

Monitoring Genotoxic Exposure in Uranium Miners

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Recent data from deep uranium mines in Czechoslovakia indicated that in addition to radon daughter products, miners are also exposed to chemical mutagens. Mycotoxins were identified as a possible source of mutagenicity present in the mines. Various methods of biomonitoring were used to examine three groups of miners from different uranium mines. Cytogenetic analysis of peripheral lymphocytes, unscheduled DNA synthesis (UDS) in lymphocytes, and lipid peroxidation (LPO) in both plasma and lymphocytes were studied on 66 exposed miners and 56 controls. Throat swabs were taken from 116 miners and 78 controls. Significantly increased numbers of aberrant cells were found in all groups of miners, as well as decreased UDS values in lymphocytes and increased LPO plasma levels in comparison to controls. Molds were detected in throat swabs from 27% of miners, and 58% of these molds were embryotoxic. Only 5% of the control samples contained molds and none of them was embryotoxic. The following mycotoxins were isolated from miners' throat swab samples: rugulosin, sterigmatocystin, mycophenolic acid, brevianamid A, citreoviridin, citrinin, penicilic acid, and secalonic acid. These data suggest that mycotoxins are a genotoxic factor affecting uranium miners.

Introduction

Uranium ore in Czechoslovakia is mined using very deep mines (1200–1600 m), which have warm temperatures and high humidity. The risk of occupational exposure in such mines has been related to the carcinogenic effect of radon daughter products, inducing lung cancer (1). To reduce this exposure to radon, ventilation in these mines was increased substantially in the period 1968–1972. As a result of this improvement the radon exposures in these mines are $1.0\text{--}1.5 \times 10^{10}$ MeV per year and infrequently are as high as $3.0\text{--}4.0 \times 10^{10}$ MeV (limit is 8×10^{10} MeV/year). In spite of the decreased exposure to radionuclides, lung cancer incidence among these uranium miners has not decreased.

In 1970, Kusak et al. (2) put forward the hypothesis that radionuclides may be accumulated in molds growing in uranium mines. Because these molds also produce aflatoxin, we initially looked for evidence of aflatoxin exposure by using an assay for measuring aflatoxin-albumin adducts (3). However, in a pilot study on 20 blood samples, no increased adduct levels were

observed (C. P. Wild, unpublished data). Samples from walls and woods in the uranium mines contained molds. Altogether, 90 types of molds were isolated, and 72% of them were embryotoxic (genus *Aspergillus*, *Penicillium*, *Fusarium*) and mutagenic using the Ames test and SOS chromotest (4). From these samples we isolated the mycotoxins sterigmatocystin, mycophenolic acid, brevianamid A, citreoviridin, penicilic acid, aflatoxin B₁ and G₁, rugulosin, and citrinin. Therefore, we hypothesized that these mutagenic mycotoxins may be another risk factor responsible for the continued high cancer risk of these miners. We report here the results of genetic and other biomonitoring studies of these uranium miners.

Materials and Methods

Subjects. The study involving blood samples was conducted in three mines in different districts in the Czech Republic (Hamr, Tachov, and Píbram) on 20–23 miners/group (mean age 38.8 for Hamr, 37.4 for Tachov, and 34.5 years for Píbram). Only healthy miners who had been working for 5–10 years in these mines were chosen for this study. They had worked in mines with a depth of approximately 1200 m, with an average temperature 26°C, and humidity 92%. Two control groups were used: a) officers from the district of Tachov, mean age 30.7 years and b) healthy volunteers from Prague, mean age 39.0 years.

Subjects for the throat swab samples were miners from Píbram (mean age 33.8 ± 4.8 years). The control throat swabs

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were taken from firemen, policemen, and iron workers in Příbram (mean age 36.1 ± 9.8 years).

Blood Samples. The blood samples for this study were taken from February to April 1990. From each subject, 20 mL of blood were collected in heparinized vacutainers. Peripheral lymphocytes were isolated immediately after delivery to the Prague laboratory and no later than 3 hr after the collection of blood.

Throat Swab Samples. Throat swab samples were taken in June 1991 using sterile wires from anticorrosive steel with cotton. Before the procedure, the swabs were touched in a sterile physiological solution. Samples were transferred for mycological cultivation in ice boxes.

Cytogenetic Analysis. The cytogenetic studies were done on short-term lymphocyte culture as described by Hungerford (5). The scheme of cultivation for 48 hr and the evaluation of chromosome aberrations were as reported by Šrám et al. (6).

