20000829

厚生科学研究研究費補助金

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」

平成 12 年度　総括・分担研究報告書

主任研究者　：　中島恵美

平成 13(2001)年　4 月

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

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 PKCYP-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、PKCYP-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、実用化への準備を薦めている。

E.結論

PKCYP-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

日本薬物動態学会（H10.11 月,仙台）

日本薬学会（H11.3 月,徳島）

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)

第 10 回日本病院薬学会年会（H12.10 月,京都）

日本薬学会第 121 年会（H13.3 月,札幌）

G.知的所有権の取得状況

特記すべきことなし。

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）

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 代謝パラメーターである Km と Vmax 値の多くは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。

本年度は昨年度に正常ラットで行った CYP1A2 のプローブと治療薬のモデルに用いた caffeine と acetanilide それぞれについての in vitro 代謝実験によるパラメーターの測定と代謝に関与するサブタイプの同定及び寄与率の算出を CYP 酵素量変動モデル動物において試みた。

B.研究方法

CYP 1 A2 の酵素誘導モデル、コリン欠乏食飼育による肝障害及び加齢モデルラットの肝ミクロゾームを酵素源とし、低基質濃度における代謝実験系を確立し、パラメーターの測定を行った。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

caffeine、acetanilide ともに CYP1A2 の酵素誘導モデルラットにおいては、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、酵素量が減少した肝障害モデル、加齢モデルにおいては、両薬物ともに代謝反応への CYP1A2 の寄与率は約 50%以下に減少した。

D.考察

CYP 酵素量が変動した場合、caffeine 及び acetanilide ともに代謝反応への CYP1A2 の寄与率が変動することが明らかになった。従って、薬物代謝に寄与する各 CYP 分子種のプローブを用いて、各 CYP 分子種の含量を診断する必要があることが示唆された。

E.結論

PKCYP-test の精度を向上させるためには、寄与率を考慮することが重要であることが明らかになった。

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)

3. 学会発表

International Conference on Drug Interaction (H11.10 月,浜松)

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日本薬学会第 121 年会（H13.3 月,札幌）

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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）

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助手

研究要旨

CYP 酵素量変動モデル動物の作製と PKCYP-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、PKCYP-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、プローブの tolbutamide を投与し、クリアランスを測定し、PKCYP-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、caffeine と acetanilide を投与し CYP1A2 についての PKCYP-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 量に減少しており、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、tolbutamide のクリアランスの変化は、比例しており、tolbutamide 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、caffeine 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 acetanilide 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 PKCYP-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、ヒトの CYP3A4 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。また種種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、個人差の大きい分子種でもある。

これらのことから、PKCYP-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 PKCYP-test が妥当であることが検証できた。また、CYP1A2 に続き CY2C11 で PKCYP-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)

第 10 回日本病院薬学会年会（H12.10 月,京都）

日本薬学会第 121 年会（H13.3,札幌）

G.知的所有権の取得状況

特記すべきことなし。

添付資料

論文別刷及び学会発表要旨

19980829

以降「薬物代謝能力診断法確立のための個体レベルでの CYP 分子種別測定方法の検討.」までは雑誌/図書等に掲載された論文となりますので、下記の「発表論文」をご参照ください。

「発表論文」

Physiologically-based pharmacokinetic analysis of grepafloxacin.
Nakajima Y, Hattori K, Shinsei M, Matsunaga N, Iizasa H, Sasabe H, Akiyama H, Miyanmoto G, Nakashima E.
Biol Pharm Bull. 2000 Sep; 23(9): 1077-83.

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.
Matsunaga N, Hattori K, Ilzasa H, Fukuhara M, Takanaka A, Nakashima E.
病院薬学. 2000 26(5): 492-504

第１３回　日本薬物動態学会年会

会期：平成１０年１１月１１日（水）～１３日（金）
会場：仙台国際センター
〒980-0856　仙台市青葉区青葉山
TEL 022-265-2211

年会長　山添　康

12B11-3　薬物代謝能力診断法確立のための個体レベルでのCYP分子種別測定方法の検討

○松永典子１)、服部研之１)、飯笹 久１)、福原守雄２)、中島恵美１)
１)共立薬大・薬剤　２)公衆衛生院・衛生薬学

【目的】薬物療法が複雑化している現在、個々の患者の薬物代謝能力を事前に診断し、これをもとに薬物療法を設計する方法の確立が望まれる。そのためには、生体のCYP量と薬物のクリアランスの関係を明らかにする必要がある。そこで、まず診断プローブのクリアランスからCytochrome P450 (CYP)量を分子種レベル別に求めるための理論を構築し、生化学的に求めた値と比較することとした。

