CYP1A2 ENZYME ACTIVITY (ANTIPYRINE TEST) IN "SHELEKHOV" FIREFIGHTERS

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Introduction

Cytochrome P4501A2 (CYP1A2) plays a key part in the implementation of the toxic effects caused by dioxin exposure. We have completed the first stage of research directed to using the antipyrine test to assess the activity of this enzyme isoform in the "shelekhov" firefighters, who liquidated a fire at a cable factor in 1992 in Russia.

Following is a report of the most recent data describing the connection between CYP1A2 activity and dioxin levels in the "shelekhov" firefighters.

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, about 1000 tons of various raw materials, basically polyvinylchloride and polyethylene, were destroyed. About 740 persons involved in fire liquidation, were exposed to the complex mixture of toxic compounds that contained dioxins. According to some estimations, from 22 to 57 g of dioxins (in I-TEQ_{DF}) formed during the fire [1]. Eleven firefighters were taken to the hospital immediately after the fire, and 59 people visited the outpatient departments of several hospitals. Previously, we described the health disorders of that cohort of firefighters in detail [2]. At present, occupational diseases connected to the fire have been registered for 104 firefighters, four of whom have already died.

The urine samples for the antipyrine tests were obtained from 1999 and to December, 2001 for firefighters who participated in a medical examination at the Institute's hospital of Angarsk. The firefighters were divided into three groups. The first group contained 17 subjects taken to the hospital after the fire with symptoms of acute intoxication. The second group contained 120 firefighters whose symptoms related to the fire were registered later - in 1994-2001. The third group included 59 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) was used as a probe for assessment of the hepatic cytochrome P4501A2 enzyme activity in the 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 [2]. The metabolite extraction was performed in two stages. 4-hydroxyantipyrine (4HAP) and nonantipyrine (NAP) were extracted at the first stage. The second stage was used to achieve optimal extraction of 3-hydroxymethylantipyrine (3HMAP) and AP.

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 21 firefighters in November 2000 in Environmental Research and Protection Center of the Republic of Bashkortostan, Ufa [3].

Results and Discussion

Table 1 shows some demographic characteristics of~~in~~ the groups.

Table 1. Summary of groups

Group	1	2	3
# subjects	17	120	59
age (years)[1]	43.8 ± 8.2 (27 - 54)	38.9 ± 9.2 (24 - 56)	33,5 ± 7,0 (21 – 51)
time spent in fire (h)[1]	35.3 ± 23.8 (8 - 90)	20.1 ± 11.8 (3 - 57)	0
smoking	never: 3 current: 13 past: 0 n.d.[2]: 1	never: 38 current: 57 past: 23 n.d.: 2	never: 13 current: 39 past: 3 n.d.: 4

[1] Values represent mean ± SD (min – max); [2] n.d.- no data.

It is necessary to note that we hold the opinion that hours spent in the fire are not regarded as the index characterizing exposure to a mixture of toxic compounds as previously. The fire area was too dissimilar in level of possible exposure.

The results are shown in Table 2 and indicate that there is significant increase of 3HMAP (as percent of the sum of the total AP metabolites) in firefighters from Group 1 compared to Group 2 and Group 3 (controls), R_w = 2.77 and 3.07, respectively. At the same time, Group 1 has a different

Table 2. AP-test results

Parameter	Group 1 (n = 17)	Group 2 (n = 120)	Group 3 (n = 59)
AP metabolites sum[1]	48.00 ± 23.66	50.83 ± 22.40	49.63 ± 21.17
NAP[1]	12.53 ± 7.57	14.42 ± 6.64	13.75 ± 6.79
4HAP[1]	20.64 ± 12.28	23.01 ± 10.67	23.54 ± 11.22
3HMAP[1]	14.82 ± 6.69	13.39 ± 7.82	12.34 ± 6.35
AP[1]	2.95 ± 1.40	3.57 ± 1.92	3.65 ± 3.59
NAP[2]	25.65 ± 7.07	29.23 ± 7.99	28.27 ± 7.36
4HAP[2]	41.54 ± 9.48^{R3}	45.33 ± 6.41	47.10 ± 6.70
3HMAP[2]	32.82 ± 10.53$^{R2R3;\ \lambda2\lambda3}$	25.44 ± 7.58	24.63 ± 7.46
3HMAP[2]- smoking[3]	32.34 ± 8.97 (n = 13)	26.81 ± 6.83 (n = 57)	25.08 ± 7.64 (n = 39)

[1] mean ± SD of metabolites NAP, 4HAP, 3HMAP and AP as % of total AP dose;

[2] mean ± SD of NAP, 4HAP and 3HMAP as % of the sum of the total AP metabolites;
[R2, R3] compared to Group 2 and Group 3, respectively: $p<0.05$, Wilcoxon rank sum test;
[λ2, λ3] compared to Group 2 and Group 3, respectively: $p<0.05$, Kolmogorov-Smirnov λ-test;
[3] only for current smoking subjects.

