厚生科学研究費補助金（医薬安全総合研究事業）

「薬物代謝能力測定キットの開発と医薬品適正使用への応用」

研究代表者：共立薬科大学　教授　中島恵美

事業実績報告書
（平成 11 年度）
総括研究報告書及び分担研究報告書

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研究報告書

厚生科学研究費補助金（医薬安全総合研究事業）
（総括・分担）研究報告書

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薬物代謝能力測定キットの開発と医薬品適正使用への応用

主任研究者　中島恵美
共立薬科大学薬剤学教授

研究要旨
　全体の総括と TDM ソフトの開発

中島恵美	共立薬科大学薬剤学
飯笹　久	共立薬科大学薬剤学
服部研之	共立薬科大学薬剤学

A．研究目的
　薬物療法の個別化を成功させ、特に小児・高齢者に対する医薬品の適正使用を推進するため、患者の薬物代謝の指標となる主な代謝酵素量を事前に診断するキットを開発する。

B．研究方法
　各種 CYP に特異的な診断プローブを用いて、in vivo での各 CYP 量を PK-CYP test で測定する。プローブの条件検討や微量定量法の確立を行う。治療薬の固有情報を組み込んで、個々の患者に最適な薬物療法を自動的に得られる薬物療法設計ソフトへの応用をはかる。

C．研究結果
　各 CYP サブタイプに特異的なプローブの組み合わせにより、主要な 6 種のサブタイプを同時に測定できるキットとして取り扱う方法論を考案した。
　また、競合阻害による相互作用を予測するための理論を構築し、動物実験から、PK-CYP test が相互作用の予測にも応用可能であることを示した。
　さらに主として TDM の対象となっている薬物について、代謝酵素、各種パラメーター、最適有効濃度、相互作用情報などを文献調査からデータベース化した。

D．考察
　これまでに、動物実験によって、我々が新たに構築した理論によってはじめて生体内の酵素量を精度良く定量できることを明らかにした。
　本年度はヒトへの臨床応用の準備段階として、各プローブをカクテルとし、キット化するための方法論を考案した。
　また、治療薬について、代謝酵素や各種パラメーターのデータベースを構築し、実用化への準備を進めている。

E．結論
　PK-CYP test の動物実験による妥当性の証明とあわせて、臨床応用に向けたデータベースの構築により、本テストの実用化の可能性が高まった。

F．研究発表
　1．論文発表
Bio. Pharm. Bull. 23. 1077-1083 (2000)
Jpn. J. Hosp. Pharm. 26. 492-504 (2000)

　2．学会発表
"Strategies for Optimizing Oral Drug Delivery: Scientific to Regulatory Approaches" (H11.4 月、神戸)
"International Conference on Drug Interaction (H11.10 月、浜松)
"Millenium World Congress of Pharmaceutical Sciences" (H12.4 月、サンフランシスコ

G．知的所有権の取得状況
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厚生科学研究費補助金（医薬安全総合研究事業）
（総括・分担）研究報告書

薬物代謝能力測定キットの開発と医薬品適正
使用への応用

　　　分担研究者　飯笹　久
　　　　　　　　　共立薬科大学薬剤学助手

研究要旨
　in vitro 酵素活性の測定と in vivo 代謝速度の変動の測定解析

A．研究目的

　過去に報告されている in vitro 代謝パラメータである Km と Vmax 値の多くが代謝物の検出限界の制約から、in vivo に投与される濃度とは異なる高濃度の範囲で測定されている。そのため、in vitro と in vivo で代謝酵素間の寄与率の見積もりに妥当性を欠くことが指摘されながら、適切な濃度範囲で測定されている報告は少ない。また、薬物は一般に複数のサブタイプにより代謝されるため、各サブタイプの寄与を正確に見積もる方法を確立することが本システムの実用化には不可欠である。

　本年度は CYP1A2 のプローブと治療薬のモデルに用いたカフェインとアセトアニリドそれぞれについて、in vitro 代謝実験によるパラメータの測定と代謝に関与するサブタイプの同定並びに寄与率の算出を試みた。

