PHASE II XENOBIOTIC BIOTRANSFORMATION IN THE "SHELEKHOV" FIREFIGHTERS

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Introduction

During evolution, some organisms adapted methods to metabolize xenobiotics. At present, particular attention is paid to the biotransformation of xenobiotics in liver. Biotransformation of xenobiotics in the liver consists of two functionally linked phases: the first phase (Phase I) is the enzyme biotransformation of lipophilic xenobiotics with cytochrome P450-dependent monooxygenases which may produce reactive metabolites. In the second phase (Phase II), the products of Phase I metabolism are conjugated with water-soluble groups. This complex of reactions, common to all living organisms, is considered to be the universal biochemical system of natural detoxification. Disturbances in this coordinated detoxification process is a common mechanism of toxicity and can lead to changes in homeostasis and pathological processes. It is essential to note that xenobiotic detoxification and activation may be the result of either first or second phases of biotransformation. The correlation between these processes depends on the activity of biotransformation enzyme isoforms for which certain xenobiotics are substrates[1,2]. The fate of electrophilic xenobiotic metabolites, formed in monooxygenase reactions with the participation of cytochrome P450, is dependent on the activity of Phase II biotransformation enzymes, which usually play a role of enzyme of detoxification. Some data suggest that dioxin-related compounds induce the enzyme activity that catalyze many common conjugation reactions[3,4]. At the same time, it is necessary to note that these enzymes are not as strongly induced as monooxygenases. Previously, we reported the results of a study of the activity of cytochrome P4501A2 (one of the indices of Phase I biotransformation) in firefighters who participated in the fire liquidation at the "Irkutskcable" factory in the city of Shelekhov in 1992[5]. We concluded that the long-term effects observed in the firefighters were caused by exposure to smoke containing dioxins that were formed during the fire.
Following is a report of data describing Phase II xenobiotic biotransformation in firefighters. The products of three conjugation reactions – glutathione, glucuronic, and sulphate, were measured in the urine and correlated with dioxin levels in blood.

Methods and Materials

Conjugation products were measured in the urine of a cohort consisting of firefighters who liquidated a fire at the "Irkutskcable" factory in the city of Shelekhov in 1992. The fire destroyed about 1000 tons of various raw materials, mainly polyvinylchloride and polyethylene. About 740 persons had varying degrees of involvement with the liquidation of the fire, during which they were exposed to a complex mixture of toxic compounds that contained dioxins. According to some estimations, from 22 to 57 g of dioxins (in $I\text{-}TEQ_{DF}$) were formed during the fire[6]. Previously, we described the health disorders of this cohort of firefighters in detail[7].

Urine samples were taken during the day on the day before the AP-test from 1999 to December 2001 during a medical examination at the hospital of the Institute of Occupational Health and Human Ecology. The firefighters were divided into three groups. Group 1 consists of 18 subjects, who, in 1993, were first taken to the hospital after the fire with symptoms of acute intoxication. Group 2 consists of 124 firefighters who had symptoms related to the fire but were registered later. Group 3 includes 63 firefighters from the same region who did not participate in the Shelekhov fire liquidation.

To examine the level of second phase of xenobiotics biotransformation in the liver, the concentration of glucuronides, mercapturic acids, and sulphates were measured in the firefighter's urine[8-10]. The concentration of creatinine[8] in urine and SH-glutathione in blood[11] were also determined. The indices were measured by absorption spectrometry.

7 polychlorinated dibenzo-p-dioxin (PCDD), 10 polychlorinated dibenzofuran (PCDF), and 12 polychlorinated biphenyl (PCB) congeners were analyzed in serum obtained from peripheral blood collected from 23 firefighters in November 2000 at the Environmental Research and Protection Center of the Republic of Bashkortostan, Ufa[12].

Results and Discussion

Characteristics of the three groups of firefighters are presented in Table 1.

Table 1: Firefighter's characteristics

Group	Group 1	Group 2	Group 3
# subjects	18	124	63
age (years)[1]	44.2 ± 8.0 (27 - 53)	39.5 ± 7.1 (25 - 63)	33.3 ± 6.9 (21 – 51)
time spent in fire (h)[1]	35.1 ± 23.0 (8 - 90)	20.0 ± 11.8 (3 - 57)	0

[1] Mean ± SD (min – max).

Table 2 shows that the firefighters had no significant difference in indices characterizing the activity of conjugation reaction. At the same time, there were several trends. Group 1 firefighters had higher urinary mercapturic acid levels than the Group 3 controls (R = 0,88, Wilcoxon rank sum test), Group 1 firefighters had higher glucuronide levels than the firefighers in Group 2 (R = 1,09), and Group 2 firefighters had higher levels of urinary sulphates than the control group (R = 1,30).

