GENETICS

Cytogenetic Study of Professional Rescue Rangers of Russian Ministry of Emergencies

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Cytogenetic studies in rescue rangers of the Russian Ministry of Emergencies has shown that this category of specialists are exposed to genotoxic factors during their professional activity. The level of induction of cytogenetic injuries of rescue team members depended on labor conditions, genotype (glutathione-S-transferase M1 and T1 xenobiotic detoxication genes), and tobacco smoking.

Key Words: *genotoxic factors; chromosome aberrations; genetic polymorphism; rescue rangers*

Professional activity of members of search and rescue teams of the Ministry of Emergencies of Russia is realized under difficult and hazardous conditions and is associated with mental stress, exposure to chemical, physical, and biological agents, including mutagens [3].

Due to progress in gene diagnosis it became possible to evaluate the individual sensitivity to mutagens and hence, liability to the development of induced tumor diseases. Many polymorphic states of various genes are known, predisposing to the development of certain diseases or preventing them [1]. The search for and detection of polymorphic states of genes encoding for resistance and/or sensitivity to extreme factors are carried out with the aim of preventing the development of cancer. The study of xenobiotic detoxication gene polymorphism is particularly interesting, because these genes largely determine the individual reaction to exogenous chemical compounds [2].

Glutathione-S-transferase (GST) is one of the main enzymes participating in detoxification of a wide spectrum of compounds with known promutagenic and mutagenic effects. Functional polymorphism of some GST genes is observed in human population, primarily GSTM1 and GSTT1 gene polymorphisms caused by long deletions in these genes. According to published data, up to 50% of the population are homozygous by deletion in GSTM1 gene and up to 38% in GSTT1 gene [6] characterized by the absence of enzyme activity. These enzymes play an important role in detoxification of chemical compounds and protection of cells from oxidative stress; the absence of GST in case of deletion polymorphism can modify mutagen metabolism in the body, which, in turn, can lead to induction of DNA aberrations and increase in the incidence of mutations.

We evaluated the mutagenic load and the impact of GSTM1 and GSTT1 genes polymorphisms for the incidence of chromosome aberrations in professional rescue rangers.

MATERIALS AND METHODS

We examined 88 workers of search and rescue teams of the Russian Ministry of Emergencies who

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participated in the search and rescuing activity and liquidation of emergencies. The mean age of examined subjects was 36.6±0.9 years, length of service in rescue teams of Ministry of Emergencies 0.5 to 15.0 years. Twenty-five male patients of hospital at the National Center of Urgent and Radiation Medicine, aged 38.0±4.0 years with corresponding health status, without history of working under difficult, hazardous, and harmful conditions, served as controls.

The blood was collected for cytogenetic analysis and evaluation of gene polymorphism; detailed information on the health status, type of work, length of service, hazardous and common habits was collected.

Analysis of chromosome aberrations in peripheral blood lymphocytes was carried out for evaluating the somatic cell genome status in control and experimental groups. Peripheral blood cell culture was prepared from cells of each examined subject [5]. Whole heparin-treated blood was cultured at 37°C for 48 h in sterile tubes or in flasks in RPMI-1640 (Biolot) with 20% FCS (Biolot), 30 mg/ml glutamine (Biolot), 10 μg/ml phytohemagglutinin (Difco), 100 μg/ml streptomycin, and 100 U/ml penicillin. Colchicine (0.3-0.5 μg/ml) was added to blood cell culture 1.5-2.0 h before the end of culturing for accumulation of dividing cells in the metaphase stage. The preparations were prepared routinely. About 200 metaphases from each individual were analyzed.

Gene polymorphism was evaluated as follows. DNA was isolated from the peripheral blood by the standard method and used for PCR of polymorphic fragments of GSTM1 and GSTT1 genes. Homozygotes (GSTM1+/+ or GSTT1+/+) and heterozygotes (genotypes GSTM1+/0 and GSTT1+/0) by normal GSTM1 and GSTT1 gene alleles were denoted (for convenience) as $GSTM1^+$ and $GSTT1^+$, respectively. The homozygotic status of the examined subject by deletion of both alleles of GSTM1 and GSTT1 genes (GSTM1 0/0 and GSTT1 0/0 genotypes) was later denoted as $GSTM1^0$ and $GSTT1^0$, respectively.

The results were processed using Statistica 6.0 software. The incidence of chromosome aberrations of different types were compared using Mann—Whitney nonparametric test. The impact of factors was evaluated using analysis of dispersions. If dispersions were unequal, the Kruskal—Wallis test was used. The genotype frequencies were compared using standard χ^2 test.

RESULTS

The total incidence of aberrations in the examined rescue team members was significantly higher than in controls ($p<0.001$; Table 1). The total incidence of chromosome aberrations increased at the expense of increased number of single fragments, which could be induced by a wide spectrum of mutagenic factors. The incidence of single fragments was almost 2-fold higher than in the control ($p<0.05$). The incidence of other types of the detected chromosome aberrations did not differ from those in the control group, but chromatid exchanges characteristic of exposure to chemical mutagens appeared in lymphocytes of rescue team members, but were not detected in controls.

Hence, the results of cytogenetic studies indicate that rescue rangers are exposed to genotoxic factors.

