Investigation of natural genetic variability and mutations in common voles (*Microtus arvalis* Pallas) and laboratory mice induced by polychlorinated biphenyls and ammonium nitrate

V. Morkūnas,
R. Lekevičius

Department of Botany and Genetics,
Vilnius University,
M. K. Čiurlionio 21,
LT-2009 Vilnius, Lithuania

The genetic variability of the common vole (*Microtus arvalis* Pallas) inhabiting the shores and island of Lake Kretuonas in the National Park of Aukštaitija was investigated by the tests of sperm abnormalities and embryo lethality. The levels of sperm abnormalities and embryo lethality were significantly higher in common voles trapped in the island than in voles inhabiting the shores. It is possible that the elevated level of genetic variability in island common voles could be caused by inbreeding taking place in an isolated population.

The male of common voles and laboratorial mice were treated with polychlorinated biphenyls and ammonium nitrate in oder to estimate the genetic susceptibility of generative cells of both species to widespread environmental pollutants. Polychlorinated biphenyls and ammonium nitrate did not increase the frequency of abnormal sperm in common voles, while the same doses of these chemical compounds in mice showed a significant effect. The lower genetic susceptibility of common vole male cells probably could be explained by adaptation of wild animals to contamination with genotoxic agents of their natural environment.

Key words: common vole, sperm abnormalities, embryo lethality, polychlorinated biphenyls

INTRODUCTION

Polychlorinated biphenyls (PCB), which have been available commercially for more than 50 years, belong to the most widespread environmental contaminants. Although PCB are known to be toxic and carcinogenic, their genotoxicity is not yet fully elucidated [1, 2]. PCB are clearly involved in the enhancement of the mutagenicity of compounds that are metabolically activated by P450 enzymes induced by PCB, but the amount of data specifically on the mutagenicity of PCBs is more limited [3]. Although Agency for Research on Cancer [4] classified PCB as non-mutagenic *in vivo*, several latest studies in rodents have shown its genotoxic effect. For example, PCB and PCB + diesel exhaust induced statistically significant increases in the legth-mutation frequency of the minisatellite PC-1 in male mice [5]. It was indicated that contamination of the environment with PCB can induce genetic damages in wild animals as well. So wild rodents *Peromyscus leucopus* and *Sigmodon hispidus* collected from a site polluted with chemicals containing PCB showed significantly higher frequencies of somatic chromosome aberrations as compared to animals inhabiting control site [6, 7].

Ammonium nitrate which has been widely used in agriculture as a mineral fertilizer is one of the sources of nitrate and nitrite. Nitrate and nitrite take place in the formation of nitrosamines, which are strong mutagens and carcinogens. More or less strong genotoxic features have been also shown by nitrite, nitrate and amonium nitrate itself [8, 9].

In the current study, we investigated the genotoxic effect of PCB or ammonium nitrate, or both, on male cells of the common vole (*Microtus arvalis* Pallas), wildly distributed throughout Lithuania, and laboratory mouse.

MATERIALS AND METHODS

Test agents. Polychlorinated biphenyls – Arochlors 1242, 1254 and 1260 (Bodega Marine Laboratory, USA) – were dissolved in sunflower oil. Ammonium nitrate was dissolved in distilled water.

Animals. Common vole (*Microtus arvalis* Pallas) male aged 8–12 weeks and weighing 20–30 g and DBF_1 hybrid mice were used in the investigation.

Chemical treatment and slide preparation. The males of common vole and DBF_1 hybrid mouse were distributed into five groups consisting of 4–6 animals. Polychlorinated biphenyls (PCB) and ammonium nitrate (AN) were administered to animals by intraperitoneal injections over a period of ten days. The control animals were treated with sunflower oil at a volume 10 ml/kg body weight per day. PCB were injected into males of the second and third groups at a dose 500 mg/kg per day and ammonium nitrate into males of the fourth group at a dose 100 mg/kg per day. The fifth group was treated with PCB and AN at the above-mentioned doses with a one hour interval between injections.

The animals of all treatment groups were sacrificed at day 35 following the first injection, because sperm abnormalities induced by mutagenic agents are most pronounced at this time [10]. The procedures of slide preparation were as described in [11]. Sperm, 800–1200 per animal, was analysed for the presence of abnormal cells.

The genetic variability of wild populations of common voles was investigated by the tests of sperm abnormalities and embryo lethality (for details of procedures, see [11, 12].

