Poster Session 2 – Pharmacokinetics

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Pharmacokinetics and bioequivalence studies of clozapine

K. Nasseri and V. Bicharanloo

Mashhad University of Medical Sciences, Iran

Clozapine is an atypical neuroleptic which has recently been included in the Iranian National Formulary and has been formulated by two pharmaceutical firms (Tehran Chimi (T) and Sobhan (S)). The aim of this project was to investigate whether the new formulations (25 mg and 100 mg tablets) were bioequivalent to the reference product (Leponex tablets (L)) and can achieve the required therapeutic plasma levels. For in-vitro studies, the criteria for acceptance were based on those suggested by the British Pharmacopoeia. For each experiment, the required number of tablets was randomly selected from each batch. The in-vivo studies were conducted on the patients of the chronic units of two psychiatric hospitals who were routinely taking the drug. The studies had the approval of the Ethics Committee of both hospitals.
Patients (n = 30) were all males, non-smokers, aged 18–48 years, had no condition other than the psychological problems and were taking no other medications. All patients met DMS-IV criteria for diagnosis of schizophrenia and were administered reference tablets every 12 h (daily doses 300–900 mg) for a period up to 2 years. Blood samples were collected from each patient before the morning dosing on two consecutive days. The patients then switched to generic clozapine tablets under the same dosing schedule and after three weeks being on the new product, one blood sample was collected from each patient on two consecutive days and the patients were switched again to the reference tablets. Clozapine was measured by a sensitive HPLC method. Analysis of assay indicated that the amount of clozapine in the reference and the two generic products were similar and were in the range of 90–110% of labels, recommended by the pharmacopoeia. Measurement of dosage units in terms of weight and content of active ingredient suggested that the three products met the criteria of uniformity; they were in the range of 85–115% of labels and %RSD in all cases was less than 3%.
Dissolution testing demonstrated that dissolution profiles of the two generic products were comparable with that of the reference standard and more than 80% of the tablets was dissolved in less than 30 min. In-vivo studies indicated that changing clozapine tablets from reference to generic products did not produce any significant change in clozapine steady-state plasma concentration (Css) in the patient population studied. The trough drug Css for L, T and S were 371, 350 and 361 ng mL^{-1}, respectively. The large interpatient variation found to Css was partly attributed to the differences in patients' drug regimens. The average values calculated for total clearance, volume of distribution and elimination half-life were 60 L h^{-1}, 600 L and 7 h, respectively. Significant correlations were observed between dose and Css. In contrast to reports from a few other countries, laboratory data for hundreds of Iranian patients revealed that clozapine was not associated with major haematological disorders.

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Sandwich culture of dog hepatocytes to study in-vitro biliary transport by hepatic transporters

P. Sharma, P. Annaert*, P. Verboven, K. Lavrijsen and H. Bohets

Preclinical Pharmacokinetics-Drug Evaluation and *Full development, Johnson & Johnson Pharmaceutical Research and Development, Turnhoutseweg 30, 2340 Beerse, Belgium

Drug transporters are carrier proteins involved in translocation of chemical substances across the biological membranes in the body. Transporters could be involved in pharmacokinetics, pharmacodynamics, drug–drug interactions and aetiology of some diseases (Faber *et al* 2003). Physiological expression of transporters in the hepatocytes coupled with CYP 450 mediated metabolism and entero-hepatic circulation of drugs has resulted in the need for development of an in-vitro model for drug transporters in hepatocytes. In this perspective, an in-vitro model utilizing dog hepatocytes was developed in which cells were grown between two layers of collagen (sandwich culture). Regeneration of bile excretory function as well as formation of canaliculi could be monitored as a function of culture time.
Hepatocytes were isolated from liver of sacrificed Beagle dogs by in-situ perfusion with collagenase, seeded onto collagen-coated 12-well plates, overlaid with gelled collagen, and cultured up to 15 days. In-vitro biliary secretion was characterised by monitoring accumulation of ^{3}H-taurocholate (1 μM) as substrate to active transport and ^{3}H-inulin (1 μM) as passive diffusion marker in bile canaliculi. Additionally, light microscopy was employed to examine the cellular and canalicular morphology. Effect of media additives (10%v/v foetal bovine serum (FBS) versus ITS/5% v/v FBS) and time of collagen overlay were investigated.
It was observed that taurocholate excretion was clearly higher (59-fold) in sandwich cultures compared to primary hepatocyte cultures. Furthermore, biliary excretion of taurocholate increased successively from 28% on 1st day to 55% on 3rd day of culture and decreased subsequently to 3.4% on sixth day. Presence of FBS (10%v/v) was found to be essential and displayed different taurocholate excretion pattern compared to ITS/FBS (5%v/v). Furthermore, collagen overlay after 4 h of cell plating compared to collagen overlay after 24 h of cell plating showed higher and a more regular pattern of biliary excretion of taurocholate. Light microscopic observation revealed the gradual development of bile canaliculi-like structures into an anastomotic network. In 24-h cell culture, the formation of bile canaliculi was observed and as time progressed, the bile canaliculi became more clear and the network was established at 120 h. Hepatocytes could be maintained in sandwich culture configuration until the 15th day, without loss of morphological characteristics and integrity.
It is concluded that sandwich cultures of dog hepatocytes represent a good model for biliary excretion of drugs. Additional studies are performed to characterise the functional expression of specific transporters in this model. Assessment of (hepatic) transport properties of a new drug will result in a better understanding of the pharmacokinetic and toxicological profiles observed at preclinical stage.