B．研究方法

　ラット肝ミクロソームを酵素源とし、低基質濃度における代謝実験系を確立し、パラメータの測定を行った。また、in vivo での基質濃度において、各サブタイプに特異的な阻害抗体を用いて、各サブタイプの寄与率を測定した。

C．研究結果

　カフェインでは、代謝反応のほぼ 100% が CYP1A2 によるものであることが明らかとなった。一方、治療薬のモデルに用いたアセトアニリドでは、代謝反応の約 60% が CYP1A2 によるものであることが明らかとなった。

D．考察

　ラットではカフェインの代謝に CYP1A2 以外に CYP2E1、3A2 の関与を示唆する報告があったが、本研究の条件下では、CYP1A2 以外の分子種はほとんど関与しないことが明らかとなった。一方、アセトアニリドは CYP1A2 でのみ代謝されると言う報告があったが、CYP1A2 の寄与率は約 60% であった。in vitro パラメータについては報告値とほぼ一致する値が得られた。

E．結論

　寄与率を考慮することにより、PK-CYP test の精度を向上させることができた。次年度は、ヒトリコンビナント酵素を用いた代謝実験系を構築することにより、種々の薬物における各 CYP サブタイプの寄与を正確に見積もるためのシステムの構築を行う予定である。

F．研究発表
1．論文発表
Jpn. J. Hosp. Pharm. 26, 492-501 (2000)
2．学会発表
"International Conference on Drug Interaction" (H11.10 月、浜松)
"Millenium World Congress of Pharmaceutical Sciences" (H12.4 月、サンフランシスコ

G．知的所有権の取得状況
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厚生科学研究費補助金 （医薬安全総合研究事業）
（総括・分担）研究報告書

薬物代謝能力測定キットの開発と医薬品適正使用への応用

分担研究者　服部研之
共立薬科大学薬剤学教助手

研究要旨
CYP 酵素量変動モデル動物の作製と PK-CYP test の検証

A．研究目的

　肝 CYP 誘導、病態モデル及び加齢動物を作製し、CYP 酵素量を定量し、PK-CYP test の検証を行う。

B．研究方法

　昨年度に作製した CYP1A2 の酵素誘導モデル及びコリン欠乏食飼育による肝障害モデル動物に加え、本年度は CYP2C11 の検証のため、四塩化炭素投与による急性肝障害モデルを作製した。本ラットに対して、フローブのトルブタミドを投与し、クリアランスを測定し、PK-CYP test の検証を行った。

　また、本研究も主目的の一つである加齢変動要因の解析のため、加齢モデル動物を作製し、酵素量を定量した。本モデルに対して、カフェインとアセトアニリドを投与し、CYP1A2 についての PK-CYP test の検証を行った。

C．研究結果

　急性肝障害モデルラットでは、CYP2C11 量が約 1/4 に減少しており、加齢モデルラットでは、CYP1A2 量が約 1/2 に減少していた。

　CYP2C11 量に対し、トルブタミドのクリアランスの変化は、比例しており、トルブタミドのクリアランスから CYP2C11 量の推定が可能であることが明らかとなった。

　また、カフェインのクリアランスから推定される酵素量及び推定された酵素量から予測されるアセトアニリドのクリアランスと実測値はほぼ一致しており、加齢動物に対しても PK-CYP test が妥当であることが明らかとなった。

D．考察

　本年度は CYP1A2 と CYP2C11 について検証を行った。CYP2C11 はラットでは最も主要なサブタイプで、人の CYP3A4 と基質特異性が似ていることが知られている。これら二つのサブタイプは、アミノ酸配列には多型の報告がないことから、酵素量が酵素活性を規定する因子であると考えられる。また、種々の環境因子により誘導されることから遺伝多型の解析だけでは、生体中の酵素活性を推定することができない。さらに、CYP3A4 は治療に使われる薬物の約半数の代謝に関与していると言われており、個体差の大きい分子種でもある。

　これらのことから、本年度の結果から、PK-CYP test の臨床への応用の可能性が高まったと考えられる。

E．結論

　昨年度の酵素量変動モデルに引き続き、急性肝障害モデル、加齢動物においても PK-CYP test が妥当であることが検証できた。また、CYP1A2 に続き、CYP2C11 で PK-CYP test が妥当であることが示された。

