

Poster Session 1 – Pharmacokinetics

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Dispersion model of hepatic elimination: studies on diclofenac in hepatic cirrhosis

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The aim was to study the influence of hepatic cirrhosis on membrane permeability and uptake of a lipophilic model substance, diclofenac. Using five non-eliminated markers (erythrocytes, albumin, sucrose, urea and water), the permeability and exchange in the isolated perfused control and cirrhotic rat livers were characterised. After injection into the portal vein of markers and diclofenac, outflow data were collected and analysed using moment analysis and the dispersion model (DM). Various parameters, including hepatic volume of distribution (V_H), mean transit time (MTT), the relative spreading (CV^2), dispersion number (D_N), efficiency number (R_N), intrinsic clearance (CL_{int}), permeability (PS) and membrane permeability (ρ) were calculated (Table 1). The output profiles of markers in cirrhotic livers, displayed a sharper appearance. The V_H values of markers were reduced which was reflected in reduced MTT. The reduction was variable among the markers. While the significant increase in the D_N value of markers in cirrhotic livers was indicative of changes in the hepatic vascular arrangements in cirrhosis. The increase in the CV^2 value indicated that the relative spreading of markers is increased in cirrhotic livers. These changes implied that due to parenchymal and microcirculatory alterations, the blood-liver exchange is progressively limited in cirrhosis. These findings were confirmed by the histological evaluations. The unaltered values of R_N and CL_{int}, and the reduced values of PS and ρ for diclofenac suggest that while the hepatic metabolic activity is not changed notably in experimental cirrhosis, the permeability of hepatocyte membrane, as a consequence of reduced diffusion in the space of Disse, is reduced substantially. The formation of this new barrier further impedes the non-instantaneous cellular transport of diclofenac, reducing its MTT while increasing CV^2. Of the mechanisms that may account for the observed changes (change in protein binding, metabolism, membrane permeability, diffusion), reduced permeability leads to a decrease in k_{12} and a relatively smaller amount of material can access the peripheral compartment during organ transit. Thus a large fraction appears in the hepatic outflow without having left the central compartment. Hence, the output profile is composed mainly of the throughput component and the shape of the profile is similar to that of a non-eliminated tracer that is confined to the extracellular space. The two-compartmental axial dispersion model introduced by Rowland and co-workers (Rowland et al 1984; Rowland & Roberts 1985, 1986) still describes adequately the output profile of DC.

Table 1 Effect of hepatic cirrhosis on disposition kinetics of DF

	Controls	Cirrhotics
MTT (s)	73	47*
CV^2	1.40	3.13*
V_H (mL g^{-1})	1.9	1.2*
D_N	0.18	0.20
R_N	0.35	0.27
CL_{int} (mL min^{-1} g^{-1})	69	69
ρ	0.86	0.71*
PS (mL min^{-1} g^{-1})	422	220*

*$P < 0.005$ vs controls

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Rowland, M., Roberts, M. S. (1986) *J. Pharmacokinet. Biopharm.* **4**: 227–260

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Inadequacy of urinary data in bioequivalence study of rifampin

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Urinary excretion data is a noninvasive method for estimation of bioequivalence. The main requirement for using urine in bioequivalence study is that, the drug should be excreted 10–30% unchanged in urine.

In bioequivalence evaluation of rifampin, urinary data has been used in several cases (Brechbuhler et al 1978; Gurumurthy et al 1999; Pillai et al 2001).

It has been reported that up to 25–30% of rifampin is excreted unchanged in urine. Most of the analytical methods used to determining the percentage of urinary excretion of rifampin are microbiologic or spectrophotometric and not suitable for differentiating rifampin and its microbiologically active metabolite, desacetylrifampin. In the studies using HPLC methods, there was not any attention to the chemical instability of rifampin. Therefore the results obtained seemed not to be precise and accurate.

In this study, urinary excretion of rifampin was investigated using an accurate and precise HPLC method (unpublished data), by the addition of ascorbic acid for prevention of oxidative degradation of rifampin. Five healthy subjects were orally administered 450 mg rifampin after an overnight fasting. Urine sampling was performed until 10 h after dose administration.