Unscheduled DNA Synthesis. The level of DNA repair in the lymphocytes was estimated by *in vitro* unscheduled DNA synthesis (UDS) induced by the alkylating agent 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) (7). The ratio of increased incorporation of radiolabeled nucleoside [methyl-³H]thymidine induced by MNNG treated as compared to control is reported here as T/C. The radioactivity was measured in a scintillation counter.

Lipid Peroxidation. The lipid peroxidation (LPO) level in lymphocytes and plasma was determined by the modified thiobarbituric acid assay (8,9). The data were expressed as nmole malondialdehyde (MDA)/mg protein or mL plasma using 1,1,3,3-tetraethoxypropane as a standard. The content of protein in the suspension of lymphocytes was determined according to Bradford (10).

Vitamins Analysis. Ascorbic acid was estimated by a colorimetric method at 500 nm using its reaction with 2,4-dinitrophenylhydrazine. α -Tocopherol (vitamin E) and retinol (vitamin A) in plasma were determined after extraction into n-heptane by HPLC (11). The plasma level of vitamin B₁₂ was estimated according to the competitive protein-binding analysis with a commercially available radioimmunoassay kit (Laborchemie, FRG).

Mycotoxin Identification. All miners' throat swab samples were cultivated on Czapek-Dox agar, Sabouraud's agar, and Sabouraud's agar with 7% NaCl. Colonies of molds were estimated according to genus *Aspergillus*, *Penicillium*, and *Fusarium*. The mold isolates were cultivated for 14 days at 25 °C on 10% saccharose and 1% yeast autolysate and subsequently extracted into chloroform. One part of the extract was used to determine the embryotoxicity on 40-hr-old chick embryos (12). The other part was used for thin-layer chromatographic determination of 23 mycotoxins (12): aflatoxin B₁ and G₁, brevianamid A, citrinin, citreoviridin, cytocholasin E, cyclopiazonic acid, deoxynivalenol, diacetoxyscirpenol, fusaric acid, griseofulvin, luteoskyrin, mycophenolic acid, ochratoxin A, patulin, penicilic acid, PR-toxin, rubratoxin B, rugulosin, sterigmatocystin, secalonic acid, T-2 toxin, and zearalenon.

Results

The biomonitoring data from the blood study are summarized in Table 1. The cytogenetic analysis indicates a statistically significant increase of aberrant cells in miners from Hamr compared to both control groups. The other two mining groups

Table 1. Biomonitoring data for three exposed groups of uranium miners and two control groups.

Parameter ^a	Controls		Miners		
	Příbram	Prague	Hamr	Tachov	Příbram
No. of subjects	23	33	20	23	23
Age	30.7 (4.6)	39.0 (9.8)	38.8 (6.8)	37.4 (7.1)	34.5 (7.4)
Aberrant cells, %	2.16 (0.34)	1.52 (0.30)	3.44 [†] (0.41)	2.69* (0.36)	2.65* (0.34)
No. of breaks per cell	0.025	0.018	0.036	0.031	0.032
No. of dicentrics per cell	1.0	0.7	1.7	2.3	2.6
UDS (treated/control)	3.91 (0.82)	4.73 (0.98)	3.24 [†] (1.06)	3.49* (0.75)	3.76* (0.85)
LPO plasma, nmole MDA/mL	1.38 (0.37)	1.09 (0.26)	2.44 [†] (1.66)	3.59 [†] (1.91)	2.52 [†] (1.61)
LPO lymphocytes, nmole MDA/mg	1.24 (0.37)	1.23 (0.31)	1.43* (0.30)	2.14 [†] (0.78)	1.35 (0.37)
Vitamin C, ng/L	5.7 (1.8)	12.2 (4.9)	5.9* (3.1)	5.6* (2.6)	4.8* (2.0)
Vitamin E, mg/L	10.8 (2.2)	9.8 (3.2)	12.7 (3.6)	11.5 (2.6)	12.3 (3.2)
Vitamin A, mg/L	0.84 (0.19)	0.70 (0.19)	0.93 (0.14)	0.94 (0.14)	0.85 (0.18)
Vitamin B ₁₂ , ng/L	301 (152)	538 (250)	160 [†] (94)	202 [†] (133)	271* (110)

Abbreviations: UDS, unscheduled DNA synthesis; LPO, lipid peroxidation; MDA, malondialdehyde.

^aData are means; SD in parentheses.

