

HEPATIC XENOBIOTIC METABOLISM OF BANK VOLE (*Clethrionomys glareolus*) IN SAWMILL AREA CONTAMINATED BY POLYCHLORINATED DIBENZO-*p*-DIOXINS AND -FURANS

Murtomaa, M.¹, Viitala, P.², Pelkonen, O.², Rautio, A.¹,

¹Centre for Arctic Medicine, P.O.BOX 5000, FI-90014 University of Oulu, Finland; ²Department of Pharmacology and Toxicology, P.O. Box 5000, FI-90014 University of Oulu, Finland

Abstract

The aim of this study is to determine the hepatic xenobiotic metabolism in two wild vole species, bank vole (*Clethrionomys glareolus*) and field vole (*Microtus agrestis*). The old sawmill area contaminated by chlorinated dibenzo-*p*-dioxins and -furans (PCDD/Fs) was used as a study area. Concentrations of PCDD/Fs in bank voles were predictably much higher in the study area, whereas PCDD/F concentrations in field vole were unexpectedly much lower. To study the metabolism of PCDD/Fs we measured the activities of CYP enzymes in liver microsomes of bank and field voles. The purpose of this study is to clarify if the great interspecies variation in the effects of contaminants is due to differences in xenobiotic metabolism. So far, only bank voles has been caught (May 2007). Preliminary results of the study show that activities of dioxin-induced CYP1A dependent enzymes EROD and ECOD were increased notably in voles caught in polluted sawmill area, although the difference was significant only in males.

Introduction

Previously studies in bank and field vole living at the old sawmill area contaminated by chlorinated dibenzo-*p*-dioxins and -furans (PCDD/Fs) showed that these two relatively close species have a significant difference in their body burden of PCDD/Fs¹. Concentrations of PCDD/Fs in bank voles were predictably much higher (240 pg WHO-TEQs g⁻¹ fat) in the contaminated area, whereas PCDD/F concentrations in field vole were unexpectedly much lower (25 pg WHO-TEQ g⁻¹ fat). This may be at least partly explained by different diet of the two vole species. In addition, high variation in local PCDD/F concentrations in soil and age-dependent accumulation of PCDD/Fs may have contributed to the observed difference. Nevertheless, there is a great inter- and intraspecies variation in the sensitivity to PCDD/Fs², and the aim of this study is to clarify if the great interspecies variation in the effects of contaminants is due to differences in xenobiotic metabolism.

Many of the effects of dioxin like compounds are caused by their ability to bind a specific protein, aryl hydrocarbon (Ah) receptor, in the cytoplasm of body cells. Once bound, the dioxin/Ah receptor complex can bind to the DNA in the nucleus resulting in induction of cytochrome P450 (CYP), particularly CYP1A1, whereupon the production of P450-dependant enzymes increases. One of the consequences of the Ah-mediated response to dioxin exposure is that some of the resulting enzymic activity might be used as a first warning signal for contaminant levels.

This paper is a preliminary report of a study that aims at further characterization of metabolism of PCDD/s by the liver of bank vole and field vole. Furthermore, the use of Ah-mediated enzymic activation as a biomarker of dioxin exposure will be evaluated.

Material and Methods

Old sawmill used as a study area is situated in the city of Oulu, Northern Ostrobothnia in Finland. The area is highly contaminated by chlorophenol mixture, Ky-5, which has been used for over 40 years until 1984, to protect timber against mould and fungi. Botanical Gardens of University of Oulu was used as an unpolluted reference area. Bank voles and field voles were live-trapped (model Ugglan Special) between August and November in 2006, and soon after capture killed by cervical dislocation. Directly after sacrificing a vole, the liver was preserved and frozen in liquid nitrogen. Livers were stored in - 80°C until analysis.

Induction of CYP1A and CYP2B were tested by measuring the hepatic microsomal ethoxyresorufin *O*-deethylase (EROD), ethoxycoumarin *O*-deethylase (ECOD) and pentoxyresorufin *O*-deethylase (PROD). The CYP1A1-mediated EROD and CYP2B –mediated PROD activities were measured as described by Burke³, using 1 μ M ethoxyresorufin and 1 μ M pentoxyresorufin as substrates. The activity of coumarin 7-hydroxylase (COH) was measured as described by Aitio⁴, using 100 μ M coumarin as a substrate.

Results and Discussion

Only bank voles has been caught so far (May 2007), and there are no data to compare between two species yet. The numbers of bank vole caught from polluted sawmill and control area was 15 and 14, respectively.

There were no differences between sexes in the induction of measured enzymes. As expected, activities of dioxin-induced CYP1A dependent enzymes EROD and ECOD were increased in voles caught in polluted sawmill area, although the difference was significant only in males (Fig.1). Phenobarbital induced PROD was also notably higher in the voles of the sawmill area, indicating the exposure to mixture of chemicals, which Ky-5 definitely was. The number of female in both study and control group was relatively small, 6 and 4, respectively. This increases uncertainty which is always present in the wild life data. Nevertheless, in spite of variation and uncertainty of data, the results of this study have surprisingly clear trend, and are definitely worth of further studies.

