DIOXIN EXPOSURE AND GENE EXPRESSION IN FIREFIGHTERS

Grassman JA[1], Chernyak YI[2,3], Merinova AP[2] , Brodsky ES[4], Shelepchikov AA[4]

[1]Health and Nutrition Sciences, Brooklyn College CUNY, 2900 Bedford Avenue, Brooklyn, New York 11210; [2]Institute of Occupational Health and Human Ecology of Siberian Branch of Academy of Medical Sciences, P.O. Box 1170, Angarsk, 665827, Russia; [3]Institute of Biophysics of ASTA, P.O. Box 4380, Angarsk, 665830, Russia; [4]Severtsov Institute of Ecology and Evolution of Russian Academy of Sciences, 33, Leninskiy prosp, Moscow, 119071, Russia

Introduction

This paper reports our continued investigation of dioxin exposure and effects among a group of firefighters who participated in the 1992 Shelekhov fire. The Shelekhov fire was a catastrophic three day event where more than 1000 tons of polyvinyl chloride, polyethylene and other plastics burned. Much of the fire suppression was performed inside a warehouse where the use of respiratory protective equipment was minimal. Afterwards, many of the firefighters became ill with an array of neurological symptoms known as "Shelekhov syndrome".[1] A cohort of 165 firefighters stratified for early and late onset Shelekhov syndrome, and participation in the Shelekhov fire has been followed since 2002. Measurement of dioxins in a subcohort of 20 firefighters found an average of 153 pg/g lipid adjusted total TEQ. However, both the firefighters who were involved in the Shelekhov fire as well as the firefighter controls had high mean levels of exposure (169 *vs* 105 pg/g). This compares with an estimated upper 95% confidence reference value of 11.8-16.9 for similarly aged US adults.[2] In an effort to understand the consequences of this exposure, we investigated the effect of dioxin exposure upon the dioxin-dependent AHR pathway. Using the metabolic probe, antipyrine, we found that CYP1A2 expression to be significantly correlated with Total TEQ.[3] Here, we report on the expression of the genes AHR, CYP1A1 and CYP1B1 and their relationship to dioxin exposure.

Materials and Methods

Diagnosis of Shelekhov Syndrome The neuropathologist and psychiatrist at the Occupational Health Clinic in Angarsk identified the collection of symptoms known as Shelekhov syndrome. Shelekhov syndrome consists of toxic encephalopathy accompanied by a distinct array of psychological abnormalities and sensory neuropathies.[4]

Selection of 20 firefighters The project was approved by each respective institution's Human Subjects Committees and informed consent was obtained prior to enrollment. The full cohort consists of 165 firefighters recruited from local professional organizations and illness registries. Due to budgetary limitations, serum dioxin levels were measured on a subcohort of 20 firefighters selected from among the larger cohort on the basis of having a complete set of data consisting of questionnaires, gene expression analysis and antipyrine tests. In addition, candidates for dioxin analyses were over 35 years old, and had weights between the 10th and 90th percentile for the cohort. The subcohort was stratified to include 5 firefighters with early onset of Shelekhov syndrome, 5 firefighters with late onset of Shelekhov syndrome, 5 firefighters who participated in the Shelekhov fire but did not become ill and 5 firefighters who had not participated in the Shelekhov fire.[1]

Dioxin analysis 7 polychlorinated dibenzo-p-dioxin (PCDD), 10 polychlorinated dibenzofuran (PCDF), and 12 polychlorinated biphenyl (PCB) congeners were analyzed in serum obtained from peripheral blood. Measurement of serum dioxin level has been previously described.[1]

Measurement of gene expression Approximately 30 ml of peripheral blood mononuclear cells (PBMs) were aseptically obtained from each subject using heparin. Blood was layered onto 12 ml of Histopaque 1077 (Sigma Aldrich) for centrifugal separation of the buffy coat. After two washes in Hank's Balanced Salt Solution, a 4-fold excess of RNAlater™ stabilization reagent (Ambion) was added and the samples were transferred to 4 C. After

