3. スタチン系薬剤の添付文書におけるcyclosporineとの相互作用に関する情報提供の現状

現在日本で市販されている6種すべてのスタチン系薬剤の添付文書で，cyclosporineとの相互作用についての注意喚起がされている．Table.2には，相互作用一覧表における血中濃度の変化及び機序に関する記載状況のみを抽出して示した．

Atorvastatin及びsimvastatinの添付文書では，cyclosporineとの相互作用の機序が本剤の（CYP3A4を介する）代謝阻害であるとされているが，これはCYP3A4で代謝を受けないスタチン系薬剤についてもCYP3A4で代謝を受けるスタチン系薬剤と同様にcyclosporine併用によるAUCの著しい増加が認められたこと，及び上述のラット肝ミクロソームを用いた研究においてcyclosporineによりlovastatinの代謝阻害が認められなかったこととは矛盾する内容である．また，cyclosporine併用によりどの程度血中濃度（AUCなど）に影響があるかについての記載はなかった．Fluvastatin及びpravastatinの添付文書では，cyclosporineの併用により横紋筋融解症があらわれるおそれがある，またはあらわれやすいとの記載のみで，発現機序や血中濃度（AUCなど）への影響に全く言及していない．これら4種のスタチン系薬剤の添付文書については，発売当時または発売後極初期の情報に基づいた記載が残っており，その後の新しい情報が組み入れられていないと考えられる．それに対し，最近（2003～2005年）承認・発売されたpitavastatin及びrosuvastatinの添付文書にはAUC及びCmaxの増加率の情報提供がなされている．さらに，薬物動態の項には詳細な試験条件や文献も引用されており，十分な情報提供と考えられる．薬物動態学的相互作用に関しては，AUC及びCmaxを含む薬物動態学的指標の変化に関する情報が重要と考えられることから，他のスタチン系薬剤の添付文書においてもpitavastatinやrosuvastatinと同様にこれらの情報が適切に提供されることが望まれる．一方，相互作用の機序については，pitavastatinの添付文書には記載されていなかったものの，rosuvastatinの添付文書にはOATP-Cを介した取り込み阻害を示した最近の研究成果を反映した記載が認められた．Rosuvastatin及びcerivastatin以外のスタチン系薬剤については直接的な証拠はないものの，cyclosporineによるOATP-Cを介した肝細胞取り込み阻害は，少なくとも現在までに開発されたスタチン系薬剤には共通した作用である可能性が示唆される．従って，添付文書においても，cyclosporineによる肝への取り込み阻害が示唆される旨情報提供することが望ましいと考えられる．

最後に，cyclosporineは，移植領域のみではなく，皮膚科領域を含む自己免疫疾患にも使用されるようになってきているので，スタチン系薬剤との相互作用には十分に注意を払うべきであると考えられる．臓器移植及び自己免疫疾患等に使用される免疫抑制剤には，cyclosporineの他にtacrolimusがある．Tacrolimusとスタチン系薬剤の臨床薬物動態学的相互作用については2つの報告があり，臓器移植患者においてsimvastatinの血中濃度に全く影響が認められなかったこと[14]，また，cerivastatinのAUCが35％増加したこと[29]が示されている．従って，tacrolimusのスタチン系薬剤への影響はcyclosporineより明らかに少ないと推定されることから，スタチン系薬剤との相互作用に関しては，tacrolimus併用時の方がcyclosporine併用時よりも問題が少ないと考

Table.2　日本のスタチン系薬剤の添付文書におけるシクロスポリンとの相互作用に関する記載状況

薬剤名	相互作用一覧表における記載	
	血中濃度の変化	機序
アトルバスタチン	[記載なし]	シクロスポリンによるHMG-CoA還元酵素阻害剤の代謝，胆汁中排泄に対する競合阻害に基づく相互作用が示唆されている．
シンバスタチン	[記載なし]	CYP3A4を阻害し，併用により本剤の代謝が抑制されるおそれがある．
フルバスタチン	[記載なし]	[記載なし]
プラバスタチン	[記載なし]	[記載なし]
ピタバスタチン	シクロスポリンにより本剤の血漿中濃度が上昇(Cmax 6.6倍，AUC 4.6倍)する．	[記載なし]
ロスバスタチン	シクロスポリンを投与されている心臓移植患者に併用したとき，シクロスポリンの血中濃度に影響はなかったが，本剤のAUC0-24hが健康成人に単独で反復投与したときに比べて約7倍上昇したとの報告がある．	シクロスポリンにより本剤の肝への取り込みが阻害されるためと考えられる．

えられる.

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安全性の問題で市場撤退となったセリバスタチンの最新情報と米国の市販後安全性監視システムの解析

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Withdrawal of Cerivastatin Revealed a Flaw of Post-marketing Surveillance System in the United States

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Cerivastatin, a lipid-lowering agent, was voluntarily withdrawn from the market because of high risk of rhabdomyolysis when used as monotherapy and as comedication with fibrates, especially gemfibrozil. Thereafter, investigators found a five-fold increase in the area under the curve (AUC) when cerivastatin was used as comedication with gemfibrozil and theorized that the increase was associated with inhibition of the hepatic uptake and metabolism. By contrast, a number of pharmacoepidemiological investigations - one of which involved evaluation of the Food and Drug Administration (FDA) database for suspected adverse drug reactions and 11 cohort studies of statin and fibrate users in United States - showed the risk of rhabdomyolysis to be greater in cerivastatin than in other statins used in either monotherapy or in comedication with fibrates, especially gemfibrozil.