【方法】薬物代謝能力を診断するための理論を生理学的薬物速度論に基づき、細胞膜内外の非結合型薬物濃度勾配を考慮して構築した。この理論の妥当性をCYP1A2のプローブとしてカフェインを選びラットを用いて検討した。

【結果及び考察】カフェインの全身クリアランスは0.80 ml/min、血中非結合型分率は0.85、肝スライスメディウム比は1.07であった。Vmax、Km値として、それぞれ216 nmol (drug)/min/nmol (enzyme)、200 nmol (drug)/mlを用いて計算した肝臓中のCYP1A2量は875 pmol/bodyであった。一方、ウエスタンブロッティング法により求めた肝臓中のCYP1A2量は844 pmol/bodyであり、ほぼ一致していた。

以上の結果から、診断プローブのクリアランスを用いて肝臓中のCYP量が算出可能であることが示唆された。

ISSN 0918-9823

日本薬学会

第119年会

29 [PG] 14-028　個体レベルでの薬物代謝能力診断法確立とラットにおけるCYP1A2誘導予測への応用

共立薬大　○松永貴子，服部研之，飯塚久，中島恵美；国立公衆衛生院　稲垣守生

【目的】我々は，個々の患者の薬物代謝能力診断法の確立を検討してきた。これまでに，in vivoでの肝・血中非結合型薬物濃度勾配(qg)を導入して，診断プローブのクリアランスからCytochrome P450 (CYP)活性を分子種別に求めるための理論(以下PKCYP test)を構築した。また，PKCYP testにより肝機能が低下した状態のラットにおいて肝重量の算出が可能であることを明らかにした。今回，肝重量の減少した状態のラットについて検討を行った。

【方法】ラットをコリン欠乏食で飼育することにより，脂肪肝のモデル動物を作製した。このラットにカフェインを投与し，体内動態パラメータを求めた。

【結果及び考察】PKCYP testに必要なパラメータである$t_{1/2}$は正常ラットと脂肪肝モデルラットではそれぞれ0.8, 0.8であり，CL_{int}は3.3, 1.9 ml/min/kgであった。これより，PKCYP testから算出したCYP1A2活性は，正常ラットと比べ脂肪肝モデルラットでは約40 %に減少していた。また，脂肪肝モデルラットでは，Western blottingの定量限界以下にCYP1A2量が減少していた。現在，PKCYP testで検出したCYP1A2活性の減少が，他の薬物のクリアランス変動の予測に応用できるかについて検討している。

1999
徳島

講演要旨集4

■会期
3月29日(月)～3月31日(水)

薬剤学・製剤学部会
分析化学部会
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アイソトープ・放射線部会
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Z. Nakashima, Kyoritsu-Yk. Tokyo

ABSTRAC

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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.

Noriko Matsunaga, Kenji Hattori, Hisashi Iizasa, and Emi Nakashima
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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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E-mail: van@bcasj.or.jp

P-022

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.

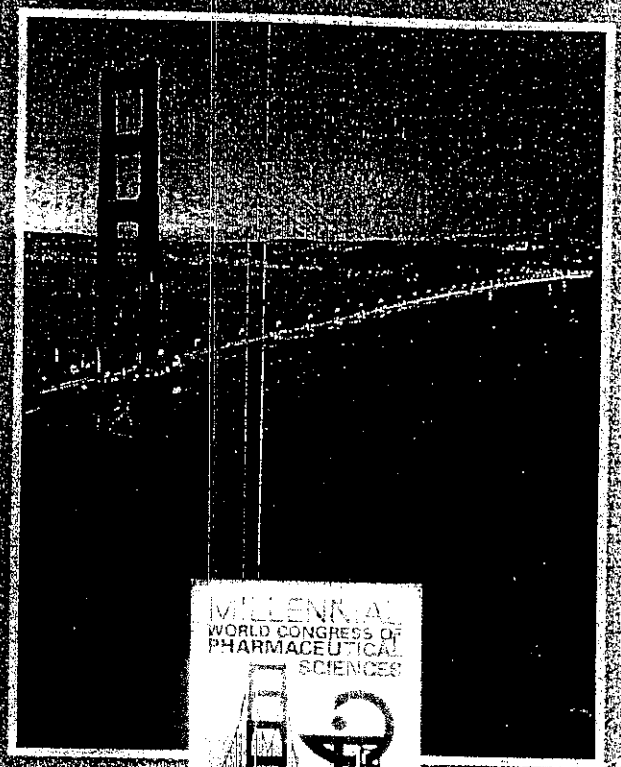
Results and Discussion: The qg value of theophylline was estimated as approximately 10 by PKCYP-test after the IV bolus administration. The effect of theophylline on the clearance of caffeine was studied. CL_{obs} was compared with the value (CL_{pred}) estimated from the PKCYP-test by using the qg value 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.

