [2]Curriculum in Toxicology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA. [3]NIEHS/NTP, Morrisville, North Carolina, USA

Introduction

Studies demonstrate that exposure to 2,2',4,4'-tetrabromodiphenyl ether (BDE 47) and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) decrease serum thyroxine (T_4) levels in laboratory animals [1,2,3]. The T_4 decrease in rodents is thought to occur through the induction of UDP-glucuronoysyltransferases (UGT) resulting in enhanced catabolism of T_4. Although, studies exposing UGT1A-deficient Gunn rats to phenobarbital (PB) or polychlorinated biphenyl (PCB) demonstrated that serum total T_4 decreases were not necessarily glucuronidation dependent[4, 5]. This suggests that other mechanisms must be involved in the thyroid hormone decreases. Similar to UGTs, sulfotransferases (SULTs) are regulated by nuclear receptors[6,7]; however, unlike UGTs, most prototypical nuclear receptor activators do not markedly induce SULTs in rodents[8]. The lack of significant inductions by nuclear receptor activators suggests that rodent sulfation is not as essential as glucuronidation in thyroid hormone catabolism. Although the induction of human T_4 sulfation by xenobiotics has yet to be examined, studies have compared thyroid hormone sulfation in human and rat. While human and rat SULTs catalyze thyroid hormones, there appears to be species differences in SULT activity. For example, even though there is an 80% amino acid sequence homology between human and rat SULT1A1[9,10], human SULT1A1 catalyzes thyroid hormones while the rat isoform does not. Human SULT1A1 was also identified as a low Km sulfotransferase with similar Kms and thyroid hormone substrate specificities as human hepatic and renal sulfotransferases[11,12]. This correlation between human SULT1A1 and sulfotranserase activites toward thyroid hormones suggests that human SULT1A1 is a prominent sulfotransferase in liver and kidney. This study focuses on the enhanced formation of T_4-glucuronide (T_4G) in rat and human hepatocytes following exposure to PCB 153and BDE 47. This study also highlights the utility of primary hepatocytes in evaluating potential species differences in the effects of thyroid hormone disruptors.

Materials and methods

Rat and human hepatocyte treatment.
Fresh sandwich-cultured rat and human hepatocytes were obtained from CellzDirect (Durham, NC). Cells were sandwich culture plated in 24-well collagen I coated culture plates overlaid with Geltrex®, and cultured at 37°C in a humidified incubator with 95% O_2:5% CO_2 in serum-free Williams' E media. 48 hours after plating, male Sprague-Dawley rat or human hepatocytes were treated daily for 72 hours with PCB 153 or BDE 47 at a concentrations of 0, 0.3, 3.0 or 30uM in 0.1% DMSO supplemented Williams' E media. 24 hours after final dosing, cell culture media was replaced with media containing [^{125}I]-T_4 at median serum concentrations observed in rats and humans; 0.05uM and 0.1uM, respectively. 24 hours after [^{125}I]-T_4 administration, media and cells were collected.

Metabolite analysis
Media is dried at 40ºC under N_2 gas. After drying, samples are reconstituted in 40ul of acetonitrile: 0.02M ammonium acetate (16%:84%). After vortexing, samples are centrifuged at 10,000xg for 10 minutes at 4ºC. After centrifugation, supernatant is removed and placed in an amber vial for UPLC analysis. Media was analyzed for iodothyronines and their conjugates using an established UPLC method with fraction collection[13].

Data analysis.
All data are represented as the mean ± standard deviation. The statistical intergroup comparisons were determined by using a one-way analysis of variance (ANOVA) followed by Dunnet's Multiple Comparison post test (GraphPad Prism 5.0, GraphPad Software, San Diego, CA). The levels of probability of statistical significance are $p < 0.05$.

