TCDD-TCDF comparison of the mouse liver transcriptome by Percellome analysis – a search for TEF gene by time and dose-dependent responses -

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Abstract

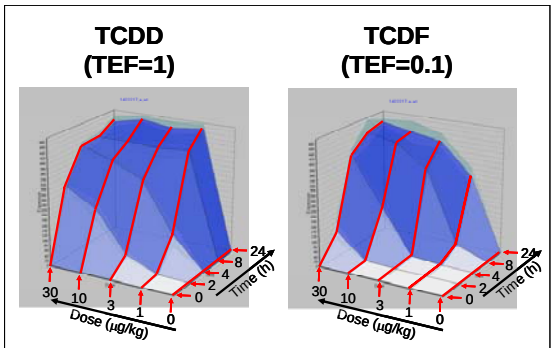
2,3,7,8-tetrachlorodibenzofuran (TCDF) can be more effective than 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in some conditions, especially *in vitro* disobeying its Toxicity Equivalence Factor (TEF) of 0.1. To investigate the molecular mechanism of TCDF with respect to TEF dependent and independent effects, we applied Percellome Toxicogenomics approach, where Percellome normalization method is used to generate mRNA expression values as "copy numbers per one cell" from Affymetrix GeneChip and the 3-D expression (X=time, Y=dose, Z=copies per cell) ("MilleFeuille" surface data, cf. http://toxicomics.nihs.go.jp/db/) that leads to biologist-friendly data analysis. TCDD (0, 1, 3, 10, 30 microg/kg, single gavage) was given to C57BL/6 male mice (n=3) and the liver was sampled 2, 4, 8, and 24 hours after the treatment (a total of 60 samples). Same protocol was performed for TCDF at the same dosages. By our original software handling the shape of the surface of each probe sets, up to 140 probe sets were shown to obey TEF and about 20 that do not obey TEF were identified.

Introduction

TCDD and TCDF are known as AhR ligands. As a part of the Percellome project, we monitored these two chemicals for the early gene response profiles in mouse liver after a single oral administration. Although, it has been shown that TEF[1] of TCDF is 0.1 against TCDD (TEF=1), our previous experience in some *in vitro* occasions showed that TCDF is as toxic as TCDD or more, indicating not all effect might obey the TEF value. However, there are seldom report on the comparison of the biological effect of TCDD and TCDF in a qualitative as well as quantitative aspect, especially those that do not obey the TEF. This study was conducted to obtain insight from the transcriptomic Percellome analysis on the molecular mechanism of TCDF with special interests on genes (probe sets) those obey the TEF value and those do not.

Materials and Methods

Sixty male C57BL/6 Cr Slc (SLC, Hamamatsu, Japan) mice maintained in a barrier system with a 12 h photoperiod (starts at 8:00) were given a single oral dosage of TCDD at 0, 1, 3, 10, 30 microg/kg at 10:00, and the liver was sampled at 2, 4, 8 and 24 hr post-gavage (three animals from each dosages groups). Another 60 male mice were given the same dosages of TCDF following the same protocol.