MILLENNIAL WORLD CONGRESS OF PHARMACEUTICAL SCIENCES

MOSCONE CENTER, SAN FRANCISCO, CALIFORNIA, USA

8–20 APRIL 2000



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ABSTRACTS

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

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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 ± 7.6 μM. After the first dose, the plasma concentration of LC reached a maximum of 1138.55 ± 240.21 μM, decreasing in a triexponential manner to 68.40 μM prior to the next dialysis session. During the next haemodialysis session the plasma concentration of LC decreased to 16.71 μM. 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

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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 80%.

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

Funding Source: Grants-in-Aid from the Japanese Ministry of Health and Welfare.

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

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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.

This work was supported by Glaxo-Wellcome and the BBSRC.

References:

[1] Berry and Rowland (1999). Eur. J. Pharm. Sci. 8 (2), xvii.
[2] Ho et al (1998). Pharm. Res. 13, 1873–1878 and Ho et al personal communication.

2-10056 THE PHARMACOKINETICS OF CEFTRIAXONE ADMINISTERED AT DIFFERENT 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 programs (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 = 0.008, amplitude = 60.4%) of ceftriaxone were determined. Evidence of possible temporal variations in the area under the concentration-time curve (PR = 88, 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.

Funding sources: Proyect AV05, SCyT, University of Buenos Aires, Argentina.

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.

Funding Source: This research was supported in part by Ministry of Health and Welfare and Grants-in-Aid from the Japanese Ministry of Education.

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-K^1 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

Hiroyuki Sasabe[1], Yukio Kato[2], Takashi Suzuki[1], Minoru Itose[1], Hitoshi Akiyama[1], Gohachiro Miyamoto[1], Yuichi Sugiyama[2]. [1]*Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., Tokushima;* [2]*Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan*

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. Pharmcol. Exp. Ther. 290, 81, 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

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講演要旨集

新世紀にのぞむ医療薬学

～科学的実践から社会的実践へ～

O-124　薬物動態学的肝代謝酵素量の測定：PKCYP-test の CYP2C11 への応用

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【目的】薬物療法が複雑化している現在、個々の患者の薬物代謝能力を事前に診断し、これをもとに薬物療法を設計する方法の確立が望まれる。我々は、診断プローブのクリアランスから生体内の CYP 量を測定する理論（PKCYP-test）を構築し、動物モデルにおいて生化学的に求めた値と比較することとした。すでに CYP1A2 については、診断プローブにアセトアニリドを test drug にカフェインを用い、PKCYP-test の妥当性を確認している。今回は、CYP2C11 について検討した。

【方法】CYP2C11 の診断プローブにトルブタミドを用いた。CYP 変動モデルは、Sprague-Dawley rat ♂に 20% 四塩化炭素（2.5mL/kg）を腹腔内投与して作成した。トルブタミド濃度は HPLC により測定し、CYP2C11 量は Western blotting により測定した。

【結果・考察】CYP 変動モデルにおける、トルブタミドの全身クリアランスは control rat の約 1/3 に減少していた。control rat の各種パラメータから算出した qg 値は 1.34 であった。PKCYP-test により CYP 変動モデルにおける CYP2C11 量を算出した結果、予測値と実測値はそれぞれ 10.5、9.1nmol/body であり良く一致し、CYP2C11 についても PKCYP-test の妥当性が確認された。

