Antipyrine metabolism in firefighters participating in the 1992 Irkutskcable factory fire in Shelekhov, Russia

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Introduction

This paper reports an ongoing investigation of dioxin exposure, biomarkers and illness among firefighters who participated in the 1992 suppression of a major fire at the Irkutskcable factory in Shelekhov, Russia. We previously reported that participation in the Shelekhov fire resulted in exposure to dioxin and the induction of CYP1A2[1]. In 2004, comparison of the serum lipid-adjusted dioxin levels in a random sampling of 15 Shelekhov firefighters and 5 firefighter controls suggested participation in the Shelekhov fire resulted in higher levels of PCDDs. Shelekhov firefighters whose responsibilities were fire suppression had nonsignificantly higher total TEQ (p=0.06) than did the super-vising officers. There was also a trend for higher serum concentrations of PCDDs among firefighters with the neurological syndrome "Shelekhov syndrome" although the role of other exposure combustion products must be considered. Overall, the firefighters were highly exposed to dioxins having an average lipid-adjusted total TEQ of 153 pg/g[2]. Here we present evidence for the dioxin-dependent induction of CYP1A2 activity among the firefighters based on the measurement of metabolites after the administration of antipyrine.

Materials and Methods

Selection of the cohort and urine donors - After obtaining informed consent, 165 firefighters from local professional organizations and illness registries were enrolled as previously described[2]. Human subjects approval was obtained and the collaborating institutions strictly complied with the associated requirements. In the years from 2002-2004, the antipyrine metabolism test (AP-test) was performed in 158 of the 165 participating firefighters. The firefighters were divided into four groups. Group 1 (n = 15) consists of subjects who were first hospitalized in 1992-1993 with acute intoxication. They also developed a pattern of symptoms which typically include toxic encephalopathy, personality and behavioral abnormalities, and acute distal sensory polyneuropathy that later became known as "Shelekhov syndrome". Group 2 (n = 45) consists of firefighters who had symptoms related to the fire but registered with Shelekhov syndrome one to two years later. Group 3 (n = 53) includes Shelekhov firefighters who may or may not have had acute intoxication but did not develop Shelekhov syndrome. Group 4 (n = 45) consists of firefighters who did not participate in the Shelekhov fire.

CYP1A2 enzyme activity - Antipyrine (AP) was used as a probe for the assessment of hepatic cytochrome P4501A2 enzyme activity in the firefighters. Urine samples were collected for 24 hours after the ingestion of 18 mg AP/kg body weight. In the first of a two part extraction, dichloromethane–n-pentane (3:7, v/v) was used to separate the metabolites 4-hydroxyantipyrine (4HAP) and norantipyrine (NAP). 3-hydroxy-methylantipyrine (3HMAP) and AP were extracted in the second stage with dichloromethane. AP and the associated metabolites were analyzed in the urine by HLPC ("Milichrom-A02", EcoNova, Russia) using a Silasorb SPH C18, 5 µm, 2 x 75 mm column. Detection was at λ = 244 nm at 45˚C. Phenacetin was used as internal standard. Eluent A was a mixture of methanol with 0,05 M phosphate buffer, pH 6,7 (10:90) whereas eluent B was 90% methanol. The eluent flow rate was 200 µl/min. The components were resolved with 7% eluent B for 10 minutes under isocratic conditions followed by a gradient of 7 to 100% eluent B for 4.5 minutes. In some samples, an unidentified impurity interfered with the resolution of the phenacetin peak. When that was the case, repeated isocratic chromatography with 20% eluent B for 10 minutes successfully resolved the phenacetin and the impurity. The AP metabolites are presented as proportion of the initial dose (% /AP) or proportion of the sum metabolites formed (% /m). The major steps in the oxidative metabolism of antipyrine metabolism are 4-hydroxylation, N-demethylation and 3-methylhydroxylation. Of these, CYP1A2 contributes

to roughly 50% of the formation of 3HMAP, 20-25% of NAP and 30% 4HAP[3].

Measurement of serum dioxin concentrations - A total of 51 dioxin analyses were used for the current report. 20 blood samples were obtained in 2004 and analyzed at the Severtsov Institute of Ecology and Evolution (Moscow) as previously described[2]. The remaining analyses were performed at the Environmental Research and Protection Centre (Ufa, Russia). Of these, 3 firefighter blood samples were obtained and analyzed in 1998, 14 in 2000 and 14 in 2002. 7 polychlorinated dibenzo-p-dioxin (PCDD), 10 polychlorinated dibenzofuran (PCDF), and 12 polychlorinated biphenyl (PCB) congeners were analyzed in each of the samples with the exception of the 1998 analyses which did not measure PCBs. The method used for the analysis of the 1998, 2000, and 2002 samples is similar to that used for the analysis of the samples obtained in 2004 and has been previously described[1]. Analyses of duplicate samples performed at each of the two laboratories provided comparable results.

