PIPERAQUINE

Bioanalysis, drug metabolism and pharmacokinetics

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska Akademin vid Göteborgs Universitet kommer att offentligen försvaras i Arvid Carlsson salen, Academicum, Medicinaregatan 3, Göteborg, fredagen den 5 oktober 2007 kl 13.00

av

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Avhandlingen baseras på följande delarbeten:

I. Singtoroj T.*, **Tarning J**.*, Annerberg A., Ashton M., Bergqvist Y., White N.J., Lindegardh N., Day N.J.P. A new approach to evaluate regression models during validation of bioanalytical assays.
Journal of Pharmaceutical and Biomedical Analysis. 2006 Apr 11;41(1):219-27.

II. **Tarning J**.*, Singtoroj T.*, Annerberg A., Ashton M., Bergqvist Y., White N.J., Day N.J.P., Lindegardh N. Development and validation of an automated solid phase extraction and liquid chromatographic method for the determination of piperaquine in urine.
Journal of Pharmaceutical and Biomedical Analysis. 2006 Apr 11;41(1):213-8.

III. **Tarning J**., Bergqvist Y., Day N.J.P., Bergquist J., Arvidsson B., White N.J., Ashton M., Lindegardh N. Characterization of human urinary metabolites of the antimalarial piperaquine.
Drug Metabolism and Disposition. 2006 Dec;34(12):2011-9.

IV. **Tarning J**., Lindegardh N., Sandberg S., Day N.J.P., White N.J., Ashton M. Pharmacokinetics and metabolism of the antimalarial piperaquine after intravenous and oral single doses to the rat.
Submitted

V. **Tarning J**., Lindegardh N., Annerberg A., Singtoroj T., Day N.J.P., Ashton M., White N.J. Pitfalls in estimating piperaquine elimination.
Antimicrobial Agents and Chemotherapy. 2005 Dec;49(12):5127-8.

VI. **Tarning J**., Ashley E., Lindegardh N., Stepniewska K., Phaiphun L., Day N.J.P., McGready R., Ashton M., Nosten F., White N.J. Population pharmacokinetics of piperaquine after two different treatment regimens of dihydroartemisinin-piperaquine in patients with *Plasmodium falciparum* malaria in Thailand.
Submitted

**Equal contribution*

ABSTRACT

Malaria is one of the most abundant parasitic diseases in the world affecting many of the poorest economies. The estimated prevalence is 300 to 700 million clinical episodes each year with up to 3 million deaths. Piperaquine replaced chloroquine as the first line treatment in China for *Plasmodium falciparum* malaria in the 1970s and was used as mass prophylaxis until the emergence of resistance in the 1990s. It has recently been the object of renewed interest as a partner drug in artemisinin-based combination therapy. Artekin® is a fixed oral combination of dihydroartemisinin and piperaquine showing excellent efficacy and tolerability against multi-resistant *Plasmodium falciparum* malaria. Only a limited number of studies have addressed the clinical pharmacokinetics of piperaquine, none of which have addressed metabolism. Despite its extensive use no published information is available about the non-clinical pharmacokinetics or drug metabolism in animals.

The bioanalytical tools used in this thesis were nuclear magnetic resonance, liquid chromatography, quantitative and qualitative mass spectrometry. Data analysis was conducted using conventional statistics, population based pharmacokinetic modeling, individual pharmacokinetic modeling and non-compartmental analysis.

The results present a new systematic approach for choosing a regression model during bioanalytical method validation that can be a useful tool for finding the optimal regression model (paper I). It incorporates predictability of independent quality control samples as well as the calibration curve fit. This approach was used to find the best regression model during the development and validation of a sensitive and selective bioanalytical method for quantification of piperaquine in urine by automated solid-phase extraction and isocratic liquid chromatography (paper II).

Five human urinary metabolites of piperaquine were identified and their molecular structures characterized in two healthy male volunteers after an oral single dose of the dihydroartemisinin-piperaquine combination (paper III). The major metabolites are a carboxyl acid cleavage product and a N-oxidated piperaquine product.

The rat appears to be a suitable animal model for non-clinical *in vivo* studies since piperaquine pharmacokinetic properties and metabolites are similar to those found in humans (paper IV). The absolute oral bioavailability was estimated to approximately 50%. The low between-animal variability in plasma concentrations after intravenous administration suggests absorption to be critical for between-animal variability in drug exposure. Piperaquine displayed a low biliary clearance with less than 1% of the total dose excreted by this route. Enterohepatic circulation would not contribute significantly to a prolongation of the terminal half-life.

Piperaquine elimination half-life might be underestimated due to inadequate assay sensitivity and/or duration of sampling in published information (paper V). This should be considered when establishing the duration of follow-up and the assessment of relapse in clinical studies. The population pharmacokinetics of piperaquine was characterized in 98 patients in Thailand with uncomplicated *Plasmodium falciparum* malaria, ranging from 3 to 55 years of age (paper VI). The study confirms that piperaquine exhibits considerable inter-individual pharmacokinetic variability, has a very large apparent volume of distribution, and a slow elimination phase. Pharmacokinetic modeling suggests that despite having a smaller central volume of distribution and slower elimination than adults, the children in this study had lower piperaquine concentrations in the therapeutically important period immediately following treatment. If this is confirmed in other malaria affected regions, then consideration should be given to increase the weight adjusted dosage in children. No pharmacokinetic differences could be seen between the two investigated study treatment regimens and further support the use of a simplified, once daily treatment, regimen to improve treatment adherence and efficacy.

Overall, this thesis has contributed to a better understanding of the bioanalysis, drug metabolism and pharmacokinetics of piperaquine which may contribute to its future safe and efficacious clinical use as an antimalarial.

Key words: Piperaquine; malaria; *Plasmodium falciparum*; antimalarials; ACT; pharmacokinetics; NONMEM; population pharmacokinetics; NCA; bioavailability; enterohepatic circulation; metabolism; metabolites; animal models; bioanalysis; regression models; validation; HPLC; LC-MS/MS; H-NMR; FTICR-MS

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