Acknowledgements

The authors are grateful for Päivi Tyni for technical assistance and Jari Ylönen and staff of Biological Research Facility, Botanical Gardens and Department of Biology of University of Oulu for work premises and generous help.

References

1. Murtomaa, M, Tervaniemi, O-M, Parviainen, J, Ruokojärvi, P, Tuukkanen, J, Viluksela, M. *Chemosphere* 2007; 68: 951.
2. Pohjanvirta, R, Tuomisto, J. *Pharmacological Reviews* 1994; 46: 483.
3. Burke, MD, Prough, RA, Mayer, RT. *Drug Metabolism and Disposition* 1997; 5: 1.
4. Aitio, A. *Analytical Biochemistry* 1978; 85:488

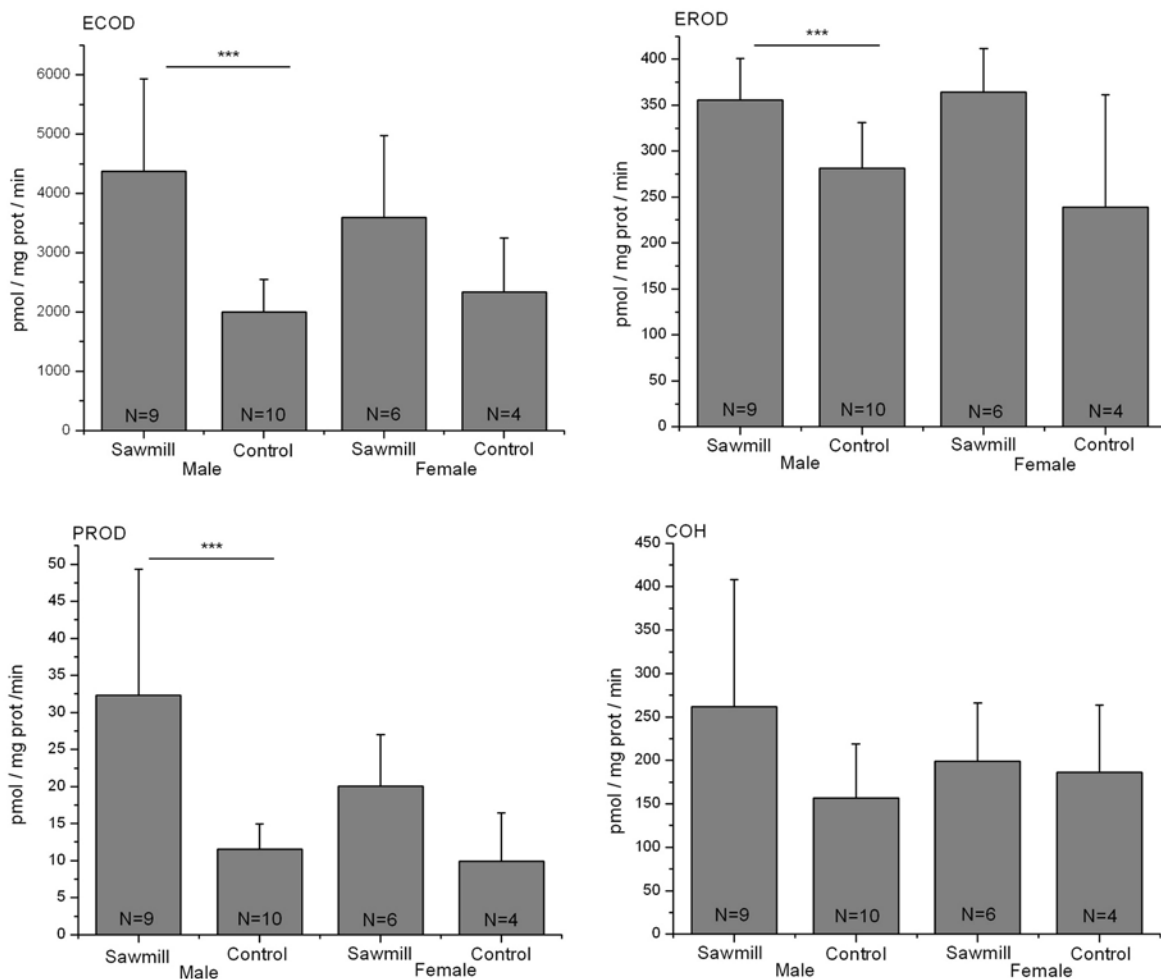


Fig.1. Ethoxyresorufin *O*-deethylase (EROD), ethoxycoumarin *O*-deethylase (ECOD), pentoxyresorufin *O*-deethylase (PROD), and coumarin 7-hydroxylase (COH) activities in bank voles from the contaminated sawmill area and control area. Activities are presented as mean \pm SD and N as the number of individuals. Significance of difference between two areas is indicated with asterisks.