Data analysis - Exposure results were reported as TEQ using WHO TEFs[4].Statistical analysis was performed SPSS 11.5.0. For the exposure data, results below the detection limits were analyzed as “0” values. Variables related to lipid-adjusted serum dioxin levels required log transformation to achieve normality.

Results and Discussion

Table 1 compares the age, disability status, and proportion of smokers in the four groups. Groups 1 and 2 are significantly older than groups 3 and 4. The firefighters in groups 1 and 2 are predominantly disabled whereas most of the firefighters in groups 3 and 4 are currently employed as firefighters.

Table 1. Demographic characteristics of the Shelekhov firefighters

Full cohort	Cohort n=158	Group 1 n=15	Group 2 n=45	Group 3 n=53	Group 4 n=45	p[2]
Age, yrs[1]	41,7 (7,2)	47,3 (7,7) [3,4]	44,6 (6,5) [3,4]	39,9 (6,6)	39,0 (6,6)	*0,000*
Disabled (#, %)	56 (35%)	13 (87%)	41 (91%)	2 (4%)	0 (0%)	*0,000*
Current smokers (#, %)	83 (52%)	8 (53%)	28 (42%)	27 (53%)	27 (60%)	*NS*

[1] mean (SD)

[2]Kruskal-Wallis ANOVA, $p < 0,05$

[3, 4] compared to Group 3 and Group 4, respectively: $p < 0,0083$ (with Bonferroni’s correction), Mann-Whitney U test.

Table 2 compares the formation of antipyrine metabolites in smokers and nonsmokers (column entitled “Cohort”) and also between groups (Columns labeled Group 1, 2, 3 and 4) using separate analyses for smokers and nonsmokers. The results obtained from the full cohort demonstrate that cigarette smoking significantly affects the formation of metabolites in a manner consistent with the induction of CYP1A2. When the formation of metabolites are expressed as a percentage of the total administered AP dose, smokers form significantly more 3HMAP than non-smokers. Similarly, when expressed as proportion of total metabolites, smokers tend to form less NAP with a corresponding increase in 3HMAP compared to nonsmokers.

Comparison of antipyrine metabolism between groups shows that, in nonsmokers, Group 2 forms significantly less 3HMAP (whether expressed either as % total administered dose or % metabolites) than the nonsmokers in Groups 1, 3, and 4. In addition, their degree of metabolic transformation of the administered AP dose metabolism is lower, particularly when compared to Group 3 whose members were both exposed at the Shelekhov fire and continue to work as firefighters. The pattern of metabolite formation is similar in smokers and nonsmokers. A lower proportion of the CYP1A2-related metabolite (3HMAP) is found in Group 2 compared to the Group 1 who were also diagnosed with Shelekhov syndrome immediately after the fire and Group 3 who participated in the Shelekhov fire but did not become ill. Given the small number of firefighters in Group 1, the comparison between Group 2 and 3 is of greater importance. The lower level of 3HMAP formation in Group 2 may be due to the cessation of smoke exposure. Alternatively, metabolism of antipyrine in Group 2 may be slower so that a larger proportion of metabolites are excreted after the 24 hour urine collection.

Table 2. Antipyrine metabolism among the Shelekhov firefighters (mean ± SD)