Cumulative amount of urinary excreted unchanged rifampin (D_u), renal clearance of rifampin (CL_r) and the parameter of D_u/Dose were calculated (Table 1). According to the obtained results, the urinary excretion of unchanged rifampin in this dose is 7 ± 3% of the total administered dose — lower than the acceptable limit of 10–30%. So it could be claimed that using the urinary excretion data is not accurate in evaluating the bioequivalence of rifampin containing products.

Table 1 Pharmacokinetic parameters following administration of 450 mg oral rifampin to healthy subjects

Pharmacokinetic parameters	Mean	s.d.	CV%
D_u (mg)	29.70	14.56	49.01
CL_r (L h^{-1})	0.44	0.24	54.63
D_u/Dose	0.07	0.03	48.63

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Determination of a correlation between plasma methotrexate concentrations and the response to treatment in patients with rheumatoid arthritis

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Low-dose methotrexate (MTX) is used in the treatment of rheumatoid arthritis (RA), but information about the concentration and response relationship is inconclusive. This study has investigated the relationship between the predicted maximum concentration (Cmax) and the response to MTX therapy. Seventy-six patients with RA prescribed oral MTX (5–25 mg/week) were studied. They were arbitrarily defined as 36 good and 40 poor responders according to their disease

activity score (DAS). One blood sample was taken from each patient and the concentrations were measured by HPLC. This concentration was interpreted using Bayesian analysis to generate revised estimates of pharmacokinetic parameters. The initial pharmacokinetic parameters used in the Bayesian analysis were obtained from 24-h plasma concentration profile we measured in 24 patients. These initial estimates were: central volume (Vc, L kg^{-1}) = 0.181 + 0.0034 body mass index, clearance (CL, L h^{-1}) = 1.683 + 1.406 creatinine clearance, alpha rate constant (α, h^{-1}) = 0.834 – 0.005 age, absorption rate constant (Ka, h^{-1}) = 0.55. The revised estimates of Vc, CL, α, β, K12 and K21 obtained from Bayesian analyses were used to predict each patient's Cmax. The predicted Cmax (371.10 ± 156.42 μg L^{-1}) was in accordance with the values obtained from other studies (Jundt et al 1993). No difference was found between the pharmacokinetic revised estimates of the 2 groups (Table1). Nine covariates: dose, Cmax, sex, age, body mass index (BMI), C-reactive protein (CRP), creatinine clearance (CrCL), disease duration and MTX treatment duration were considered to explain the interpatient variability with respect to response using logistic and multiple linear regression. This analysis revealed that the odds ratio for response was associated with sex and CRP (ln odds = 0.49 + 1.296 Sex + 0.07 CRP, $P < 0.05$). These covariates were also found to correlate to the DAS (DAS = 2.644 + 0.611 Sex + 0.022 CRP, $P < 0.01$). The number of tender joints (T28) was explained by age, dose and CRP (T28 = 13.379 – 0.168 Dose – 0.135 Age + 0.04 CRP, $P < 0.01$). A linear relationship was also found between ESR (erythrocyte sedimentation rate) and CRP together with sex and disease duration (ESR = 4.335 + 8.415 Sex + 0.304 Disease duration + 0.488 CRP, $P < 0.001$). Similarly, CRP and disease duration were also significantly related to the global health status (GH = 26.448 + 0.265 CRP + 0.559 Disease duration, $p < 0.01$). However, no covariates were significantly related to swollen joint counts. These data suggest that there was no therapeutic range or any correlation between plasma MTX concentration and clinical efficacy between good and poor responders. Thus plasma MTX measurements were not helpful in defining an optimal regimen for the treatment of RA. Despite the lack of correlation with plasma drug concentrations, the efficacy of treatment measured by T28 was dose-related.

