DETERMINATION OF CYP1A2 ENZYME ACTIVITY BY ANTIPYRINE TEST AND LEVELS OF DIOXINS IN "SHELEKHOV" FIREFIGHTERS

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Introduction

Cytochrome P4501A2 (CYP1A2) is considered to be the second member of the TCDD-inducible CYP1A subfamily and has similar induction mechanism as CYP1A1. CYP1A2 activity is mostly found within the liver and can be measured in people by monitoring for metabolites of test drugs in urine[1]. Antipyrine is one of these drugs. Previously, this method was used for assessment of the activity of liver CYP1A2 in humans, who lived on South Vietnam territories sprayed with Agent Orange, as well as for firefighters who liquidated a fire at a cable factory in 1992 in Russia[2,3]. According to the latest estimations using a factory materials combustion model, from 22 to 57 g of dioxins (in I-TEQ_{DF}) formed during the fire[4].

New data about CYP1A2 activity and dioxin levels in "shelekhov" firefighters are presented in this paper.

Methods and Materials

Study population. The cohort of firefighters who liquidated a fire at the "Irkutskcable" factory in the city of Shelekhov (East Siberia) in 1992 was examined. During the fire, 12.5 tons of polyvinylchloride film, about 320 tons of polyvinylchloride plasticate, 300 tons of cable paper, 19 tons of polyester film, 30 tons of polyethylene HP, 20 tons of cable silk, 364 tons of glass silk, 5 tons of colophony, 25 tons of wax and 47 tons of coloring agents for polyvinylchloride and polyethylene, together with some other materials, were destroyed. About 740 persons involved in fire liquidation (included 229 students, 18-20 years old, from the Fire-Technical School), were exposed to the complex mixture of toxic compounds that contained dioxins.

Eleven firefighters were taken to the hospital immediately after the fire, and 59 people visited out-patients departments of the hospitals. The firefighters' health has changed for the worse. The syndrome complex in firefighters presented as toxic encephalopathy with psychoorganic syndrome and vegetative-sensory polyneuropathy of the limbs. Depressive and hypochondriacal disorders were expressed in various degrees, asthenoorganic syndrome, emotion-volitional disorders predominated in psychoorganic syndrome[5]. At present, occupational diseases connected to the fire have been registered for 103 firefighters, four of whom have already died.

The urine samples for the antipyrine test were obtained from 1999 and to March, 2001 for 149 individuals (males) from this cohort who participated in a medical examination at the Institute of Occupational Health and Human Ecology hospital of Angarsk. The firefighters were divided into three groups. The first group contained 18 subjects taken to the hospital after the fire with symptoms of acute intoxication. The second group contained 92 firefighters whose symptoms

related to the fire were registered later - in 1994-2001. Thirty-seven of firefighters from the first and second groups were examined twice with a year interval. The third group included 39 firefighters from the same region who did not participate in the Shelekhov fire liquidation. The information about the age, time spent in fire, and smoking status was obtained for each subject.

Assay of CYP1A2 enzyme activity. Antipyrine (AP) is used as a probe for assessment of the hepatic cytochrome P4501A2 enzyme activity in firefighters. 18 mg/kg body weight of AP was ingested and urine samples were collected for 24 hours. Urine samples were analyzed for AP and its metabolites by HLPC, as previously described[3]. The procedure of metabolite extraction was made in two stages. 4-hydroxyantipyrine (4HAP) and nonantipyrine (NAP) were extracted at the first stage. The optimal extraction of 3-hydroxymethylantipyrine (3HMAP) and AP was tried to achieve at the second stage.

Dioxin analysis. 7 polychlorinated dibenzo-p-dioxin (PCDD), 10 polychlorinated dibenzofuran (PCDF), and 12 polychlorinated biphenyl (PCB) congeners were analyzed in serum of peripheral blood collected from 23 firefighters in November 2000 by high resolution gas chromatography/mass spectrometry by methods USEPA 1613 and 1668. Internal standards were obtained from Wellington Lab. (Canada), calibrate solutions and extraction standards from Cambridge Isotope Lab. (USA).

Results and Discussion

Table 1 shows some demographic characteristics in the groups.

Table 1. Summary of groups

Group	1	2	3
# subjects	18	92	39
age (years)[1]	42.8 ± 8.3 (27 - 52)	39.5 ± 7.3 (24 - 56)	31,8 ± 6,3 (21 – 43)
time spent in fire (h)[1]	29.6 ± 21.3 (8 - 72)	21.0 ± 12.5 (3 - 57)	0
smoking	never: 4	never 30	never: 10
	current: 14	current: 42	current: 26
	past: 0	past: 20	past: 3

[1] Values represent mean ± SD (min – max).

It is necessary to note that hours spent in the fire are not regarded as the index characterizing

Table 2. AP-test results

Parameter	Group 1 (n = 18)	Group 2 (n = 92)	Croup 3 (n = 39)
AP metabolites sum[1]	50.95 ± 24.38	53.88 ± 23.10	52.89 ± 22.51
3HMAP[1]	16.05 ± 6.20	14.27 ± 8.17	13.39 ± 6.60
AP[1]	3.03 ± 1.57	3.72 ± 2.05	4.35 ± 4.24
NAP[2]	24.06 ± 5.69	29.56 ± 8.63	28.32 ± 8.29
4HAP[2]	41.77 ± 8.62	44.98 ± 6.14	46.61 ± 5.75
3HMAP[2]	34.17 ± 10.55*	25.46 ± 7.22	25.07 ± 7.37
3HMAP[2]- smok[3]	31.70 ± 8.75 (n = 14)	27.38 ± 6.22 (n = 42)	26.17 ± 8.01 (n = 26)

[1] Mean ± SD of metabolites sum, 3HMAP and AP in % to AP dose;

[2] mean ± SD of NAP, 4HAP and 3HMAP in % to metabolites sum, equals to 100;

* compared to Group 2 and Group 3: $p<0.05$, Wilcoxon rank sum test;

[3] only for current smoking subjects.

exposure to a complex mixture of toxic compounds. The fire area was too dissimilar in level of possible exposure. The results are shown in Table 2.