■会　期：平成12年10月7日(土)・8日(日)
■会　場：国立京都国際会館 京都市左京区宝ヶ池
■年会長：乾　賢一 京都大学医学部附属病院薬剤部

KYOTO

第121年会

日本薬学会

The Pharmaceutical Society of Japan

SAPP

28【PE】II-094　薬物代謝能力診断法（PKCYP-test）による
テオフィリンのクリアランスの解析
－3-メチルコラントレン投与ラットへの応用
○林美代子，松永典子，西島妙子，服部研之，飯笹久，
木津純子，中島恵美（共立薬大）

【目的】これまで、我々は個々の患者の薬物代謝能力をCytochrome P450（CYP）量として求めるための理論（PKCYP-test）を構築してきた。この理論ではパラメータとして、肝一血漿中遊離型濃度比（qg）を利用している。すでに、カフェインとアセトアニリドではその適応が可能であることを示した。今回、3-メチルコラントレン投与により酵素誘導したラットにおいて、PKCYP-testを用いたqg値に基づくテオフィリンのクリアランス解析を行った。
【方法】3-メチルコラントレン（MC）を投与してCYP1Aを誘導したラット（MC-treated rat）にテオフィリンを投与し、体内動態パラメータを求めた。また、Western blottingによりCYP1A2量を測定した。
【結果及び考察】MC-treated ratのテオフィリンのCLintおよびCYP1A2量は、それぞれ正常ラットの6倍および20倍であった。PKCYP-testにより正常ラットおよびMC-treated ratのqg値は、それぞれ6および3と算出された。qgの異なる原因として、CYP1A2以外のCYP分子種の関与や体内動態パラメータの誤差などが考えられ、テオフィリンの肝ミクロソームを用いた*in vitro*代謝実験を行い、CYP1A2の寄与率について検討した。

28【PE】II-095　薬物代謝能力診断法（PKCYP-test）を用いた
病態モデルラットにおけるCYP1A2量の測定と
カフェインのクリアランス変動予測
○松永典子，金谷奈美，渡部愛子，服部研之，飯笹久，
柴崎敏昭，中島恵美（共立薬大）

【目的】我々は、個々の患者の薬物代謝能力を診断プローブのクリアランスからCytochrome P450（CYP）量として求めるための理論（PKCYP-test）を構築した。また、酵素誘導したラットにおいて、PKCYP-testにより測定した酵素量からカフェインのクリアランスが予測可能であることを明らかにした。今回、病態モデルラットについて検討を行った。
【方法】コリン欠乏食で飼育して脂肪肝としたラット（CD-fed rat）、および35週齢のラット（Aged rat）を病態モデルラットとして用いた。アセトアニリドおよびカフェインをそれぞれCYP1A2のプローブおよびtest drugとして両モデルラットに投与し、体内動態パラメータを求めた。また、両薬物の肝ミクロソームにおける代謝への抗CYP1A抗体による影響より、CYP1A2の寄与率を見積った。
【結果および考察】アセトアニリドおよびカフェインの肝ミクロソームでの代謝におけるCYP1A2の寄与率は、CD-fedおよびAged ratともに正常ラットと比較して低下していた。両モデルラットにおいて、求めた寄与率を考慮することにより、アセトアニリドのクリアランスからPKCYP-testを用いてCYP1A2量を算出し、カフェインのCYP1A2によるクリアランスを2倍以内の誤差で予測することができた。以上の結果から、プローブの特異性を見積ることにより、PKCYP-testによるCYP量の測定が病態モデルラットにおいても精度良くできることが明らかとなった。

要旨集－1

Japan / Wales DDS Seminar

at

Kyoritsu College of Pharmacy

Tuesday, 5 September 2000

Scientific Programme

14:00-14:05

Opening remarks

Welcome
Masataka Mochizuki
Professor and President, Kyoritsu College of Pharmacy

Chairperson: Motoko Kanke
Professor, Kyoritsu College of Pharmacy

14:05 - 14:30
Employment of artificial neural networks to evaluate chemical enhancers in transdermal drug delivery
Kozo Takayama
Professor, Department of Pharmaceutics, Hoshi University

14:30 - 14:55
Role of pharmacists in development and practice of DDS/CR products
Emi Nakashima
Professor, Dept. of Pharmaceutics, Kyoritsu College of Pharmacy

