DETERMINATION OF CYTOCHROME P4501A2 (CYP1A2) ENZYME ACTIVITY USING ANTIPYRINE AS PROBE-TEST IN "SHELEKHOV" FIREFIGHTERS

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Introduction

A multitude of experiments aimed to determine effective biochemical markers of dioxin exposure have been carried out in recent years. Some of the most interesting studies investigated the phenomenon of dioxin induction of the products of a family of genes (CYP1A1, CYP1A2 and CYP1B1) that cause an increase in the activity of microsomal monooxygenases in mammalian organisms[1]. Particularly, the results of the studies include the determination of ethoxyresorufin-*o*-deethylase activity in peripheral blood lymphocytes of humans that were exposed to dioxins[2-4]. At the same time, there are data about the use of probe-test drugs for the same purpose. Antipyrine, for example, is used to evaluate the activity of liver CYP1A2[5,6]. Considerable correlative dependence was found between the content of antipyrine metabolites in urine and CYP1A1 activity (benzpyrene – hydroxylase) in blood lymphocytes of humans, who lived on South Vietnam territories sprayed with Agent Orange[5]. The common problems of these two approaches are ones that are connected with inter-individual activity difference of enzymes studied and influence on it by the set of other factors, such as smoking, age and etc[7].

At the same time, new data about the role of isoformes of cytochrome P450 in metabolism of antipyrine and the screening possibilities of the test that changed our understanding considerably have been reviewed recently[8-10]. It was found that CYP1A2 participates in the formation of all three major metabolites of antipyrine and determines the formation of 3-hydroxymethylantipyrine. This paper examines the determination of enzyme activity of CYP1A2 using antipyrine as a probe in firefighters who liquidated a fire at the cable factory in 1992. We informed about the beginning of that work in the frames of a profound clinical and laboratory examination before[11].

Materials and Methods

Study population. The cohort of firefighters who liquidated a fire at the "Irkutskcable" factory in 1992 in the city of Shelekhov (20 km far from Irkutsk) was examined. During the fire, which continued for three days, 12.5 tons of polyvinylchloride film and about 300 tons of polyvinylchloride plasticate, together with some other materials, were destroyed. Over 600 persons, who were exposed to the complex of toxic compounds that contained dioxins, were involved in fire liquidation.

Several studies of levels of PCDDs/PCDFs and PCBs in adipose tissue and whole blood samples have been made since the fire[12-14]. The levels of PCDDs/PCDFs in the samples of whole blood of a few firefighters were (in TEQ) in limits from 35.8 up to 42.6 ppt in lipids. These levels were slightly higher than the ones of Shelekhov and Sayansk inhabitants – 24.7 – 31.0 ppt in lipids[14]. The state of firefighters' health has changed for the worse. It is necessary to note that the high level of disability, which is typical for that cohort of firefighters (20%), is not found in other firefighters from the region[15].

The urine samples for antipyrine test were obtained in 1999 and at the beginning of the year 2000 for 67 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 two groups. The first group contained 16 subjects who were taken to the hospital after the fire with symptoms of acute intoxication. The second one had 51 firefighters who were in the hospital in 1994-2000. Almost all of the firefighters have been examined in hospital several times (up to 7 times) over these years. The information about the age, time spent in fire, and smoking status was taken 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[16] in some modification[17]. The procedure of metabolites extraction was made in two stages. 4-hydroxyantipyrine (4HAP) and norantipyrine (NAP) were extracted at the first stage. The optimal extraction of 3-hydroxymethylantipyrine (3HMAP) and AP was tried to achieve at the second stage. The chromatographic experiments were carried out with a Milichrom-4 liquid chromatograph (Russia) and Silasorb SPH C18, 5 μm, 2 x 64 mm column (Chemapol, Praga, Czech Republic).
Chemicals. AP was obtained from Darnica Co. (Kiev, Ukraine). AP metabolites: 4HAP, NAP, and 3HMAP were generous gifts of Professor Sergey Kolesnikov (East-Siberian Scientific Center of Siberian Branch of Academy of Medical Sciences, Irkutsk, Russia). Phenacetin (as internal standard) was produced by Pharmacon Co. (St. Petersburg, Russia). Type H-3 β-glucuronidase from Helix pomatia from Sigma Chemical Co. (St. Louis, MO) was kindly gifted by Dr. Mike DeVito and Janet Diliberto (National Health and Environmental Effects Research Laboratory, RTP, NC).

Results and Discussion
Table 1 shows some demographic characteristics in the groups. We would like to note that the percent of current smoking subjects is higher in the first group than in the second one, 81% and 51%, respectively.

Table 1. Summary of groups

Group	1	2
# subjects	16	51
age (years)[1]	41.7 ± 8.3 (27 - 52)	39.0 ± 5.9 (25 - 55)
time spent in fire (hours)[1]	29.5 ± 19.7 (8 - 72)	23.6 ± 10.3 (1 - 72)
smoking	never: 3	never: 19
	current: 13	current: 26
	past: 0	past: 6

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

We would like to note that the information we have about the time spent in the fire (the index of possible dioxin exposure) requires verification. We haven't had any information about the places and duration of time (in terms of event chronology) spent by firefighters in fire liquidation yet. The lack of these data and control group (which is formed at the present) made us compare two selected groups. The results are shown in Table 2.

Table 2. AP-test results

Parameter	Group 1 (n = 16)	Group 2 (n = 51)
AP metabolites sum[1]	53.78 ± 24.73	63.91 ± 20.52
3HMAP[1]	15.51 ± 6.15	16.01 ± 6.83
AP[1]	3.11 ± 1.58	3.42 ± 1.27
NAP[2]	23.56 ± 7.06	27.44 ± 6.88
4HAP[2]	44.91 ± 7.62	47.69 ± 4.51
3HMAP[2]	31.53 ± 10.91*	24.87 ± 6.27
	(n = 13)	(n = 26)
3HMAP[1]- smok[3]	16.25 ± 6.38	18.51 ± 6.91
3HMAP[2]- smok[3]	30.75 ± 9.93	26.42 ± 5.72

[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: $p<0.05$, Wilcoxon rank sum test;
[3] only for current smoking subjects.

The significant increase of 3HMAP (in % to antipyrine metabolites sum) was found in the first group of firefighters compared to the second 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. Collectively, 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.

Of course, this is only the first step. It is necessary to assess the effect of smoking and also some other factors, which are able to modify CYP1A2 activity of subjects of that group. The preliminary analysis of the data showed that active smoking (consumption of about 20 cigarettes a day) not always had the increase of indices that characterize CYP1A2 activity. The data for current smoking subjects given in Table 2 show the same. Probably, we should think to substitute antipyrine for a more specific CYP1A2 substrate, such as theophylline or caffeine[10]. However, the results substantiate the usefulness of the continuation of research in the direction chosen. We hope that we can go further in our understanding of "shelekhov firefighters phenomenon" during the next step with the data for the control group, with a larger number of examined subjects, and the use of adequate statistics methods.

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