The significant increase of 3HMAP (in % to antipyrine metabolites sum) was found in the first group of firefighters compared to the second and third one. Consider the fact that CYP1A2 plays the primary part in formation of that metabolite, the difference discovered can affirm the increase of functional activity of that isoform of cytochrome P450 in liver. These data can be connected with this group of firefighters exposed to the complex mixture of toxic compounds, which contained dioxins, to a large extent. The absence of differences between the second and the third groups is interesting. The analysis of the data showed that smoking did not always increase the indices that characterize CYP1A2 activity. The data for current smoking subjects given in table show the same.

The follow-up data on 37 firefighters (5 individuals from the first and 32 from the second group, respectively) who were examined twice with a year interval are presented in Table 3.

Table 3. Dynamics AP-test results (n = 37)

Time examination, year	3HMAP[1]	NAP[2]	4HAP[2]	3HMAP[2]
1999 – 2000	15.97 ± 9.06	26.17 ± 8.89	48.56 ± 5.84	25.27 ± 8.82
2000 – 2001	14.62 ± 7.37	29.13 ± 8.09	43.45 ± 5.80	27.42 ± 8.10

[1] Mean ± SD of 3HMAP in % to AP dose;
[2] mean ± SD of NAP, 4HAP and 3HMAP in % to metabolites sum, equals to 100.

Table 3 shows that any changes of the studied indexes over time were not found in the examined group. In addition, the decrease in 3HMAP (in % to metabolites sum) was recorded in 15 firefighters and increase in 22 individuals. Eight subjects had increase by more than 50%, while the maximum decline was by 36%. Probably, the reduction of this index will be noted with increasing the size of the group with follow-up data. On the one hand the reason for this can be a decrease in cytochrome P450-dependent monooxygenases activity with age (that is typical for men), on the other hand it can be gradual elimination of "shelekhov" dioxins, the content of which in humans we do not exclude. However, the time period between the examinations was too short for age and change in dioxin burden could really contribute much to the differences in the examined groups.

The data from Table 4 demonstrates the levels of PCDD/PCDFs (in TEQ) for 3 subjects (1 from the first and 2 from the second group, respectively) who were examined twice in one lab (Environmental Research and Protection Centre of the Republic of Bashkortostan, Ufa) can serve as the confirmation of "shelekhov" dioxins' version.

Table 4. The levels of PCDD/PCDFs in the samples of whole blood of the 3 firefighters (in TEQ), ppt in lipids

Subject no.	August, 1998	November, 2000	% of decrease
1 (33, past)[1]	42.88 (7.75; 5.6; 16.2)[2]	31.69 (5.9; 6.32; 12.61)	26.1
2 (44, past)	40.86 (7.48; 11.26; 15.59)	24.92 (5.79; 6.51; 1.07)	39
3 (53, never)	39.9 (5,5; 8.9; 15.37)	20.31 (5.33; 4.02; 1.16)	49.1

[1] Age (years) and smoking status;
[2] 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF.

It is interesting that the decrease in dioxin levels is found for all the subjects. Very large declines in 2,3,4,7,8-PeCDF were observed for subjects 2 and 3. The most contribution to both terms of

examination were made by 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, and also 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF. Besides, the levels of other congeners considerably increased compared to the first examination. Such growth could be expected as it occurs with inhabitants of industrial developed regions. Found decrease is one more argument for dioxin's participation in effects observed in firefighters and the degree of effect expression.
Table 5 shows the results of examination of 5 firefighters from the first group, held at the end of 2000.

Table 5. The levels of PCDD/PCDFs and PCBs in the samples of whole blood (in TEQ, ppt in lipids) and of AP-test results of the 5 firefighters from group 1

Subject no.	Age	Smoking status	PCDDs/PCDFs	PCDDs/PCDFs/PCBs	3HMAP[2]
1	52	Never	62.77	98.6	57.34
2	51	never	40.33	64.5	50.08
3	45	10-15 /day[1]	21.58	44.2	37.51
4	41	10/day[1]	18.6	39.6	36.05
5	53	never	20.31	65.9	29.63

[1] Cigarettes a day; [2] mean ± SD of 3HMAP in % to metabolites sum, equals to 100.

These data demonstrate a strong correlation between the dioxin level in lipids of peripheral blood serum and indexes of AP-test, showing the CYP1A2 activity in liver, for the first four subjects. It is needed to note that the basic contribution to total value of dioxins for the fifth subject was made by PCBs (nearly 69%). At the same time, this correlation was not observed for 13 firefighters from the second group who had the same examination.
In general, the data indicate that long-term effects, observed in "shelekhov" firefighters, can be caused by exposure to dioxins which were contained in the complex mixture of toxic compounds.

Acknowledgments
The authors are grateful to Dr. Nina I. Portyanaya (Branch 5 of the SRC - Institute of Biophysics, Angarsk) and Dr. Oleg L. Lakhman (Institute of Occupational Health and Human Ecology, Angarsk) for their help in medical examination of the firefighters. We would like to thank Dr. Scott A. Masten (NIEHS, RTP, NC, USA), Dr. Russ B. Hauser (HSPH, MA, USA), Dr. Olga A. Timofeeva (NHEERL, RTP, NC, USA) for their comments and encouragement, and Ruphina Y. Chernyak for technical assistance.

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