Rodenticide resistance

Structural mechanism of anticoagulant rodenticides resistances: Identification of a hydrophobic cluster responsible for the affinity between inhibitors and the target protein

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Vitamin K antagonists (VKAs) rodenticides have been used for the past decades for rodent populations management. These molecules act by inhibiting the vitamin K epoxide reductase (VKORC1) enzyme, an endoplasmic reticulum resident protein. VKORC1 catalyzes vitamin K epoxide reduction to vitamin K quinone then hydroquinone, the latter state being the exclusive cofactor of gamma-glutamyl-carboxylase (GGCX) enzyme. GGCX is responsible for the activation of clotting factors II, VII, IX and X. Inhibition of VKORC1 by VKAs thus prevents the reduction of vitamin K epoxide to quinone, inducing a disruption of the blood coagulation cascade. However, two main issues have arisen from the use of VKAs: resistances and ecotoxicity. Many anticoagulant resistances were described for both rats and mice, that led to the development of second generation VKAs. Although they were more potent, these molecules have caused some wildlife poisoning cases: secondary poisoning of rodent predators – such as foxes and raptors – was reported. Despite the use of VKAs since the 1950s, the *Vkorc1* coding gene was only identified in 2004, so VKAs resistances have been first characterized at genetic and enzymatic levels over the last decade. Many VKORC1 mutations have been identified as resulting in resistances, with frequencies that can locally reach 100%: L120Q, Y139C and Y139F mutations have been widely described in rats, as well as W59G, L128S and Y139C in mice. Nevertheless, the structural mechanisms of resistances induced by these mutations remained unclear since no 3D data was available for VKORC1 enzyme, due to its membrane location. But the publication of the experimentally solved VKORC1 structure in 2021 has provided reliable and valuable new data for exploring and explaining the structural mechanisms of VKAs resistances induced by VKORC1 mutations.

In the present work, we investigated the effects of the five VKORC1 mutations mentioned above on the enzyme structure: W59G, L120Q, L128S, Y139C and Y139F. Molecular modelling studies led us to identify a hydrophobic cluster ensuring VKORC1 structure integrity and encompassing W59, L120, L128 and Y139 residues. The mutation of a single amino acid in this structural region totally disrupts the hydrophobic pattern, leading to a loss of structure of VKORC1-VKAs binding site. The W59G mutation even triggers a disruption of the whole enzyme, also preventing the binding of vitamin K epoxide, the VKORC1 natural substrate. These results allowed us to explain the structural consequences of VKORC1 mutations and to characterize the mechanisms that decrease affinity between the mutated enzyme and VKAs. In an other hand, we also studied the role of the phospholipidic membrane towards VKAs. Variations of membrane permeability to anticoagulants was investigated, considering two types of lipids (phosphatidylcholine and phosphatidylserine, the major species in endoplasmic reticulum membrane). The obtained results indicated that lipid bilayers are less permeable to second generation VKAs, but show higher variation towards first generation VKAs depending on their lipid composition. These preliminary results might therefore imply a role for the endoplasmic reticulum membrane in VKAs selectivity/retention, thus its involvement in ecotoxicity issues – the lipid profile being variable over species, mammals and raptors for example. Taken all together, our results help at understanding structural mechanisms underlying resistances and ecotoxicity issues relative to VKAs. It also paves the way for the development of new, efficient and less ecotoxic VKORC1 inhibitors, which would allow the management of rodent populations while preserving wildlife.