Accumulation of chlorophacinone in susceptible and resistant Norway rat strains

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Introduction

Anticoagulant rodenticides (AVK) are known to accumulate substantially in the liver of exposed animals. Their half-lives are very long; up to 350 days for brodifacoum in rats (Erickson and Urban, 2002). As a consequence, secondary poisoning occurs in predators and scavengers feeding regularly on poisoned animals (Berny et al., 1997, Shore et al., 1999). This has been observed in many species, including endangered species such as the red kite (*Milvus milvus*) (Berny and Gaillet, 2008). In Europe, rodenticide resistance appears to be widely distributed. There is strong evidence supporting the role of the Vkorc1 gene in the resistance of the Norway rat (*Rattus norvegicus*) (Rost et al., 2004) and several SNPs' associated with resistance have been identified (Pelz et al., 2005). The objective of this work is to analyze the potential for bioaccumulation of given anticoagulant compounds in resistant rats from various European strains. The hypothesis tested was that resistant strains should accumulate anticoagulants to a larger extent than susceptible rats.

Materials and methods

Resistant rats were obtained from Germany (Dr. Jens Jacob), United Kingdom (Dr. Alan McNicoll) and France, carrying respectively the Y139C, L120Q (Berkshire strain) and Y139F resistance mutations. Susceptible rats were obtained from our own breeding colony. The genetic status of each strain was verified with DNA sequencing (homozygous resistant animals). The resistance status of each strain has already been published (Pelz et al., 2005). Rats were fed *ad libitum* on a wheat-bait containing chlorophacinone (50 mg/kg). This anticoagulant was chosen because it is one of the most commonly used in France and also because all strains are known to be resistant to a significant extent to this AVK. Four animals of each strain (2 males, 2 females) were euthanized after 1, 4, 9 or 14 days of continuous exposure. A second series of exposure was conducted on rats exposed 3 days on day 3, 8 and 13 and euthanized at days 4, 9 and 14. PT time was determined on the day of euthanasia for each rat. Chlorophacinone and its major hydroxyl metabolites were analyzed by HPLC with UV detection in plasma and liver samples for all animals.

Results

The resistance status of the strain had a definite effect on the survival rate at day 14 and on the time-to death. Resistance was L120Q > Y139C=Y139F, as confirmed by mean time to death and elevation of PT. Daily consumption of chlorophacinone was fairly consistent for all strains and during the entire study period with a mean of 3.0 mg/kg/day (LD50: 2.1 mg/kg). Accumulation of chlorophacinone was clearly dose-dependent for the first week (i.e. day 4), at least for the parent compound (Figure 1).

Accumulation seemed to stop during the second week in all strains except L120Q. No significant differences could be detected between strains after 4 days. At Day 9 or 14, differences were noted, especially related to the time of death, which was clearly strain dependent. Overall, L120Q rats accumulated higher concentrations of chlorophacinone in the liver than other resistant strains and susceptible rats (incomplete data at the time of abstract submission). Metabolites accumulated somewhat in the liver but with no apparent dose-dependent relationship. Among the four different durations of exposure (days 1, 4, 9 and 14) accumulation occurred to a less significant extent. (Plasma and some liver samples are still being analyzed at the time of submission of this abstract).

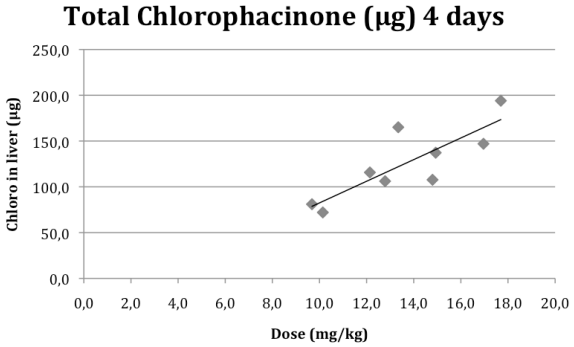

Fig. 1 Liver accumulation of chlorophacinone (Chloro) after 1 week in resistant rats (data combined for all strains).

Discussion

Published work regarding AVK accumulation in resistant rats is very limited so far. Atterby et al. (2005) compared whole-body residues of the L120Q strains (both Hampshire and Berkshire, the latter being resistant to difenacoum) and found no significant difference between susceptible and resistant strains. In their study, the authors showed that whole body concentrations peaked after 3 days and remained constant over 20 days of feeding. These results are quite different from ours, but the study design was also very different: we chose chlorophacinone, because all the strains were known to be strongly resistant and we analyzed only the liver, known to be the cumulative site of contamination of many mammalian species, rather than whole bodies. Since the liver is also rapidly consumed by predators, this choice appeared more appropriate to investigate potential ecotoxicological impacts of the anticoagulant. Our results clearly show that resistant rats may carry more AVK in their body than susceptible rodents, therefore they represent a greater risk to predators and scavengers feeding on them.

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