F．研究発表
1．論文発表
Jpn. J. Hosp. Pharm. 26. 492-504 (2000)
2．学会発表
"Millenium World Congress of Pharmaceutical Sciences" (H12.4 月、サンフランシスコ

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これ以降「International Symposium—Strategies for Optimizing Oral Drug Delivery」まで雑誌/図書等に掲載された論文となりますので.「研究成果の刊行に関する一覧表」をご参照ください。

「研究成果の刊行に関する一覧表」

Nakajima Y ,Hattori K ,Shinsei M ,Matsunaga N ,Iizasa H ,Sasabe H ,Akiyama ,Miyamoto G and Nakashima E. **Physiologically-based Pharmacokinetic Analysis of Grepafloxacin.** Biol Pharm Bull. 2000;23(9):1077-1083

Matsunaga N, Hattori K, Iizasa H, Fukuhara M, Takanaka A and Nakashima E **A Simplified Diagnostic Approach for Estimating in vivo Hepatic Drug Clearance ; Its Preliminary Application for the Drug Caffeine, Using CYP1A Probe in a Rat Model.** 病院薬学. 2000; 26(5): 492-504

ABSTRACTS

International Symposium

Strategies for Optimizing Oral Drug Delivery:
Scientific to Regulatory Approaches

ODD '99
KOBE-JAPAN

April 19-21, 1999
International Conference Center, Kobe, Japan

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B-38

ESTABLISHMENT OF DRUG METABOLISM ABILITY DIAGNOSIS: THE APPLICATION TO CLEARANCE PREDICTION FOR ACETANILIDE BASED ON *IN VIVO* CYP1A2 LEVEL.

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Minato-ku Shiba-koen 1-5-30, Tokyo 105-8512, Japan

Objective: It might be possible to estimate the clearance of newly administered drugs on the basis of the diagnostic value of metabolism enzyme level of individual patients, if it becomes possible to measure the metabolism enzyme level as a diagnostic value beforehand. In the previous report from our laboratory, a method for measuring the *in vivo* CYP level (PKCYP-test) was derived based on the clearance theory. Then, we have been trying to establish a system which estimates the clearance of the drug based on this measured value. In this study, acetanilide was chosen as a model drug, and the clearance was estimated by the model animal in which the enzyme level fluctuated. Then the relationship between the measured and observed values was examined.

Method used: The features of PKCYP-test are to consider the free-concentration gradient (qg) of a drug between the intracellular and blood spaces based on the physiological pharmacokinetics. The validity of PKCYP-test was confirmed by administering caffeine, which was chosen as a CYP1A2 probe in the rat. 3-Methylcholanthrene (MC) treated rats were used as an induced metabolic enzyme model. In MC treated rats, the clearance of acetanilide was estimated using the *in vivo* CYP1A2 level which was calculated from PKCYP-test by using caffeine as a probe. This predictive value was compared with the measured value which was obtained by administering acetanilide to the MC treated rats.

Result: The total body clearance (CLt) of caffeine and the free fraction in blood were measured in normal rats and MC treated rats. The level of CYP1A2 in the liver was measured by Western blotting. By using the reported values of Vmax, and Km, the qg value of caffeine was determined as almost unity. The estimated CYP1A2 quantity in MC treated rats using PKCYP-test was in good agreement with the measured level by Western blotting. The clearance of acetanilide which was estimated using the *in vivo* CYP1A2 level which was calculated from PKCYP-test using caffeine as the probe agreed well with the measured value.

Conclusion: The amount of CYP in the liver could be calculated by using the clearance of the diagnosis probe from the above results. In the model animal in which the enzyme level fluctuated, it was proven that the clearance of the drug is predictable by using the *in vivo* CYP level which was calculated from PKCYP-test. In future, the possibility of universally obtaining the clearance of an administered drug will be examined using CYP diagnostic value in the diseased state.

International Conference on Drug Interaction
(Acronym: ICDI)

Date: October 21-23, 1999

Place: Act City Hamamatsu

111-1 Itaya-machi, Hamamatsu City, Sizuoka-ken, 430-7790, Japan

Access: The venue, Act City, stands next to JR Hamamatsu Station, which is approximately 1.5 hours (230 km) from JR Tokyo Station by Hikari, the Shinkansen. Through the underground path, it takes about 3 minutes on foot from Hamamatsu station to the venue.