Table 2: Urinary indices of Phase II biotransformation among the Shelekhov firefighters

Index	Group 1 (n =18)	Group 2 (n = 124)	Group 3 (n = 63)
Mercapturic acids, mmol SH/mol creatinine	13.02 ± 7.57	11.37 ± 6.07	10.11 ± 3.77
Glucuronides, g glucuronic acid /day	1.15 ± 0.93	0.91 ± 0.62	0.93 ± 0.50
Sulphates, g/day	2.97 ± 1.08	3.12 ± 1.21	2.78 ± 1.04

Mean ± SD (min – max).

Table 3 presents the biotransformation Phase II data from 72 firefighters in Groups 1 and 2 who were examined twice in a one year interval. Table 3 shows that over a one year interval, the concentration of mercapturic acids in the firefighter's urine decreased (T = 3,43) while the concentration of glucuronides increased (T = 3,95) In the same interval, the decrease in the concentration of mercapturic acids in urine was accompanied by a decrease in the concentration of reduced glutathione in the firefighter's blood (T = 2,11).

Table 3: The concentration of mercapturic acids, glucuronic acid and sulphates in urine and SH-glutathione in the blood of 72 firefighters over a one year interval

Year of exam	Mercapturic acids, mmol SH/mol creatinine	Glucuronides, g glucuronic acid/day	Sulphates, g/day	SH-glutathione, μmol/ml
First exam 1999 – 2000	13.44 ± 9.08	0.64 ± 0.37	3.27 ± 1.51	1.49 ± 0.76
Second exam 2000 – 2001	9.44 ± 5.22*	1.04 ± 0.71*	3.04 ± 1.21	1.31 ± 0.51*

All values presented as Mean ± SD; * p<0.05, T- Wilcoxon criterion for linked examination pairs.

It is necessary to note that in Table 2 the analyses for Groups 1 and 2 of the "shelekhov" firefighters were received during the time that the examinations for Group 3 controls were given. Therefore, Table 2 includes data for the first as well as for the second measurement time of Table 3. The cross-sectional data shown in Table 2 does not reveal any change in the Phase II indices. In contrast, the Phase II indices in Table 3 varied when sampled at two difference times. It should be noted that additional data is needed to draw conclusions regarding the nature of the trend. Also, since the firefighters in Table 3 were not divided into groups, the observed outcome is from the entire cohort.

The firefighters were exposed to a mixture of compounds including polycyclic aromatic hydrocarbons, carbon oxide, vinylchloride, phthalates, and dioxins. This mixture may be responsible for the observed effects in Phase II biotransformation. It is likely that the levels of endogenous glutathione were depleted due to protective reactions involving antiperoxides that could cause the inhibition of glutathione conjugation reaction. At the same time, there was a compensatory increase in other conjugation reactions such as those that produced the observed increase in glucuronic acid. The decrease in reduced glutathione in the firefighter's blood is consistent with this observation.

In accordance with this, Table 4 data are of interest. These data suggest that the dioxin congeners that cause the CYP1A2 induction in the Group 1 firefighters[5] inhibit the clearance of mercapturic

Table 4: Correlation coefficients for selected variables

Variables		Group 1 (n = 5)	Group 2 (n = 18)
MA[a]	3HMAP[b]	- 0.80	- 0.21
MA	PCDD[c]	- 0.70	- 0.28
MA	PCDF[c]	- 0.80	- 0.28
MA	PCDD/PCDF[c]	- 0.90*	- 0.32
MA	TOTAL TEQ[c]	- 0.60	- 0.37
S[d]	TOTAL TEQ[c]	- 0.70	- 0.36

Values represent Spearman's rank correlation coefficient: * p < 0.05.
[a] here and below MA - mercapturic acids. [b] 3HMAP as % of the sum of the total AP metabolites.
[c] made in ERPC, Ufa[12]; this value and the values below are adjusted for lipid and concentrations are based on the WHO-TEQ. [d] S – sulphates.

acids.
A recent long-term study of exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin found that rat livers display a pattern of altered Phase II biotransformation similar[13] to that presented in Table 3. During the course of the rat study, there was an increase in the urinary excretion of mercapturic acids followed by a decrease 90 days after the cessation of treatment. As with the firefighters, there was a concomitant decrease in the concentration of glutathione conjugates in the urine of the experimental animals. The sulphate content, which was not different from control during the first examination period, increased.
The results provide evidence that exposure to a complex dioxin-containing mixture of toxic compounds formed during the fire alters Phase II xenobiotic biotransformation in firefighters. The data can be considered to be an expression of the compensatory-adaptive in response to a complex mixture of exposure.

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