distribution from Group 2 and Group 3 for 3HMAP (as percent of the sum of the total AP metabolites), $\lambda = 1.50$ and 1.83, respectively. On this background, the decrease of 4HAP content (as percent of the sum of the total AP metabolites) was found in the Group 1 firefighters with acute intoxication compared to firefighters of the Group 3 - control group ($R_w = 2,03$). Since CYP1A2 is responsible for the formation of 3HMAP, the appearance of 3HMAP confirms the activation of that enzyme isoform in liver. Since the contribution of CYP1A2 to 4HAP formation is about 30 % [4], it is possible to assume that the decline of that metabolite is connected to a decrease in CYP3A4(5) activity which can contribute up to 65 % [4]. These data can be connected with the firefighters exposed to the mixture of toxic compounds, which were likely to have contained more dioxins. The absence of differences in metabolite concentration and percents between the Group 2 and Group 3 is interesting to note. The data for current smoking subjects (also shown in Table 2) demonstrate that smoking does not always increase indices that characterize CYP1A2 activity.

Table 3 compares a sampling of the "shelekhov" firefighters in whom dioxins levels were determined and the AP-test was made.

Table 3. 3HMAP[a] and dioxins[b] level characteristics in firefighters

Group	1	2
# subjects	5	16
age (years)[c]	49.20 ± 3.89* (41 - 53)	40.61 ± 7.52 (28 - 54)
smoking	never: 3 current: 2 past: 0	never: 4 current: 9 past: 3
3HMAP[a]	41.53 ± 10.31* (29.63 – 57.34)	25.86 ± 7.76 (11.06 – 38.94)
PCDD[b]	19.69 ± 8.74 (13.33 – 32.89)	17.73 ± 5.60 (6.60 – 27.67)
PCDF	13.50 ± 9.91 (6.44 – 29.91)	10.71 ± 4.04 (5.66 – 18.25)
PCDD/PCDF	32.72 ± 18.96 (18.60 – 62.77)	28.34 ± 7.80 (12.93 – 43.85)
PCB	29.84 ± 10.60 (20.90 – 45.60)	29.05 ± 10.76 (17.01 – 60.00)
PCDD/PCDF/PCB	62.56 ± 23.34 (39.60 – 98.60)	56.26 ± 8.52 (40.50 – 69.40)

[a] 3HMAP as % of the sum of the total AP metabolites;
[b] made in ER & PC, Ufa[3]; here and below values represent for the congener groups in WHO-TEQ, ppt lipids;
[c] here and below values represent mean ± SD (min – max);
* compared to Group 2: $p<0.05$, Wilcoxon rank sum test.

It should be noted that CYP1A2 activation in the Group 1 firefighters increases with age (Table 3). However, previous studies indicate that AP metabolism speed declines with age [5]. Thus, the current observation suggests that dioxins may be responsible for the observed effects.

Furthermore, the correlation of the presented indices were analyzed in each of these groups. The most interesting of them are shown in Table 4.

The data demonstrate a correlation between 3HMAP and PCDF, 3HMAP and PCDD/PCDF, age and PCB in the Group 1 of firefighters, and the absence of a correlation in the Group 2. It should be noted that the basic contribution to total value of dioxins for one of 5 subjects of the Group 1 was made by PCBs (nearly 69 %). The data demonstrate strong correlations between 3HMAP and the level of all

dioxins in lipids of peripheral blood serum ($p < 0.01$) for the other four subjects.

In the next stage, we tried to assess exposure of firefighters smoking on CYP1A2 activity. To make the assessment we combined 7 smoking and 11 non smoking subjects from each groups. It was determined that age and PCDD/PCDF/PCB of non smoking subjects were higher, than that of smoking subjects (correlation coefficient ñ = 0.63). At the same time, there were no significant differences in 3HMAP levels (as percent of the sum of the total AP metabolites) between the two groups. Then, we split up the Group 2 (from Table 3) into two groups: 4 smokers

Table 4. Correlation coefficients for 3HMAP[a] and selected variables.

Variables		Group 1 (n = 5)	Group 2 (n = 16)
3HMAP	Age	- 0.05	0.19
3HMAP	PCDD	0.70	- 0.42
3HMAP	PCDF	0.99**	- 0.05
3HMAP	PCDD/PCDF	0.90*	- 0.27
3HMAP	PCB	0.00	- 0.09
3HMAP	TEQ	0.40	- 0.34
PCDD	Age	0.67	- 0.16
PCB	Age	0.98**	0.02
PCDD/PCDF/PCB	Age	0.87	- 0.06

Values represent Spearman's rank correlation coefficient (ñ): * $p < 0.05$, ** $p < 0.01$.
[a] 3HMAP as % of the sum of the total AP metabolites.

and 9 non smokers. Smoking firefighters had a significantly higher levels of 3HMAP (as percent of the sum of the total AP metabolites) compared to the nonsmoking firefighters (R_w = 2,01), 18.86 % and 30.05 %, respectively.

In conclusion, we would like to note that the highest values of 3HMAP in Group 1 of firefighters (Tables 2 and 3) were determined in non smoking subjects. Thus, 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.

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