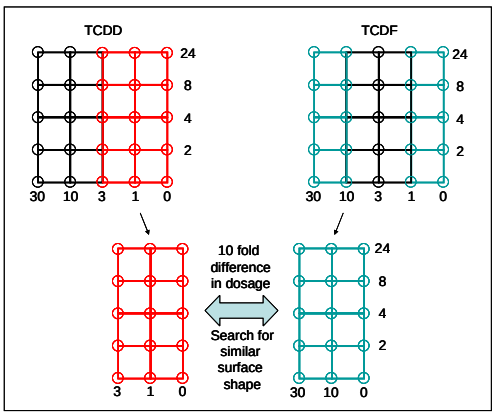


Time and dose-dependent-induction of Cyp1a1 expression by TCDD and TCDF

As reported previously[2], liver tissue blocks soaked in RNAlater (Ambion Inc., TX) were kept overnight at 4°C or until use. RNAlater was replaced with RLT buffer (Qiagen GmbH., Germany), and homogenized. A small aliquot from each sample homogenate was treated with DNAse-free RNase A followed by Proteinase K, and the DNA concentration was measured by PicoGreen fluorescent dye (Molecular Probes Inc., USA). In proportion to the DNA contents, each sample homogenates were spiked with the grade-dosed spike cocktail (GSC; cocktail of five *Bacillus subtilis* RNA sequences selected from the gene list of Affymetrix GeneChip arrays (AFFX-ThrX-3_at, AFFX-LysX-3_at, AFFX-PheX-3_at, AFFX-DapX-3_at, and AFFX-TrpnX-3_at), and then processed by the Affymetrix Standard protocol to apply to Mouse 430 2.0 GeneChip. The raw read outs were converted to Percellome data, and were plotted onto 5x5 (5

dose levels and 4 time points with virtual 0 hr using 2 hr vehicle value as a surrogate) 3-D surface graphs to visualize dose- and time-dependent alteration of each genes (cf. http://toxicomics.nihs.go.jp/db/).

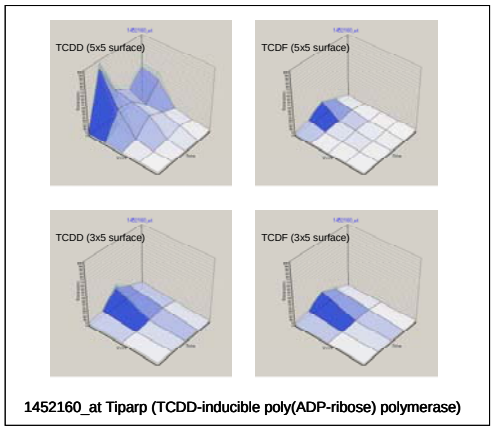
To search for TEF genes and non-TEF genes, from the TCDD surface data, the lower two doses and vehcile controls were used to draw 3x5 3-D surface graphs (0, 1, 3 microg/kg and virtual 0, 2, 4, 8, 24 hrs). From the TCDF data, the higher two doses and vehicle controls were used to 3x5 draw 3-D surface graphs (0, 10, 30 microg/kg and virtual 0, 2, 4, 8, 24 hrs). If the TCDF-responsive genes obey the TEF (0.1 versus 1), then the 3x5 surfaces which is 10 fold higher in dose level should give the same surface shape. Using our original surface comparison software, named MF-Compare, probe set-wise comparison of the shape of the 3x5 surfaces was performed and the TEF gene list (or probe set list) was generated. Likewise, non-TEF list was generated.



Results and Discussion

Up to 140 probe sets were shown to obey the TEF and more than 20 that do not obey TEF were identified.

Among TEF genes some were just a similar shape with less magnitude that fits the 10 fold dose level shift alone and without shift in time. Other patter includes that TCDD has faster induction (i.e., peak at 2 hr) at highest dose and TCDF and the lower dose of TCDD has later induction (peak at 4 hr) as shown in the right figure for 1452160_at Tiparp (TCDD-inducible poly(ADP-ribose) polymerase). Cyp1A1 shown above has the same characteristics. These two types of response may have fundamental differences in the gene cascade events controlling the timing or time-wise pattern of gene expression.



1452160_at Tiparp (TCDD-inducible poly(ADP-ribose) polymerase)

The non-TEF gene list has at least two types, "same shape same magunitude" as if TEF of TCDF is 1, and those showing different patters or more precisely only significant inductio by TCDF and not by TCDD.

The regulatory mechanisms behind those gene lists (probe set lists) awaits further analyses, such as promoter analysis by Chromatin immunoprecipitation (ChIP), ChIP on Chip, *in silico* enhancer-promoter search, and others including DNA methylation analysis by bisulfite method, etc. Some of them are underway.

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References

1. Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Tohyama C, Tritscher A, Tuomisto J, Tysklind M, Walker N, Perterson RE, Toxicol Sci. 2006, 93:223.
2. Kanno J, Aisaki K, Igarashi K, Nakatsu N, Ono A, Kodama Y, Nagao T. BMC Genomics. 2006; 7: 64.