Chairperson: Tadahiko Mashino
Associate Professor, Kyoritsu College of Pharmacy

ROLE OF PHARMACISTS IN DEVELOPMENT AND PRACTICE OF DDS/CR PRODUCTS

Emi NAKASHIMA

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During the past decade, one of the most striking event in pharmacotherapy has been the rapid development and the commercialization of DDS/CR products. The role of pharmacists is getting bigger not only to use these formulations properly but also to develop new products for each patient in order to solve the patient's problem. Interindividual differences in pharmacotherapy are commonly observed, and, it is important to analyze the mechanism of drug delivery process in whole body. Genetic and molecular analysis in pharmacokinetics has produced new insights into how drug molecules are delivered to the target tissue and/or organ to show the appropriate effect. We describe two topics with analyses in vitro-in vivo correlation related to the interindividual differences in pharmacokinetics.

A Novel Diagnostic Method for Drug Metabolism Capacity: The Application to Clearance Prediction for Drugs Based on *in vivo* CYP Level.

We devised a new approach to diagnosing the drug metabolism capacity, based on the physiological pharmacokinetics for estimation of *in vivo* cytochrome P450 (CYP) quantity (PKCYP-test). During the devising process of the PKCYP-test, liver to blood free concentration gradient *in vivo* (qg) was introduced. The validity of the PKCYP-test was examined using CYP1A2 probe and test drugs. Moreover, theophylline clearance was investigated by the PKCYP-test in rats with elevated levels of CYP enzyme.

The liver enzyme elevation was induced by 3-methylchoranthrene (MC) administration (MC-treated rats). Choline deficient diet fed rats (CD-fed rats) were used as a model of decreased CYP enzyme level in the liver. The effect of anti-CYP1A serum on the microsomal metabolism of theophylline was investigated using microsomes from control rats.

By applying the qg value of control rats to MC-treated and CD-fed rats, the CYP1A2 quantity was calculated based on the clearance of caffeine. The estimated clearance of acetanilide in MC-treated and CD-fed rats from the predicted level of the CYP1A2 agreed with the observed value. There was a 6-fold difference in the CLint of theophylline between control and MC-treated rats. In control rats, when the microsomal metabolism of

theophylline was inhibited by anti CYP1A serum, the residual activity was about 60%.

It appears to be possible to predict interindividual differences in metabolic clearance in patients with different amounts of CYP.

Establishing a conditionally immortalized cell line from rat brain pericytes and it's application in vitro blood-brain barrier model: Co-culture system of conditionally immortalized rat cell lines.

The blood brain barrier (BBB) plays an important role in drug delivery or metabolism in the brain. It consists mainly of endothelial cells, astrocytes and pericytes. In this study, we tried to establish a brain capillary pericyte cell line derived from temperature-sensitive SV40 large T antigen transgenic rats (Tg-rat). We tried to establish a new model system using conditional immortalized cell lines derived from temperature-sensitive SV40 large T antigen transgenic rat BBB.

Brain capillaries were prepared from homogenates by density gradient centrifugation. After enzyme digestion of capillaries, isolated cells were evaluated by immunostainings for PDGFRbeta, Thy-1, western blotting for alpha-smooth muscle actin and RT-PCR for mural cell marker, angiopoietin-1. Pericytes were isolated from the brain capillary of Tg-rat. The colonies were incubated at 33°C and passaged through single-cell cloning. The expression of PDGFRbeta, Osteopontin, ICAM-1, and angiopoietin-1 were evaluated by RT-PCR analysis and alpha-smooth muscle actin (alpha-SMA) was determined by Western blot analysis in the presence of cytokines. Cell calcification was examined by VonKossa staining.

A BBB derived glial-endothelial cell line (TR-BBB13), an cell line (TR-AST4) and a pericyte cell line (TR-PCT) were used to construct the in vitro co-culture system. In a non-contact co-culture system, TR-BBB13 and TR-AST4 were cultured on the inside of transwell-type dishes , with or without TR-PCT1, respectively, at the bottom of a 6-well plate. The enzymatic activities of alkaline phosphatase(ALP), g-glutamyl transpeptidase(gGTP) were measured using commercial kits as specific markers of BBB function. Interestingly, the TR-BBB13 enzymatic activities are similar to brain capillary in the presence of TR-PCT1.

We have been able to establish a brain capillary pericyte cell line, TR-PCT. The BBB function in co-culture systems may be improved in the presence of pericytes.

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