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APPLICATION OF PKCYP-TEST TO DRUG-DRUG INTERACTION FOR CAFFEINE AND THEOPHYLLINE

N. Matsunaga[1], K. Hattori[1], T. Nishijima[1], H. Iizasa[1], M. Fukuhara[2], and E. Nakashima[1]

[1]Kyoritsu college of pharmacy, Tokyo, 105-8512, Japan
[2]National Institute of Public Health, Tokyo, 108-8638, Japan

Introduction: We have constructed a theoretical basis for obtaining in vivo Cytochrome P450 (CYP) quantities of molecular species from the clearance of the diagnosis probe (PKCYP-test) by introducing liver-to-blood free concentration gradient in vivo (qg). In the present study, the possibility of the PKCYP-test to quantitatively predict the drug interaction was examined.

Experimental Methods: Male Sprague-Dawley rats were used. During the constant intravenous infusion of theophylline, caffeine was administered as an IV bolus dose to the rat. The concentration of theophylline and caffeine was measured by HPLC. The clearance of caffeine (CL_{obs}) was obtained by moment analysis.

Results and Discussion: The qg value of theophylline was estimated as approximately 10 by PKCYP-test after the IV bolus administration. The effect of theophylline on the clearance of caffeine was studied. CL_{obs} was compared with the value (CL_{pred}) estimated from the PKCYP-test by using the qg value of theophylline. CL_{pred} was also estimated by assuming that metabolism of caffeine by CYP1A2 was competitively inhibited by theophylline. CL_{obs} was close to CL_{pred} by PKCYP-test rather than the predicted value by assuming that the free concentration of theophylline in the liver was equal to the free concentration in blood. However, some diminished inhibitory effects were observed.

Conclusion: Above results indicated that the introduction of qg was useful for considering not only liver-to-blood free concentration gradient in vivo but also the error of the estimation of Km and Vmax between in vitro and in vivo experiments.

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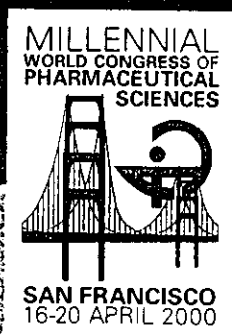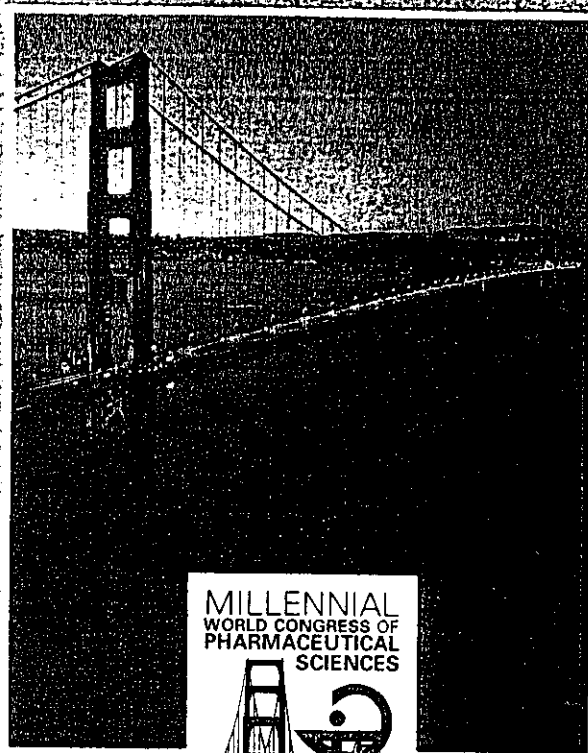
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ABSTRACTS

2-10041 PHARMACOKINETICS OF L-CARNITINE (LC) AFTER SINGLE AND MULTIPLE INTRAVENOUS ADMINISTRATION TO CHRONIC HAEMODIALYSIS PATIENTS

A.M. Evans[1], R.L. Nation[1], G.-F. Fornasini[2], S. Pace[2], E.F. Lemanowicz[2], R. Faull[3]. [1]Centre for Pharmaceutical Research, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, South Australia, 5000, Australia; [2]Sigma-Tau Pharmaceuticals, Gaithersburg, Maryland, United States; [3]Royal Adelaide Hospital, Adelaide, South Australia, 5000, Australia

Purpose: The objective of the study was to evaluate the pharmacokinetics of LC following single and multiple i.v. administration of LC (Carnitor®) in 12 chronic haemodialysis patients.