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Drug level determination in the paediatric population using blood spots: applications in unlicensed and off-label drug use

J. S. Millership, P. S. Collier and J. C. McElnay

Children's Medicines Research Group, School Of Pharmacy, Queen's University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK

For over forty years, dried blood spots (DBS) have been used for the study of inborn errors of metabolism in children. Blood spots from a heel prick are spotted onto a Guthrie Card and the spots are allowed to dry. Once dried the cards are placed in a wallet and transported to the lab for analysis. Early studies were involved in investigations of phenylketonuria but now a wide range of other metabolic disorders can be investigated (Mei *et al* 2001). More recently, DBS have been investigated for other uses (e.g. investigation of nutritional problems such as vitamin A deficiency (Craft *et al* 2000), drug abuse in sport (Peng *et al* 2000), determination of blood levels of anti-malarials from samples taken at geographically remote sites (Kolawole & Mustapha 2000) and drug level determinations in paediatrics (Oliveira *et al* 2002)).
Within our group we have been involved in drug level measurement in paediatric plasma samples for a number of years. This work is part of our continuing studies into unlicensed and off-label drug use in children. One of the problems we have encountered in this work is the difficulty associated with the collection of venous blood samples. We have, therefore, commenced investigations into the utilisation of DBS for routine measurement of drug (diclofenac) levels in paediatric patients. Screened whole blood spiked with diclofenac (30 μL: 5–50 μg mL^{-1}) was spotted onto a Guthrie Card. The spot was allowed to dry in the air for 2 h while protected from light. A 6-mm circle was punched from the centre of the DBS and this was extracted using 60/40 methanol:water. The cut circle was placed in the extraction solvent and this was allowed to stand for 1 h. The extraction solvent was decanted from the circle. Following evaporation of the solvent the sample was reconstituted in

mobile phase and analysed by HPLC. This HPLC analysis was conducted using a Waters Spherisorb S5 ODS 1 column (4.6 × 125 mm) column fitted with a Waters Spherisorb S5 ODS 1 column (4.6 × 10 mm) guard column. The mobile phase consisted of MeOH/water (adjusted to pH 3.3 with H_3PO_4) (63:37) pumped at 1 mL min^{-1}. UV detection was at 280 nm. The chromatograms obtained from unspiked whole blood were relatively free from interfering peaks with no interference with the diclofenac peak. The extracted DBS indicated that that determination of diclofenac was possible, in DBS prepared from whole blood spiked with this drug, at levels found in paediatric plasma samples (5–50 μg mL^{-1}).

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Pharmacokinetics of clozapine in long-term therapy

K. Nasseri and V. Bicharanloo

Mashhad University of Medical Sciences, Iran

The pharmacokinetic profiles of clozapine and its main metabolite, norclozapine, were evaluated in 30 chronic schizophrenic inpatients during long-term treatment. The studies were conducted on the patients of the chronic units of two psychiatric hospitals and had the approval of the Ethics Committee of both hospitals. The dose–plasma level relationship and inter- and intra-individual variability of plasma levels during maintenance treatment with clozapine was also investigated. Patients were all males, non-smokers, aged 18 to 48 years, had no other condition and were taking no other medications. All patients met DMS-IV criteria for diagnosis of schizophrenia and received stable daily doses (300–900 mg) for a period up to two years. Blood samples were collected from each patient before the morning dosing in two consecutive days. Patients plasma and red blood cells (RBC) drug concentrations were determined by HPLC. The pharmacokinetic parameters were calculated from both non-compartmental and compartmental approaches with zero-order input rate using a kinetic model for simultaneous fit of clozapine and norclozapine concentrations. Large interpatient variations in pharmacokinetic parameters of the two drugs were observed. The trough clozapine steady-state concentration (Css) averaged 360 ng mL^{-1}. Plasma clozapine concentration peaked on average at 2 h. The mean elimination rate constants from compartments 1 (k10) and 2 (k20, elimination rate constant of norclozapine) were 0.085 and 0.16 h^{-1}, respectively. The rate of formation of norclozapine, k12, averaged 1.2 h^{-1}. The mean fraction of the administered dose converted to norclozapine was estimated to be 65%. The terminal elimination half-lives averaged 7.6 h for clozapine and 13 h for norclozapine. The mean RBC/plasma concentration ratios were 23% and 81%, respectively. From RBC concentration data, the mean elimination half-lives were 7.6 h for clozapine and 16 h for norclozapine. The mean value for total clearance of clozapine, uncorrected for bioavailability, was 60 L h^{-1} and the volume of distribution was 7 L kg^{-1}. Significant correlation was observed between dose and Css and between dose and area under the time–concentration curve. The pharmacokinetics of clozapine after multiple doses were linear over the range of clozapine plasma concentrations of 145–1450 ng mL^{-1}. The pharmacokinetic model designed can be used to determine the population pharmacokinetic parameters of clozapine and norclozapine in order to optimise individual dosage regimens using a Bayesian methodology. Even though clozapine plasma levels may show a significant degree of variation, this is not necessarily reflected in a change in psychopathology. The large interpatient variation found was partly attributed to the differences in patients' drug regimens. In contrast to some reports, laboratory data for these and hundreds of other Iranian patients revealed that clozapine was not associated with major hematological disorders.