	Y/N[1]	Cohort (n=75/83)	Group 1 (n = 6/ 9)	Group 2 (n = 26/ 19)	Group 3 (n = 25/ 28)	Group 4 (n = 18/ 27)	p*
As percent of total dose of AP							
NAP	N	16,2 ± 4,8	17,3 ±2,4	15,3 ±4,7	17,3 ±4,8	15,7 ±5,3	
	Y	17,8 ±5,9	16,1 ±4,8	15,5 ±4,6 [4*]	18,1 ±6,4	19,8 ±6,0	
4HAP	N	***23,5 ± 8,2***	20,5 ±9,1	21,9 ±8,2	26,2 ±6,6	23,0 ±9,5	
	Y	***29,2 ± 9,4[5]***	24,2 ±8,0	30,0 ±10,4	30,7 ±10,7	28,6 ±7,5	
3HMAP	N	***9.8 ± 5,9***	***11,4 ±2,5***[2]	***6,8 ±4,4***[3]	***12,6 ±5,6***	***9,8 ±7,4***	*0,001*
	Y	***13,7 ± 6,7[5]***	17,5 ±7,7	12,4 ±6,6	13,6 ±7,8	13,5 ±4,9	
AP	N	3,1 ± 1,5	2,8 ±1,7	2,8 ±1,0	3,2 ±1,2	3,4 ±1,1	
	Y	2,8 ± 1,2	2,3 ±1,0	3,0 ±1,3 [4*]	3,0 ±1,4	2,7 ±0,9	
Sum met	N	***49,5 ± 16,0***	***49,2 ±8,4***	***44,0 ±14,9***[3]	***56,1 ±13,2***	***48,5 ±20,1***	*0,030*
	Y	***60,7 ± 18,1[5]***	57,8 ±14,4	57,9 ±18,5	62,4 ±21,9	61,9 ±14,9	
As percent of total metabolites							
NAP	N	***33,7 ± 6,8***	36,2 ±8,3	35,8 ±7,2 [3*]	31,1 ±6,2	33,4 ±5,7	*0,028*
	Y	***29,7 ± 6,4[5]***	***28,4 ±7,5***	***27,9 ±7,0*** [4*]	***29,3 ±6,3*** [4*]	***32,0 ±5,2***	
4HAP	N	47,5 ± 7,0	40,4 ±11,8	49,2 ±5,6	47,0 ±6,4	47,9 ±7,0	*0,002*
	Y	48,2 ± 6,4	***42,3 ±8,4*** [2*, 3*]	***51,8 ±5,7***[4]	***49,4 ±5,0*** [4*]	***46,4 ±5,7***	
3HMAP	N	***18,8 ± 7,6***	***23,4 ±5,5***[2]	***14,9 ±6,4***[3]	***21,9 ±7,7***	***18,7 ±7,6***	*0,003*
	Y	***22, 1 ± 7,5[5]***	29,4 ±10,9 [2*, 3*, 4*]	20,3 ±7,4	21,3 ±6,9	21,7 ±5,8	

* Kruskal-Wallis ANOVA, p < 0,05

[1] Smoking status: N = nonsmoker ,Y = smoker

[2, 3, 4] compared to Group 2, Group 3 or Group 4, respectively: p < 0,008 (Bonferroni's correction), Mann-Whitney U test.

[2*, 3*, 4*] compared to Group 2, Group 3 or Group 4, respectively: 0,008 < p < 0,05, Mann-Whitney U test.

[5] Student's t test, p < 0,05

The dioxin-dependence of the group differences found in Table 2 is evaluated in Table 3 which shows the age-adjusted correlations between AP metabolites, and lipid-adjusted serum levels of dioxins among the 51 members of the cohort for whom dioxin measurements are available. No significant relationships between dioxins and the formation of NAP were detected. The only significant association detected in non-smokers was the negative correlation between the percentage of AP metabolized and PeCDD.

Table 3. Correlation of antipyrine metabolites and lipid-adjusted serum dioxins[1]

AP or	Smoking	Lipid adjusted serum levels

metabolite[2]		TCDD	PeCDD	PCDDs	PCDFs	PCBs	TEQ
As percent of administered dose:							
4HAP	No	-,145	-,093	-,137	,103	,033	-,123
	Yes	-,159	-,053	-,133	-,140	-,132	-,039
3HMAP	No	-,136	-,242	-,177	,086	,198	-,042
	Yes	,060	-,279	,013	,256	**,444**[3]	**,434**[3]
AP	No	-,173	**-,482**[3]	-,385	-,167	,188	-,119
	Yes	,099	,133	,181	-,069	-,208	-,012
As percent of total AP metabolites:							
4HAP	No	-,103	-,062	-,158	-,006	-,326	-,274
	Yes	-,258	,117	-,158	-,181	**-,490**[3]	**-,471**[3]
3HMAP	No	,054	-,143	-,047	,040	,153	,047
	Yes	,115	-,302	,068	,317	**,452**[3]	**,443**[3]

[1]24 smokers and 27 nonsmokers with recent dioxin analyses from 2000 -2004; for PCB, 48 firefighters with 24 smokers and 24 nonsmokers

[2]Age adjusted Pearson's correlation

[3] $p < 0,05$

Among dioxin congeners, PeCDD approaches TCDD in biological effect and has a long half life of nearly 16 years[5]. Hence, this observation may be the consequence of accumulating earlier exposures. Among smokers, both PCB and Total TEQ exposure were positively correlated with the formation of 3HMAP and negatively correlated with 4HAP. This pattern is consistent with the induction of CYP1A2 and is quite possibly the consequence of exposure from both occupational and environmental exposures. Although earlier results showed that Shelekhov firefighters, as well as those with Shelekhov syndrome, tended to have higher levels of PCDDs, no association between PCDDs and AP metabolites were found among the 51 firefighters shown in Table 3.

In summary, these data indicate that cigarette smoking has a large impact of AP metabolism. When the effect of smoking is excluded, exposure to dioxins, expressed as Total TEQ or as Total PCBs, produces a pattern of AP metabolites that is consistent with an increase in CYP1A2 activity. Subsequent work will increase the number of dioxin measurements, correct for the different times associated with the acquisition of the exposure data, and explore the impact of exposure latency upon CYP1A2 expression.

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