Methods: Patients undergoing three dialysis sessions/week received i.v. LC (20 mg.kg−1) at the end of each dialysis session, for 9 weeks. Blood and dialysis samples were collected during the inter- and intra-dialysis periods after the first and last dose of LC. Pre- and post-dialysis blood samples were collected each week. Samples were analysed for LC by HPLC.

Results: The mean pre-dialysis plasma concentration of LC prior to the first dose of LC was $21.28 \pm 7.6 \mu M$. After the first dose, the plasma concentration of LC reached a maximum of $1138.56 \pm 240.21 \mu M$, decreasing in a triexponential manner to $68.40 \mu M$ prior to the next dialysis session. During the next haemodialysis session the plasma concentration of LC decreased to $16.71 \mu M$. During repeated dosing, there was accumulation of LC, and after 9 weeks the pre- and post-dialysis plasma levels of LC were about 190 and 40 μM, respectively. The pre- and post-dialysis plasma levels of LC decreased once LC dosing was ceased.

Conclusions: LC supplementation results in the movement of LC into the slowly equilibrating carnitine pool. This stored LC is lost from the body via haemodialysis, once LC administration is stopped.

2-10042 ANALYSIS OF THEOPHYLLINE CLEARANCE USING THE PKCYP-TEST IN CONTROL AND 3-METHYLCHOLANTHRENE-TREATED RATS

N. Matsunaga, K. Hattori, T. Nishijima, H. Iizasa, M. Fukuhara[1], E. Nakashima. Kyoritsu College of Pharmacy; [1]National Institute of Public Health, Tokyo, Japan

Purpose: We have established a method for characterizing drug metabolism capacity based on physiological pharmacokinetics to estimate the amount of in vivo cytochrome P450 (CYP) (PKCYP-test). In setting up this PKCYP-test, a liver-to-blood free concentration gradient in vivo (qg) was introduced. In the present study, theophylline clearance was investigated by the PKCYP-test in rats with elevated levels of CYP enzyme.

Methods: Male Sprague-Dawley rats, 6 weeks of age, were used. The raised liver enzyme was induced by 3-methylcholanthrene (MC) administration (MC-treated rats). Control and MC-treated rats were given theophylline intravenously and its pharmacokinetic parameters were determined. Theophylline concentrations were measured by HPLC. The effect of anti-CYP1A serum on the microsomal metabolism of theophylline was investigated using microsomes from control rats.

Results: There was a 6-fold difference in the CLint of theophylline between control and MC-treated rats. In addition, the amount of CYP1A2 varied 22-fold between control and MC-treated rats. In control rats, the qg value of theophylline calculated from the PKCYP-test was 13.4. Since the Kp, f of theophylline was about 1.5, the high qg value may be due to several factors including another metabolic pathway and errors in estimating the pharmacokinetic parameters. In control rats, when the microsomal metabolism of theophylline was inhibited by anti-CYP1A serum, the residual activity was about 60%.

Conclusions: A considerable part of theophylline metabolism is mediated by CYP isoforms, except for CYP1A.

2-10043 VEROTOXIN 2 OF ESCHERICHIA COLI O157 DECREASES THE HEPATIC DRUG-METABOLIZING ENZYME ACTIVITY WITH THE APOPTOSIS IN HEPATOCYTE

Kiyoyuki Kitaichi[1], Yuki Nishio[2], Cai Shao Hui[1], Masayuki Nadai[3], Hiroichi Nagai[2], Michio Ohta[4], Hideo Yoshizumi[3], Takaaki Hasegawa[1]. [1]Dept. Med. Technol., Nagoya Univ. Sch. Health Sci., Nagoya; [2]Dept. Pharmacol., Gifu Pharmaceu. Univ., Gifu; [3]Facul. Pharm., Meijo Univ., Nagoya; [4]Dept Bacteriol., Nagoya Univ. Sch. Med., Nagoya, Japan

Purpose: Verotoxin (VT) of E. coli O157 infection may cause the life-threatening organ failures. Thus, we tried to investigate the effect of VT type 2 (VT2) on the hepatic drug-metabolizing enzyme activity in rats by using antipyrine as a probe drug.