Results and discussion:

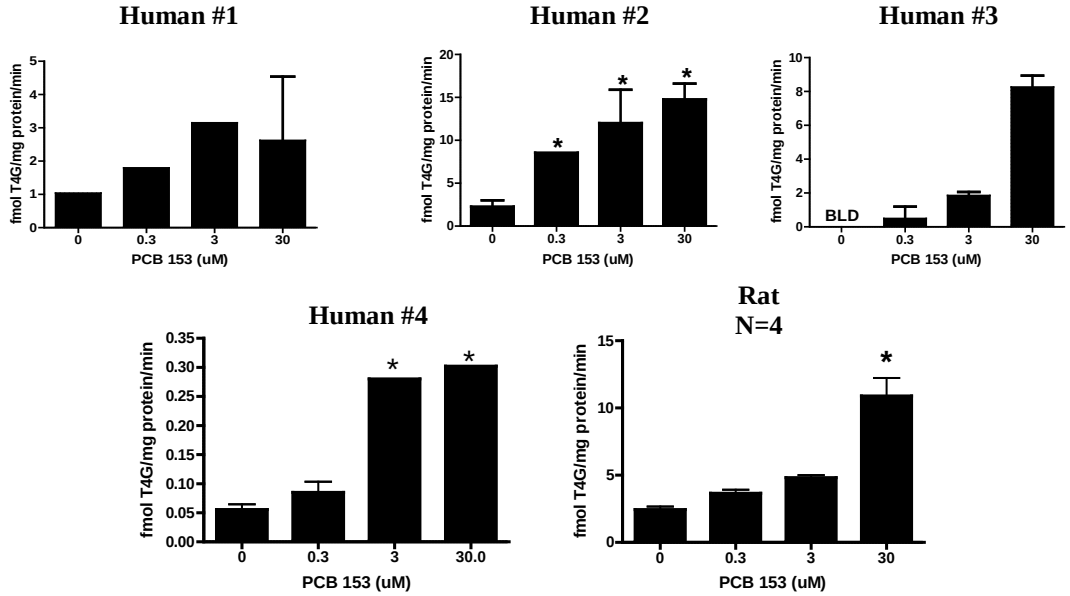
Metabolite Summary (Table 1). Thyroid hormone metabolite percentages in media of rat and human hepatocytes. Hepatocytes were cultured in treated with media containing [^{125}I]-T_4 at median serum concentrations observed in rats (0.05uM) and humans (0.1uM) for 24 hours. Overall, T_4 metabolic rates are faster in rats than in humans.

Table 1

Metabolite	Rat	Human
T_4G	16.90%	0.06%
T_4S	0.67%	0.05%
T_3/rT_3	0.88%	1.02%
-I	16.55%	4.90%

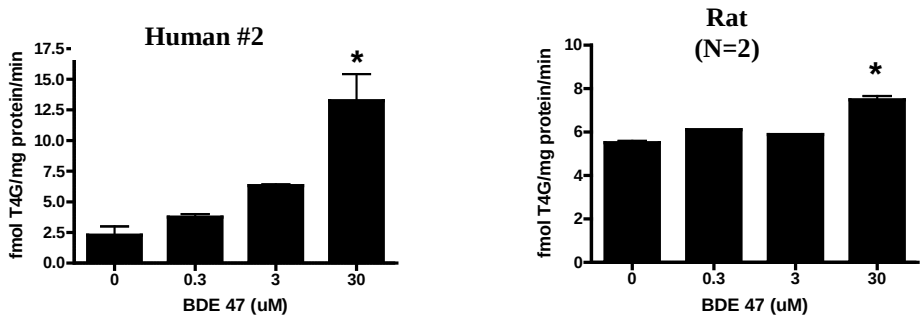
T_4 glucuronidation following PCB 153 treatment (Figure 1). Comparison of T_4G appearance in media of rat or human hepatocytes following pretreatment of hepatic enzyme inducers PCB 153. T_4G formation was increased with pretreatment of rat hepatocytes with PCB153. T_4G appearance increased with all human donor hepatocytes except for donor #1. T_4G appearance in media of human donor #3 was undetectable in controls. Data shows variability between human donors. (*Indicates statistical significance compared to control; $p<0.05$; BLD=below limits of detection [0.05 fmol/mg protein/min]).

