The use of TGCE for mutation detection in VKORC1 in warfarin resistant rats (Rattus norvegicus)

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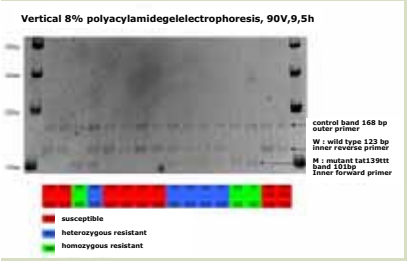
Introduction

Rodenticide resistance in the brown rat is a well-known problem in rodent control. It is caused by an altered enzyme, namely vitamin K epoxide reductase (VKOR), in the liver of these rats whereby there is no or less inactivation of this enzyme by rodenticides based on anticoagulants. As a result the recycling of vitamin K and the activation of coagulation factors are not inhibited and so the rodenticide fails to work. The alteration of the enzyme is caused by a mutation in the VKORC1 gene. Until now, eight different mutations, which are related with anticoagulant resistance are found in Europe. In Belgium as in France the TAT-139-TTT mutation is present.

Materials & Methods

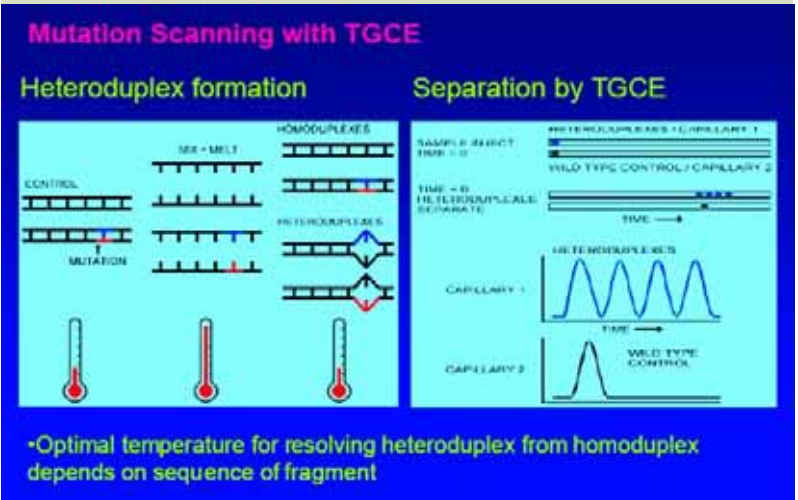
Animals

The rats, fifty in total, used in this study were parents and offspring of crosses between wild and albino rats, additional some extra wild rats were used.



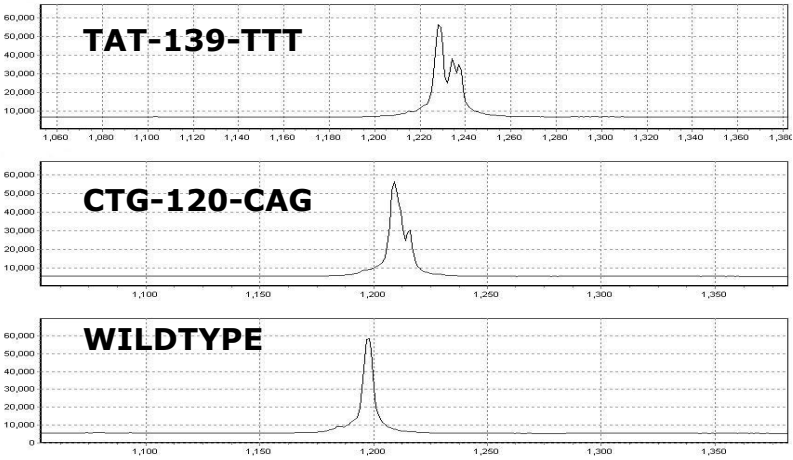
Technique

All the rats were tested with a blood clotting response (BCR) test and PCR designed for the mutation present in Belgium (Rost et al., 2004). Mutation detection is based on heteroduplex formation and temperature gradient capillary electrophoresis (TGCE).



TGCE is performed after amplification of the VKORC1 gene and is based on homo- and heteroduplex formation, after denaturation (heating) and renaturation (cooling) of a DNA sample. DNA was extracted from blood samples. TGCE separates these duplexes and makes them visible by different peaks in the electrophoresis curve. The form of the curve is specific for each mutation and gives us information about the genotype : homo- or heterozygous resistant resulting in respectively, one or more than one peak. In case of doubt or just to confirm the results one can work with mixed samples (unknown sample and homozygous wildtype or homozygous known mutant)

Results



TGCE confirmed all our BCR results and most of the PCR- findings. There were indeed some incompatibilities between BCR and PCR. Wild rats who were resistant according to BCR did not carry the typical Belgian-French mutation. Samples of these rats were tested with TGCE which revealed another mutation in Belgium. Sequence analysis learned us that it was CTG-120-CAG, previously only found in Great-Britain namely in Berkshire and Hampshire.

Conclusion

TGCE as a technique can be used for the screening of known mutations in a wild rat population, but can also be used for the detection of new mutations. It is a reliable technique, perhaps somewhat labour intensive but this is compensated by the low cost per test. The technique is especially interesting when dealing with a great number of samples

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