Table 1 Comparison of the pharmacokinetic parameters (mean ± s.d.) between the good and poor responders

Parameters	Good response	Poor response	*P* value
Cmax (μg L^{-1})	356.4 ± 155.0	384.7 ± 158.5	0.44
CL (L h^{-1})	8.52 ± 4.82	7.65 ± 4.12	0.38
Vc (L kg^{-1})	0.27 ± 0.05	0.27 ± 0.04	0.91
K12 (h^{-1})	0.101 ± 0.025	0.101 ± 0.038	0.99
K21 ($h-^{-1}$)	0.010 ± 0.263	0.010 ± 0.211	0.86
Vd (L kg^{-1})	1.50 ± 0.21	1.52 ± 0.19	0.58
Alpha (h^{-1})	0.559 ± 0.106	0.554 ± 0.134	0.85
Beta (h^{-1})	0.095 ± 0.055	0.94 ± 0.050	0.97

Student's *t*-test

Jundt, J. W., et al (1993) *J. Rheumatol.* **20**: 1845–1849

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Distribution of β-blocking drugs among tissues: role of stereochemistry and tissue phosphatidylserine

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Obtaining predictive pharmacokinetic information of new chemical entities early is important in drug discovery and helps identify compounds worthy of further study. While much attention has been paid to developing predictive methodology for drug absorption and metabolic stability, relatively little attention has been applied to drug tissue distribution. Our work studied tissue distribution for a series of β-blocking drugs in the rat, with particular interest in accounting for differences among tissues and examining the influence of stereochemistry. We determined tissue-to-plasma partition coefficients, based on both total and unbound drug in plasma (Kp and Kpu), for the individual enantiomers of acebutolol, betaxolol, bisoprolol, metoprolol, oxprenolol, pindolol and propranolol and S-timolol in male Sprague Dawley rat tissues at steady state following cassette intravenous dose infusion to dual cannulated rats, utilising a novel LC-MS assay for quantification. Values of Kp ranged from 0.2 for R&S-acebutolol in the brain to 200 for R&S-betaxolol in the lung. Those of Kpu ranged from 0.2 for R&S-acebutolol in the brain to 1400 for R-propranolol in the lung. Significant correlations ($R^2 > 0.8$) between tissue partitioning (Kp and Kpu) and phosphatidylserine content in a variety of rat tissues (Nishiura et al 1987; Yata et al 1990) were observed, indicating that this acidic phospholipid has potential for predicting much of the regional tissue distribution of β-blockers. This substantiates the findings of Yata et al (1990) with weakly basic drugs. Regarding stereoselectivity, pronounced enantiomeric differences in Kp were observed for pindolol and propranolol, but not the other compounds, with values being greater for the S- than R-enantiomer. Stereoselective differences in plasma protein binding were observed and when this was taken into account (yielding Kpu values), much but not all of the enantioselective differences disappeared, indicating other contributory factors. Possibilities include, stereoselective binding to phosphatidylserine, as demonstrated by Hanada et al (1998) for weakly basic drugs, and perhaps stereoselective transport mechanisms.

In conclusion, the potential of phosphatidylserine content to predict regional Kpu values for weakly basic compounds, as seen with β-blocking drugs, appears promising. It suggests that information gained in one tissue can help to predict information in another, thereby improving the chances of successfully employing a full whole-body physiologically-based pharmacokinetic model from limited tissue data to predict temporal events occurring in many tissues following drug administration.

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Nishiura, A., et al (1987) *J. Pharmacobio-dyn.***10**: 135–141
Yata, N., et al (1990) *Pharm. Res.* **7**: 1019–1025

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Population pharmacokinetics of intravenous indometacin in neonates with patent ductus arteriosus

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In newborn infants, closure of the ductus arteriosus (DA) normally occurs within 48–96 h after birth (Gentile et al 1981). However, persistent patency is frequently observed in premature infants and can result in serious complications (e.g. congestive heart failure). Indometacin is a non-steroidal anti-inflammatory drug used in the treatment of patent ductus arteriosus (PDA). Response to indometacin is variable and its disposition in neonates is not well understood. The aim of this research was to investigate the pharmacokinetics of indomethacin in neonates with PDA using population analysis.