Methods: Wistar rats (280–300 g) received a bolus intravenous injection of the hepatic-metabolizing drug, antipyrine (20 mg/kg), 6, 12, and 24 hours after VT2 (2 mcg). Blood samples were collected at designated intervals after antipyrine administration, and the concentration of plasma antipyrine was analyzed by HPLC. The hepatic drug-metabolizing activity was represented as the half-life of antipyrine (t1/2). The histopathological examinations of liver as well as biochemical parameters, including nitrite/nitrate (NOx) in plasma, were taken in the same regimen.

Results: Disappearance of antipyrine from plasma was significantly prolonged in VT2-treated rats in a time-dependent manner as represented in increasing the t1/2. Significant increase in plasma NOx levels was also observed in VT2-treated rats. An iNOS inhibitor, S-methylisothiourea, as well as dexamethasone ameliorated VT2-induced delay of the t1/2 with decreasing plasma NOx levels. Histopathological examinations revealed that the hepatocyte showed the apoptosis 6 hours after VT2 injection and the apoptosis/necrosis 24 hours after the injection.

Conclusion: These results suggest that VT2-induced apoptosis in hepatocyte and/or NO may play a crucial role in the decreasing hepatic drug-metabolizing enzyme activity. Furthermore, these results may give caution the dosage regimen of hepatic-metabolizing drugs during E. coli O157 infection.

2-10044 CHARACTERIZATION OF A COMPUTER PROGRAM FOR PHARMACOKINETICS BASED ON MAXIMUM LIKELIHOOD ESTIMATION USING THE GAMMA DISTRIBUTION WITH A PROBABILITY DENSITY FUNCTION: COMPARISON WITH THE NORMAL DISTRIBUTION

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Objective: The current pharmacokinetic programs are assumed that the data error has a normal or log-normal distribution. However, clinical data often have errors of non-normal distribution. The objective of this study was to characterize a computer program which described for maximum likelihood estimation within the gamma distribution as a probability density function (p.d.f.) for non-normal distribution.

Methods: A Monte Carlo method was preformed to estimate the pharmacokinetic parameters. A one-compartment intravenous model and an oral model were assumed. The simulated drug concentrations were generated using a 10% S.D. based on the gamma or normal distribution. The gamma or normal distribution was adopted as the p.d.f. to estimate model parameters. The Powell method was used as the maximization of the logarithmic likelihood. The constraint of parameters was not adopted.

Results: There was no statistical difference among the pharmacokinetic parameters estimated arising from the difference in p.d.f. and data distributions. The parameters estimated based on the gamma and normal distributions were consistent with the same pharmacokinetic model and variance in drug concentration. However, the number that fails to calculate the parameters based on the p.d.f. with a normal distribution was five to seventy times greater than that based on the gamma distribution.

Conclusion: The estimator based on the p.d.f. with gamma distribution has a high convergence compared to that based on the normal distribution. A computer program describing the maximum likelihood estimation within the gamma distribution is thought to be useful for pharmacokinetic analysis.

2-10045 VEROTOXIN 2 OF ESCHERICHIA COLI O157 CHANGES INTESTINAL ABSORPTIVE FUNCTIONS OF DRUGS IN RATS

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Introduction: Verotoxin 2 (VT2) of E. coli O157: H7 has been identified as a worldwide cause of serious human gastrointestinal disease and the life-threatening hemolytic uremic syndrome (HUS). However, it is not clear whether VT2 modifies the intestinal absorption of nutrients and drugs in animals and man. The present study was thus designed to investigate changes in intestinal absorptive functions in rats pretreated with VT2 using cephalexin (CEX) and cefazolin (CEZ) as model drugs.