Figure 1



T_4 glucuronidation following BDE 47treatment (Figure 2). Comparison of T_4G appearance in media of rat and human liver hepatocytes. T_4G increased by as much as 13.3-fold in human as compared to rat hepatocytes (1.4-fold). (*Indicates statistical significance compared to control; $p<0.05$).

Figure 2



Conclusions

Previous rodent studies have reported decreases in circulating T_4 concentrations following exposure to PCBs and PBDEs, though the mechanism by which T_4 is reduced is not entirely clear; however, several studies suggest that the T_4 decreases are related to increased metabolism by UGTs. Data from this study shows that more T_4G appeared in the media of rat hepatocytes as compared to humans. T_4G appearance in the media increased with PCB 153 by as much as 3.8-fold, whereas in human hepatocytes the increase was as much as 12-fold. Following BDE 47 treatment, T_4G $f_4$ormation from rat and human hepatocytes increased by 1.4- and 13.3-fold, respectively. The data also shows variability between human donors with respect to T_4G appearance, suggesting polymorphisms in the nuclear receptor or UGT genes. Our data supports the idea that hepatic enzyme inducers increase the hepatic metabolism of thyroid hormones by xenobiotics which *in part* may decrease circulating thyroid hormone concentrations in rats and humans; however, the mechanisms are still unclear.

Acknowledgements

This is an abstract of a proposed presentation and does not necessarily reflect EPA or NIEHS/NTP policy.

References:

1. Craft, E.S., DeVito, M.J., Crofton, K. M. (2002) *Toxicol. Sci.* 68: 372-80
2. Richardson, V.M., Staskal, D.F., Ross, D.G., Diliberto, J.J., DeVito, M.J., Birnbaum, L.S. (2008) *Toxicol. Appl. Pharmacol.*226: 244-50
3. Hallgren, S. , Darnerud, P.O. (2002) *Toxicology* 177(2-3), 227-43
4. Kato Y, Suzuki H, Ikushiro S, Yamada S, Degawa M, (2005) *Drug Metab Dispos* 33:1608-12
5. Maglich JM, Stoltz CM, Goodwin B, Hawkins-Brown D, Moore JT, Kliewer SA, (2002) *Mol Pharmacol* 62: 638-46
6. Sonoda, J., Xie, W., Rosenfeld, J. M., Barwick, J. L., Guzelian, P. S., Evans, R. M. (2002) *Proc Natl Acad Sci U S A* 9**9**(21): 13801-6

7. Saini, S. P., Sonoda, J., Xu, L., Toma, D., Uppal, H., Mu, Y., Ren, S., Moore, D. D., Evans, R. M., Xie, W. (2004) *Mol Pharmacol* 65(2): 292-300
8. Alnouti, Y., Klaassen, C. D. *J Pharmacol Exp Ther* (2008);324(2): 612-21
9. Yamazoe, Y., Nagata, K., Ozawa, S., Kato, R. *(1994) Chem Biol Interact* 92(1-3): 107-17
10. Weinshilboum, R. M., Otterness, *D.* M., Aksoy, I. A., Wood, T. C., Her, C., Raftogianis, R. B. (1997). *Faseb J* 11(1): 3-14
11. Visser, T. J., Kaptein, E., Glatt, H., Bartsch, I., Hagen, M.,Coughtrie, M. W. (1998) *Chem Biol Interact* 109(1-3): 279-91
12. Kester, M. H., Kaptein, E., Roest, T. J., van Dijk, C. H., Tibboel, D., Meinl, W., Glatt, H., Coughtrie, M. W., Visser, T. J. (1999) *J Clin Endocrinol Metab* 84(4): 1357-64
13. Rutgers, M., Pigmans, I.G., Bonthuis, F., Docter, R.,Visser, T.J. (1989) *Endocrinology* 125: 2175-86