transport, the RNAlater™ was removed for storage at -70 C. An average of 1500 ng total RNA was obtained per sample (range <100 to 5400 ng) using RNAeasy kits (Qiagen). For each subject, 1000 ng of total RNA was reverse transcribed using random primers and Multiscribe reverse transcriptase™ (250 U/reaction) supplied by the High Capacity cDNA archive kit (Applied Biosystems). Gene expression was measured in 25 ng of cDNA using ABI Assay on Demand Gene expression assays with unlabeled PCR primers and TaqMAN probes labeled with FAM, a minor groove binding dye. All reactions were performed in triplicate using TaqMAN Universal PCR master mix with Amperase UNG activity (Applied Biosystems). Real-time PCR was performed on a ABI 7500 thermocycler at 50 C for 2 minutes, 95 C for 10 minutes followed by 40 cycles of 95C for 15 seconds and 60 C for 1 minute. β actin was measured as the endogenous control for each sample. Every PCR analysis contained a calibrator consisting of pooled human and HEPG2 cell cDNA. Results are expressed as relative quantification (RQ) which is the ratio of the normalized test cDNA to normalized calibrator cDNA.

Data analysis Exposure results were reported as TEQ using WHO TEFs.[5] Statistical analysis was performed SPSS 11.5.0. For the exposure data, results below the detection limits were analyzed as "0" values. Nonparametric methods were used because of the small sample size and the non-normality of the RQ results.

Results and Discussion

Table 1 shows the demographics and the response characteristics for the complete subcohort of 20 firefighters and by tercile of exposure based on their Total TEQ. Highly exposed firefighters were more likely to be disabled and had a higher BMI than firefighters with the lowest Total TEQ. Although there were 9 non- or past smokers and 11 current smokers, their dispersion among the terciles reduces the likelihood that smoking confounds the relationship between Total TEQ and gene expression. When compared to non-smokers, smokers were found to have higher levels of AHR and CYP1A1 expression but not CYP1B1 (AHR p=0.037, CYP1A1 p=0.053; CYP1B1 p=0.239).

Table 1. Characteristics of the firefighters by Total TEQ category

		Total TEQ-lipid adjusted (pg/g)		
	Cohort	Low 27.2-85.1	Medium 85.2-152.4	High 152.5-476.5
N (%)	20	6 (30)	7 (35)	7 (35)
# Officer (%)	7 (35)	3 (50)	3 (43)	1 (14)
# Current smokers (%)	10 (50)	3 (50)	4 (57)	4 (57)
# Disabled (%)[1]	11 (55)	1 (17)	6 (85)	4 (57)
Age ± SD (min, max)	43.9 ± 35.1 (35.7, 53.5)	42.4 ± 6.2	43.5 ± 3.0	45.7 ± 6.0
BMI ± SD (min, max)[2]	26.6 ± 3.1 (21.3-33.5)	24.2 ± 2.3	27.8 ± 2.3	27.5 ± 3.6
Yrs firefighting (min, max)	14.8 ± 5.5 (8, 30)	17.2 ± 7.5	16.3 ± 3.9	11.3 ±3.3
Yrs since firefighting (min, max)	4.6 ± 4.1 (0, 11)	2.3 ± 3.8	6.1 ± 3.1	5.0 ± 4.9
# Shelekhov syndrome (%)	10 (50)	1 (17)	5 (71)	4 (57)