Statistical analysis of questionnaires failed to identify harmful factors responsible for increased incidence of chromosome aberrations, as the spectrum of potentially mutagenic factors to which the rescue workers were occupationally exposed was extremely wide. The respondents noted contacts with mercury, ammonium, chlorine, phenol, heavy metals, ionizing radiation, exposure to high concentrations of harmful gas, high temperatures. Many of these factors are characterized by mutagenic activity. The synergistic effect cannot be ruled out,

TABLE 1. The Incidence of Various Types of Chromosome Aberrations in Peripheral Blood Cells of Rescue Rangers and Controls

Group	Number of analyzed metaphases	Total incidence of aberrations, %	Incidence of aberrations, %			
			chromatid type		chromosome type	
			single fragments	exchanges	paired fragments	dicentric chromosomes
Control	5874	1.24±0.21	0.94±0.16	0.00+0.02	0.28±0.10	0.02±0.02
Rescue rangers	18672	2.46±0.18**	1.82±0.18*	0.09±0.02	0.50±0.06	0.02±0.01

Note. *$p<0.05$, **$p<0.001$ compared to the control.

TABLE 2. Relationship between Incidence of Chromosome Aberrations and Tobacco Smoking in Rescue Rangers

Group	*n*	Total incidence of chromosome aberrations	Incidence of aberrations, %			
			chromatid type		chromosome type	
			single fragments	exchanges	paired fragments	dicentric chromosomes
Nonsmokers	18	1.85±0.36	1.13±0.31	0.05±0.04	0.56±0.14	0.07±0.07
Tobacco smokers	61	2.59±0.22*	1.95±0.22*	0.10±0.03	0.49±0.08	0.03+0.01

Note. *$p<0.05$ compared to nonsmokers.

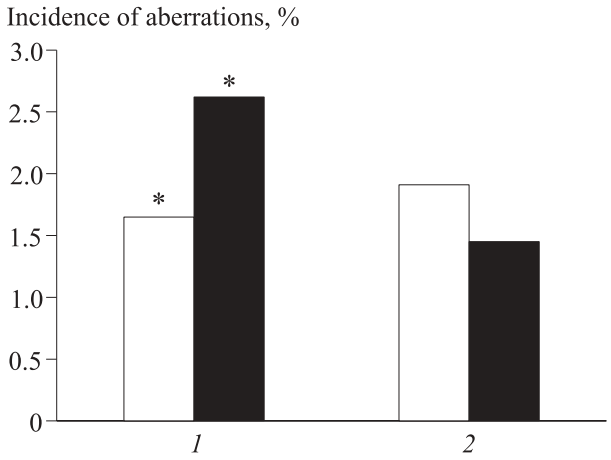

Fig. 1. Deletion polymorphism of GSTT1 gene and incidence of chromosome aberrations in tobacco smoking (*1*) and nonsmoking (*2*) rescue rangers. Light bars: $GSTT^0$; dark bars: $GSTT1^+$. *$p<0.05$ significant difference between the groups.

when a combination of several potentially mutagenic factors leads to a strong mutagenic effect.

An additional mutagenic factor, highly prevalent in this population, is tobacco smoking. Mutagenic and carcinogenic effects of tobacco smoking are well known [7]. However, at the cytogenetic level the negative effect of tobacco smoking manifests mainly against a provoking background, *i.e.* in subjects exposed to radiation and/or chemical mutagenic factors [4]. No effect of the tobacco smoking factor on the number of chromosome aberrations was detected in the control sample. A significant increase in the total incidence of chromosome aberrations ($p<0.05$) and incidence of single fragments ($p<0.05$) were detected in the group of tobacco smoking rescue workers ($n=61$) in comparison with nonsmokers ($n=18$) (Table 2). Five subjects were excluded from this study as they abandoned tobacco smoking just recently and could not be referred to any of the groups.

Hence, increased incidence of chromosome aberrations in tobacco smoking rescue rangers in comparison with nonsmokers and the absence of the effect in the control group indicate the synergism of tobacco smoking and occupational mutagenic exposure of the rescue workers.

By the distribution of the studied GSTM1 and GSTT1 genotypes the group of rescue rangers corresponds to the European population (Table 3) [11].

The study of individual sensitivity to genotoxic factors showed no relationship between GSTM1 and GSTT1 gene polymorphisms and cytogenetic parameters in the total group of rescue rangers. However, the incidence of chromosome aberrations in tobacco smoking rescuers with the $GSTT^0$ genotype was significantly lower than in those with the $GSTT1^+$ genotype (Fig. 1). Hence, homozygotic status by the GSTT1 gene deletion to a certain measure promoted resistance to this complex of mutagenic factors.

The effects of polymorphic status of GSTM1 and GSTT1 genes on the incidence of chromosome aberrations in individuals exposed to environmental mutagenic factors were described not once [9,12]. As GSTM1 and GSTT1 genes participate in xenobiotic detoxification, presumably, $GSTT1^0$ genotype prevents activation of the genotoxic constituents of harmful factors to which the rescue rangers are exposed [8].

Hence, rescue team members of the Russian Ministry of Emergencies are occupationally expo-\sed to genotoxic factors. The level of induction of cytogenetic damage is determined by the genotype and tobacco smoking. As high level of cytogenetic markers is an indicator of carcinogenic risk [10], it

TABLE 3. Incidence of Genotypes by GSTM1 and GSTT1 Genes in Rescue Rangers

Genotype	*n*	Incidence, %
$GSTM1^0$	27	45.0±6.4
$GSTM1^+$	33	55.0±6.4
$GSTT1^0$	12	20.0±5.1
$GSTT1^+$	48	80.0±5.2

is desirable to minimize the prevalence of tobacco smoking and to carry out measures aimed at prevention of cancer in professional rescue rangers.

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