Methods: VT2 (2 mcg) isolated from E. coli O157: H7 was administered intravenously to male Wistar rats. After 24 h of VT2 injection, the absorption of CEX and CEZ from small intestine (upper and middle parts) was evaluated by in situ closed loop method. After the absorption experiment had finished, the mucosa from each intestinal segment was separated immediately to measure

2-10054 CONFIRMATION OF A PORE SIZE DISTRIBUTION THEORY USING PARACELLULAR PROBES IN THE PERFUSED RAT INTESTINE

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As part of an extensive program to characterise paracellular absorption, studies have been performed in the in situ perfused rat jejunum and ileum with the aim of predicting in vivo oral bioavailability.

In order to confirm data obtained previously (1) using ^{14}C PEG 400 as a probe molecule, a series of D-peptides and a group of drug molecules, known to permeate via the paracellular route, have been investigated.

Absorption was assessed from a recirculating isotonic media, over a period of 1 hour, by collection and HPLC assay of mesenteric blood. The fractional rate of absorption of each species, together with morphometric data, was used to calculate apparent permeability (P_{app}) and, subsequently, the predicted bioavailability.

A good correlation between P_{app} and Stokes radius was observed for all of the molecules studied in both the jejunum and ileum but the same over-prediction of the oral bioavailability was observed with the peptide and drug molecules as had been observed for the PEG 400 data.

However, when the predicted bioavailabilities of the peptide and drug molecules were calculated from their Stokes radii, incorporating the size and abundance of the tight junction in the rat ileum and jejunum, obtained from a modelling exercise using the PEG 400 P_{app} data (1), the values obtained were an accurate prediction of the oral bioavailability of the peptide and drug molecules obtained in vivo (2).

The data confirms the pore size and abundance values obtained using PEG 400 and confirms that Stokes radius alone is sufficient to predict the bioavailability of paracellularly absorbed molecules.

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2-10056 THE PHARMACOKINETICS OF CEFTRIAXONE ADMINISTERED AT DIFERENT TIMES OF THE DAY

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Purpose: The aim of this study was to evaluate possible daily variations in the kinetics of ceftriaxone administered at different times of the day.

Methods: Sixty female Sprague Dawley rats (weight = 100 g, n = 6 rats per sampling time point) maintained under controlled environmental conditions (12 h light/12 h dark regimen) received a single intraperitoneal injection of ceftriaxone (100 mg·kg^{-1}) at 4.00, 10.00, 16.00 and 22.00 hs. Blood samples were taken at 0.08, 0.16, 0.5, 0.75, 1, 2, 4, 6, 9 and 12 hs after drug administration. Plasma levels of ceftriaxone were determined by microbiological assay. Pharmacokinetic analysis was made by a computerized programe (TOPFIT 2.0) and the cosinor method was used for the chronobiological analysis of the pharmacokinetic parameters.

Results: The best fit of all data was to a one-compartment model. Dosing-time dependent variations in the mean absorption time (PR = 100, p = 0.032, amplitude, maximun-minimun/mean * 100 = 86.4%) and total body clearance (PR = 100, p = 0.006, amplitude = 60.4%) of ceftriaxone were determined. Evidence of possible temporal variations in the area under the concentration-time curve (PR = 98, p = 0.12, amplitude = 51.4%) and lag time (PR = 98, p = 0.14, amplitude = 180.4%) of ceftriaxone was revealed.

Conclusions: The present study strongly suggest that time of administration can affect the pharmacokinetics of ceftriaxone. Further studies are needed to determine the clinical significance of biological rhythms in the pharmacokinetics of antimicrobial agents.

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2-10057 APPLICATION OF THE PKCYP-TEST TO PREDICT THE AMOUNT OF IN VIVO CYP2C11 USING TOLBUTAMIDE AS A PROBE

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Purpose: We have established a novel method for measuring the in vivo level of CYP (PKCYP-test) from the clearance of a probe and have confirmed this

in an animal model. In this study, tolbutamide was chosen as a probe drug for CYP2C11, and the amount of CYP in an animal model, with a fluctuating CYP level, was estimated by Western blotting and the PKCYP-test.