**p* < 0.05 compared only to Prague control.

[†]*p* < 0.05 compared to both controls.

showed a significant increase in aberrant cells when compared to the Prague control group. The most frequent category of chromosome aberrations were chromatid breaks. Although dicentrics appear to be slightly increased in the exposed groups, this increase is not significant. Furthermore, if the chromosomal aberrations were due to radon, one would expect a much higher and significant increase in dicentrics.

Unscheduled DNA synthesis was significantly decreased in all groups of miners when compared to controls from Prague. Only UDS in the Hamr group was significantly decreased as compared to both control groups.

Lipid peroxidation was significantly increased in the plasma of all miner groups compared to both controls. Lipid peroxidation in lymphocytes was increased only in miners from the district Tachov (Table 1).

Low plasma levels of vitamin C were found in all groups from uranium districts including controls (physiological range between 5 and 15 mg/L). Plasma levels of vitamin B₁₂ were also low in the mining districts compared to Prague.

The results of mycotoxin analysis from throat swab samples are shown in Tables 2 and 3. In the miner group, 27% of samples carried molds. From these samples 60 different molds were isolated; 31 of them were embryotoxic. Spectrum of molds isolated from these samples as well as several mycotoxins produced by embryotoxic strains are presented in Table 4. Only 5% of the control samples carried molds and non of them was embryotoxic.

Table 2. Results of throat swabs.

Group (n)	No. of samples with molds	No. of isolated molds	No. of embryotoxic molds
Miners (116)	31 (27%)	60	31 (53%)
Controls (78)	4 (5%)	4	0

Table 3. Molds isolated from throat swabs.

Type	n	Embryotoxicity	
		n	Degree
Penicillium	28	8	+++
		13	++
Aspergillus	14	7	-
		3	+++
		6	++
Others	18	5	-
		1	++
		17	-

Table 4. Species of *Aspergillus* and *Penicillium* molds and mycotoxins in throat swab samples.

Type of mold	Isolated strains, n	Embryotoxic strains, n	Mycotoxin
<i>A. versicolor</i>	4	3	Sterigmatocystin
		1	?
<i>A. flavipes</i>	2	2	?
<i>A. restrictus</i>	2	1	?
<i>A. candidus</i>	1	0	0
<i>A. glaucus</i>	1	1	?
<i>A. repens</i>	1	1	?
<i>A. sydowii</i>	1	0	0
<i>A. ustus</i>	1	1	?
<i>A. wentii</i>	1	0	0
<i>P. rugulosum</i>	8	8	Rugulosin
<i>P. cyclopium</i>	3	2	Penicilic acid
<i>P. viridicatum</i>	3	2	Mycophenolic acid
<i>P. brevicompactum</i>	2	2	Mycophenolic acid
			Brevianamid A
<i>P. fellutanum</i>	2	2	?
<i>P. frequentans</i>	2	1	?
<i>P. spinosum</i>	2	0	0
<i>P. expansum</i>	2	1	Citrinin
		1	?
<i>P. citreoviride</i>	1	1	Citreoviridin
<i>P. notatum</i>	1	0	0
<i>P. thomii</i>	1	0	0
<i>P. oxalicum</i>	1	1	Secalonic acid

Discussion

Our study observed an unexpected factor in uranium mines: the increased occurrence of molds (genus *Aspergillus* and *Penicillium*) that produce mycotoxins. A possible genotoxic hazard for uranium miners may be the inhalation of dust contaminated with these molds. The improvement of ventilation in uranium mines in the period 1968-1972 decreased the concentration of radon daughter products but simultaneously may have increased the circulation of dustborne molds and thereby increased human exposure to the molds.

The cytogenetic analysis found an increase in chromatid breaks, similar to the effect of chemical mutagens. The increase of dicentric chromosomes compared to controls was not different. The significantly increased plasma LPO levels and suppressed UDS in lymphocytes may be due to exposure to mycotoxins and radon. The increase of LPO in plasma may be the result of radiation-induced free radicals (13). It is also believed that the higher plasma level

of vitamin E in uranium miners may be a natural defense against free-radical stress.

Several years ago, an increased risk of larynx cancer among uranium miners was observed (Kubat and Dolezal, unpublished report). Its localization was specific (supraglottic), and the cancer was diagnosed 10 years earlier than its usual incidence among the Czech population. We propose that the mycotoxins we have identified in the miners throat swabs support this clinical observation. The most significant is the finding of the mycotoxins sterigmatocystin, rugulosin, citrinin, and penicilic acid, which are mutagenic and have some evidence of carcinogenic activity (13,14).

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