Indometacin (0.09–0.23 mg kg^{-1}) was administered intravenously (1–3 doses) to 37 neonates with a median (range) weight of 1.16 kg (0.57–2.19 kg), gestational age of 29 weeks (25–34 weeks) and postnatal age of 14 days (1–77 days). Population analysis of the 185 plasma concentrations obtained was performed using NONMEM (a non linear mixed effects modelling program) and a one-compartment model with first-order elimination (Beal & Sheiner 1992). Estimates of the pharmacokinetic parameters, clearance (CL) and volume of distribution (V), and their variability between patients were obtained. The following covariates were examined for any significant influence on CL and V — gestational age, current

weight, postnatal age, gender, presence of respiratory distress syndrome and concomitant therapy with digoxin, diuretics or aminoglycoside antibiotics.

CL and V were found to vary exponentially with postnatal age (PNA, $P < 0.005$). The final models for CL and V were CL (L h^{-1} kg^{-1}) = 0.00585 $e^{0.0338 \times PNA}$ and V (L kg^{-1}) = 0.232 $e^{0.0145 \times PNA}$. V is multiplied by 0.726 if the neonate receives digoxin. No other covariates were found to be significant. The interpatient variability in CL and V, expressed as coefficients of variation, were 46.8% and 20.8%, respectively. The residual variability was 22.5% at the mean indomethacin plasma concentration of 0.76 μg mL^{-1}. Mean population estimates of CL, V and elimination half-life (t/) over the range of PNA included in the study, with and without concomitant digoxin therapy are shown in Table 1.

Table 1 Mean population estimates of CL, V and t/

PNA (days)	CL (L h^{-1} kg^{-1})	V (L kg^{-1})	t/ (h)
1	0.0061	0.235	26.7
1 with digoxin	0.0061	0.171	19.4
14	0.0094	0.284	20.9
14 with digoxin	0.0094	0.206	15.2
77	0.0790	0.709	6.2
77 with digoxin	0.0790	0.514	4.5

These population estimates allow an evidence-based approach to be used to predict initial doses of indometacin most likely to result in a desired plasma concentration. However, the interindividual and residual variabilities indicate that subsequent doses may need to be adjusted depending upon clinical response.

Beal, S. L., Sheiner, L. B. (1992) *NONMEM users guides*. NONMEM Project Group, University of California at San Francisco
Gentile, R. et al (1981) *J. Pediatr.* **98**: 443–448

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Investigation of the population pharmacokinetic parameters of diclofenac in paediatrics

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Many drugs used in hospital to treat paediatrics are either unlicensed or are prescribed outside the terms of their product licence, off-label usage (Conroy et al 2000). One such drug is diclofenac, a non-steroidal anti-inflammatory drug licensed for the treatment of juvenile chronic arthritis but routinely used off-label for pain relief. Traditional pharmacokinetic (PK) studies are difficult to perform in paediatrics because of the need for multiple blood samples which creates ethical and technical problems. However, population methods have been developed that allow the estimation of PK parameters from sparse data collected as part of the routine clinical care of the patient. The aim of this research was to investigate the population pharmacokinetics of diclofenac administered off-label to paediatrics.

Sparse data was collected from 123 children aged 3 months to 12 years and weighing 8.7–71.0 kg who had received diclofenac (10–50 mg) orally or rectally. The 173 plasma concentrations obtained were fitted to a one-compartment model with first order absorption and elimination using NONMEM (a non linear mixed effects modelling program) (Beal & Sheiner 1992). The pharmacokinetic parameters, absorption rate constant (Ka), clearance (CL/F) and volume of distribution (V/F), and their variability between patients were estimated. Bioavailability (F) could not be determined as diclofenac was not administered intravenously. A variety of developmental and clinical factors were investigated for any significant effect on the parameters.

Preliminary results indicate that CL/F and V/F are related to weight (WT) as shown by the following models, CL/F (L/hr) = 1.02 × $WT^{0.75}$ and V/F (L) = 0.97 × WT. The mean population estimate for Ka is 5.1 h^{-1}. Interpatient variability in CL/F and V/F expressed as coefficients of variation is 45% and 34% respectively and the residual variability is 56%. The parameter estimates are not significantly affected by route of administration indicating that there is no difference in the rate of absorption or extent of bioavailability. No other covariates had a significant effect on the PK parameters.

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