The isomeric effects of pinene on Plasmodium and Anopheles

Robyn L. Van Zyl\textsuperscript{1,2}, Natasha C. Jansen Van Vuuren\textsuperscript{1,2}, Obaidiyah Mustapha\textsuperscript{1,2}

\textsuperscript{1}Pharmacology Division, Dept of Pharmacy & Pharmacology, University of the Witwatersrand, South Africa, \textsuperscript{2}WITS Research Institute for Malaria (WRIM), Faculty of Health Sciences, University of the Witwatersrand, South Africa

Background: Pinene is a bicyclic monoterpene consisting of \(\alpha\) - and \(\beta\) -constitutional isomers, with both structural isomers having enantiomers: \((+)-\alpha\)-, \((-)-\alpha\)-, \((+)-\beta\)- and \((-)-\beta\)-pinene. Isomers and enantiomers are well known to contribute contrasting pharmacological properties. These effects were investigated to identify new antimalarial and insecticidal agents in the combat against drug-resistant malaria and mosquitoes.

Methods: The four pinenes and standard controls were commercially purchased. The antimalarial activity was determined in vitro against the asexual stages of Plasmodium falciparum (3D7, NF54), as well as the haemolytic effect. The larvicidal activity against Anopheles arabiensis (KGB) larvae was compared to the mortality of Artemia franciscana nauplii, with morphological alterations noted. The toxic effects were also evaluated on the human neuroblastoma (SH-SY5Y) and kidney epithelial (HEK-293) cell lines using the MTT cellular viability assay. The possible mechanisms of action were evaluated by determining inhibition of haemozoin formation and metal chelating properties.

Results: Of the four isomers/enantiomers evaluated, \((+)-\alpha\)-pinene was the most potent against both strains of P. falciparum, whilst its enantiomer, \((-)-\alpha\)-pinene was significantly less active against the 3D7 strain. The inhibitory effect was directed against the intra-erythrocytic parasite as indicated by minimal haemolysis by all the pinenes and the mechanism of action of \((+)-\alpha\)-pinene was due to inhibition of haemozoin formation. A differential effect of the isomers and enantiomers on the lethality of the Anopheles larvae was observed, with \((-)-\alpha\)-pinene and \((+)-\beta\)-pinene inhibiting both species (74.2; 61.0\%) and their enantiomers half as active. Whilst only \((+)-\beta\)-pinene was toxic to the Artemia nauplii and the most toxic against the HEK-293 cells. An isomeric effect was observed with the \(\alpha\)-pinenes inhibiting the neuroblastoma cell lines compared to the low inhibitory effect of the \(\beta\)-pinenes. Although \((-)-\alpha\)-pinene, \((+)-\beta\)-pinene and \((-)-\beta\)-pinene possessed favourable copper (I) chelating properties, these pinenes did not protect human red blood cells from Cu(I)-induced lysis, but the Cu(I) chelate did enhance cell death of the neuroblastoma cells.

Conclusions: The differences in the isomeric as well as enantiomeric effects of novel antimalarial and insecticidal compounds need to be investigated to develop a safer and more active product.