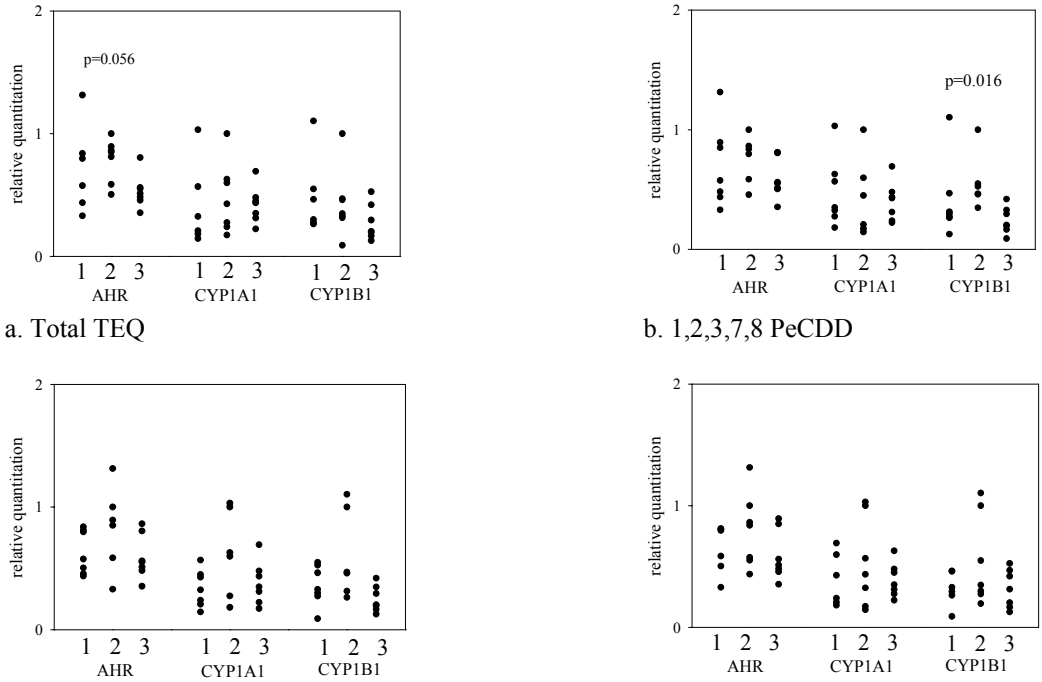
[1]Kruskal-Wallis test, comparison of terciles: p=0.044
[2] Chi-square comparison of terciles: p=0.061

As seen in Table 2, the expression of AHR, CYP1A1 and CYP1B1 were highly and positively correlated in the 20 firefighters indicating a pattern of activation consistent with the established dioxin pathway in rodents[6] and in humans in vitro.[7,8,9]

Table 2. Correlation of gene expression in 20 firefighters (Pearson's correlation)

Gene		r	P
AHR	CYP1A1	0.534	0.015
AHR	CYP1B1	0.742	0.000
CYP1A1	CYP1B1	0.682	0.001

Figure 1. Relative expression (RQ) of AHR, CYP1A1 and CYP1B1 in 20 firefighters by exposure category (1=low; 2=medium; 3=highest exposure tercile)



a. Total TEQ

b. 1,2,3,7,8 PeCDD

c.PCDFs

d. PCBs

Figure 1 shows the relative expression of AHR, CYP1A1 and CYP1B1 by tercile for Total TEQ, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD), all PCDFs, and coplanar PCBs (PCBs). This depiction shows that for each of the dose metrics except for PCBs, the levels of CYP1B1 gene expression are lowest among the firefighters with the highest exposure. However, the differences are statistically significant only for CYP1B1 and PeCDD (p=0.016). A similar pattern, where the expression is lower in dioxins but not PCBs, is observed for the expression of AHR. Expression of AHR is close to significance when exposure is measured as Total TEQ (p=0.056).
PeCDD has a half life of 15.7 years[10] and therefore, may be a more informative indicator of long term exposure and the interaction between serum dioxins and gene induction. This preliminary analysis suggests that this may be the case.

Despite the demonstrated ability of dioxins such as TCDD to alter the constituents of the pathway in human PBMs cultured *in vitro*, efforts to detect a similar activation of the pathway in humans have been largely unsuccessful.

Landi et al., 2003 found that AHR expression in PBMs was inversely correlated with serum concentrations of TCDD suggesting possible down regulation of the pathway.[11] Our analysis on this small subcohort of 20 firefighters showed a similar pattern for AHR.

Acknowledgements
The authors are grateful to Dr. Irina V. Kudaeva and Galina V. Skornyakova (Institute of Occupational Health and Human Ecology, Angarsk) for the help in collecting the blood samples, Radik A. Khismatulin, head of the disabled firefighter's union in Irkutsk, for arranging the examinations. Drs. Elena Y. Mir-Kadyrova Denis B. Feshin, Vladimir G. Zhilnikov (Severtsov Institute of Ecology and Evolution, Moscow) contributed to the dioxin analyses. This research was funded by Award No. RB1-2375-AN-02 of the U.S. Civilian Research & Development Foundation for the Independent States of the Former Soviet Union (CRDF) and a PSC CUNY 34 Research Award.

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