Methods: After an 8-hr fast, Sprague-Dawley rats were given a single dose of 20% carbon tetrachloride (2.5 ml/kg) and fasted for another 12 h. Then, they were used as a model of reduced CYP. The pharmacokinetic parameters of tolbutamide were measured in control and carbon tetrachloride-treated rats. The concentration of tolbutamide was measured by HPLC. The level of CYP2C11 in the liver was measured by Western blotting.

Results: In carbon tetrachloride-treated rats, the amount of CYP2C11 which was calculated by the PKCYP-test fell to 20% of the value in control rats. The quantity of CYP2C11 which was measured by Western blotting also fell to 20% of the value in control rats.

Conclusion: This study shows that the PKCYP-test can also be applied to estimate the amount of CYP2C11 using tolbutamide as a probe.

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2-10058 MECHANISM OF TISSUE DISTRIBUTION OF GREPAFLOXACIN (GPFX), A FLUOROQUINOLONE ANTIBIOTIC

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Purpose: GPFX is a fluoroquinolone with potent antibacterial activity against both Gram-positive and Gram-negative bacteria. The most important pharmacokinetic feature of GPFX is its higher distribution into various tissues, especially the lungs, compared with other fluoroquinolones. Because this drug is expected to be used for the treatment of respiratory infections, it is important to characterize its pulmonary distribution. The purpose of the present study is to clarify the mechanism governing the distribution of GPFX into the lung.

Methods: Lung uptake of [^{14}C] GPFX in rats was assessed in an in vivo single-pass study and by integration plot analysis. The steady-state tissue distribution kinetics of [^{14}C] GPFX was assessed following the constant infusion of [^{14}C] GPFX.

Results and Discussion: Approximately 12% of the dose of [^{14}C] GPFX was taken up during its single-pass through the lung, such distribution being much higher than that of [^{14}C] inulin (1.5%). The tissue uptake clearance of [^{14}C] GPFX was higher in lung, and was very similar to the blood flow rates in most organs. Thus, no specific mechanism was found in its tissue uptake process to govern its higher distribution to the lung although its pulmonary uptake is very efficient. [^{14}C] GPFX binds to phosphatidylserine (PhS) to a much higher degree than to other phospholipids and the steady-state tissue distribution, as well as the subcellular distribution in lung, correlates well with the PhS content. The [^{14}C] GPFX association to the PhS synthase transformant of CHO-K1 cells depended on the PhS content of these cell lines.

Conclusion: Specific binding of GPFX to PhS determines its high distribution to the lungs.

2-10059 EFFLUX TRANSPORT SYSTEMS FOR GREPAFLOXACIN (GPFX), A FLUOROQUINOLONE ANTIBIOTIC, IN THE BRAIN AND OTHER ORGANS

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Purpose: GPFX is a fluoroquinolone antibacterial agent which is highly distributed to various tissues, especially the lungs. GPFX and its 3-glucuronide, a main metabolite, are transported across the bile canalicular membrane at least partially by a primary active transport mechanism, cmoat/mrp2 (Sasabe et al., J. Pharmacol. Exp. Ther. 284, 1033, 1998). In addition, Fluoroquinolones are transported from the brain to the blood by mdr1a (Murata et al., J. Pharmacol. Exp. Ther. 290, 51, 1999). Thus, its active efflux transport mechanism has been proposed to be located in the liver and brain, but this transport system has not been investigated in other organs. The purpose of this study is to characterize the efflux transport system for GPFX in several organs since this may govern its tissue distribution kinetics.

Methods: The tissue distribution of GPFX in mdr1 gene-deficient mice (mdr1a(−/−) and mdr1a/1b(−/−)) and normal mice (mdr1a/1b(+/+)) was examined to clarify the contribution made by these transporters to GPFX efflux.

Results and Discussion: After a 5 mg/kg i.v. bolus dose of [^{14}C]GPFX, the tissue-to-plasma concentration ratio (Kp) in the brain of mdr1a(−/−) and mdr1a/1b(−/−) during its elimination phase (4–8 hr after dosing) was about 2.4 times higher than that in mdr1a/1b(+/+), whereas the Kp values in other