Statistical analysis. All statistical analyses were performed using the InStat V2.02 (GraphPad Software, USA) statistical package. The percentage of abnormal sperm and dead embryos of different animal groups and populations was compared applying the Student's t criteria. The difference was considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The genetic variability of common voles (*Microtus arvalis* Pallas) inhabiting the shore and island of Lake Kretuonas in the National Park of Aukštaitija was evaluated. Ten males and females of each population were investigated by the tests of sperm abnormalities and embryo lethality. Our previous investigation demonstrated that the concentration of such widespread environmental pollutants as DDT and its metabolites was 1.4–2 times higher in tissues of onshore common voles [13]. Therefore it was rather unexpected that sperm abnormalities (SA) and embryo lethality (EL) were significantly higher ($P < 0.001$) in the island population than in common voles inhabiting the shores. The SA and EL in shore common voles made up 0.58 ± 0.10% and 3.5 ± 1.3%, respectively, while in island common voles they made up even 1.32 ± ± 0.11% and 18.2 ± 2.9%, respectively. Similarly high levels of the SA and EL were determined only in common voles trapped near such large sources of atmospheric pollution as the Mažeikiai oil refinery, Kėdainiai chemical plant or Elektrėnai power station [14]. In our opinion, the reason for those high levels of genetic damage in common voles inhabiting the reservation, where local commercial activity is restricted or forbidden, could be inbreeding taking place in the isolated island population.

The male of common vole (trapped on the shores of Lake Kretuonas) and laboratory DBF_1 mice

Table. **Effects of polychlorinated biphenyls (Arochlors 1242, 1254 and 1260) and ammonium nitrate on sperm abnormalities in common voles and DBF_1 mice**

Treatment	Number of animals	Analysed sperm	Abnormal sperm	Abnormal sperm, % ± S.E.M.
		Common voles		
Control	6	6812	36	0.46 ± 0.10
Arochlor 1242	5	5000	30	0.60 ± 0.12
Arochlor 1254	6	5773	34	0.59 ± 0.11
Ammonium nitrate	5	5656	21	0.37 ± 0.10
A.1242 + AN	4	4180	32	0.77 ± 0.14
		DBF_1 mice		
Control	6	6030	22	0.36 ± 0.11
Arochlor 1254	5	5858	55	0.94 ± 0.12*
Arochlor 1260	4	3142	42	1.34 ± 0.21*
Ammonium nitrate	6	5783	37	0.64 ± 0.10
A.1254 + AN	5	5100	74	1.45 ± 0.17**

* $P < 0.005$. ** $P < 0.001$, Student's t test.

were treated with polychlorinated biphenyls or ammonium nitrate, or both, in the second stage of the study. The effects of these chemical compounds on the frequency of abnormal sperm in treated animals are shown in Table. PCB, ammonium nitrate and both did not increase the frequency of abnormal sperm in common voles, while a statistically significant increase of sperm abnormalities was found in mice treated with PCB and PCB+ammonium nitrate. Thus, PCB in our experiment showed some genotoxic activity, while ammonium nitrate was almost inactive in the sperm abnormality test. Interestingly, laboratory mice were more susceptible than common voles to abnormal sperm formation under the influence of usual environmental contaminants. The lower genetic susceptibility of common vole male cells probably can be explained by adaptation of wild animals to contamination with genotoxic agents in their natural environment.

Overall, our study results corroborate the data on the effects of polychlorinated biphenyls inducing genetic damages *in vivo*. The present investigation also provides evidence that different responses to genotoxic agents observed in the field monitoring among species and even populations may reflect internal population and species differences (*e. g.,* different levels of spontaneous mutations or different susceptibility to genetic damage formation) rather than differences related to exposure.

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V. Morkūnas, R. Lekevičius

PILKŲJŲ PELĖNŲ (*MICROTUS ARVALIS* PALLAS) GENETINIO KINTAMUMO BEI POLICHLORINTŲ BIFENILŲ IR AMONIO SALIETROS GENOTOKSIŠKUMO PILKIESIEMS PELĖNAMS IR LABORATORINĖMS PELĖMS TYRIMAI

S a n t r a u k a

Tirtas pilkųjų pelėnų (*Microtus arvalis* Pallas), gyvenančių Kretuono ežero pakrantėje ir jo saloje (Aukštaitijos nacionalinis parkas), genetinis kintamumas. Spermatozoidų anomalijų ir embrionų mirtingumo lygiai buvo statistiškai patikimai aukštesni salos populiacijoje. Aukštesnį saloje gyvenančių pilkųjų pelėnų genetinio kintamumo lygį greičiausiai lemia izoliuotoje populiacijoje vykstantis inbridingas.

Siekiant įvertinti pilkųjų pelėnų ir laboratorinių pelių jautrumą aplinkos teršalų genotoksiškumui, abiejų rūšių patinai buvo veikiami polichlorintais bifenilais ir amonio salietra. Minėti junginiai pastebimai nepadidino pilkųjų pelėnų spermatozoidų anomalijų dažnio, tuo tarpu pelėse spermatozoidų anomalijų aptikta statistiškai patikimai daugiau. Viena iš priežasčių, dėl kurios pilkojo pelėno vyriškų lytinių ląstelių genetinis jautrumas mažesnis negu laboratorinių pelių, gali būti šio graužiko prisitaikymas prie jo natūralios aplinkos užteršimo genotoksinėmis medžiagomis.

Raktažodžiai: pilkasis pelėnas, spermatozoidų anomalijos, embrionų mirtingumas, polichlorinti bifenilai