This incident regarding risk of rhabdomyolysis in cerivastatin monotherapy was taken to court in the United States and unpublished company (manufacturer of cerivastatin) documents were opened. The incident was then analyzed and discussed in the *Journal of American Medical Association (JAMA)* as a concern of the current US post-marketing surveillance system. The company's action and timing were judged and found to be inappropriate (although companies of this sort generally have insurmountable conflicts of interest), and the work of the US regulatory system and funding for post-marketing safety management were found to be insufficient. On the basis of the current situation, the authors and editors recommend further improvement of post-marketing regulations including the establishment of an independent drug safety board to oversee post-marketing surveillance.

Among the opened, unpublished data, was the finding that cerivastatin obviously induced myopathy in a dose-dependent manner when administrated as monotherapy. As for other statins, only limited data was available for the relationship between the dosage and the rate of myopathy. For the safety use of statins, this should be clarified by proper surveillance system.

Key Words: cerivastatin, gemfibrozil, rhabdomyolysis, withdrawal, post-marketing safety management

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はじめに

StatinはHMG-CoA還元酵素を特異的に阻害することによって，血中のコレステロール濃度を減少させ，狭心症や心筋梗塞などの心血管系疾患を予防するための薬剤である．Statinは一般には安全性が高く，非常に多くの患者に投与されているが，まれに横紋筋融解症を誘発することが問題となっている．一方，主なstatinはCYP3A4によって代謝され，CYP3A4阻害作用を有する薬剤との併用により，それらのstatinの血中濃度が著しく増加することから，特異的なCYP阻害剤の影響を受けにくいstatinとして，CYP3A4とCYP2C8の両方により代謝されるcerivastatinが開発された．実際，cerivastatinの血中濃度はCYP3A4の強力な阻害剤のitraconazoleと併用しても殆ど増加が認められない．しかし，米国において，cerivastatinの単独投与で横紋筋融解症が多発し，死亡が出たばかりか，gemfibrozilとの併用でさらに高頻度で横紋筋融解症が誘発され，多くの死亡が出たため，自主的に市場撤退した[1]．また，日本では，gemfibrozilは販売されていないものの，cerivastatin単独投与によるリスク等を考慮して市場撤退した．最近，cerivastatin単独及び

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gemfibrozilとの併用による横紋筋融解症の発現頻度の解析，薬物動態相互作用機序の研究，また，cerivastatinをモデルに米国における市販後安全性管理の状況が解析されたので，本項ではそれらを総合的にまとめる．

1. Cerivastatinの臨床薬物動態特性

CerivastatinはCYP3A4とCYP2C8の両酵素によって代謝されることから，単独のCYP3A4阻害剤との併用ではcerivastatinの代謝はほとんど阻害されないと考えられる．実際，itraconazole, erythromycin, cimetidineとの併用投与でcerivastatinのAUCは最大でも30%程度の増加[2~4]で，atorvastatin, simvastatin, lovastatinのような数倍の増加に比べて著しく影響が少ない．一方，gemfibrozilとの併用では，simvastatin[5]及びlovastatin[6]のAUCがそれぞれ2及び3倍の増加に対して，cerivastatin[7]のAUCは5倍と，2倍程度強く影響が現れることが示されているが，他のstatinに対する各種CYP阻害剤の影響が一般に数倍以上であることを考えると，必ずしも特別にリスクが高いことを示唆するわけではない．

2. 相互作用の機序に関する研究

Cerivastatinは発売初期にgemfibrozilとの相互作用に基づく横紋筋融解症の発現が認められ，2年半後にこの相互作用によるリスクを主な原因として市場撤退した．その後，gemfibrozilがcerivastatinの薬物動態に影響を及ぼすことが発表され[7]，その機序に関する研究が行われた．Prueksaritanontらはヒト肝ミクロソームを用いた実験でatorvastatin及びsimvastatinの酸化はgemfibrozilにより軽微に抑制されるが，cerivastatinのM1及びM2代謝物への酸化は中等度に抑制されること，一方，atorvastatin及びsimvastatinのグルクロン酸抱合化はgemfibrozilにより軽度に抑制されるが，cerivastatinのグルクロン酸抱合化は中等度に抑制されることを示した[8]．このように，gemfibrozilはcerivastatinの代謝をより強く抑制することから，これがcerivastatinの血中濃度の増加を引き起こし，副作用発現頻度の増加の原因の一つであると推定された．

一方，Shitaraらは肝におけるcerivastatinの代謝にはCYP2C8の方が重要であり，相互作用の機序として，gemfibrozil及びその代謝物（グルクロン酸抱合体）がCYP2C8を強く阻害するとともに，cerivastatinの肝への取り込みに関与するOATP-Cも阻害することを示した[9]．CYP2C8により代謝を受けないpravastatin[10]やrosuvastatin[11]のAUCもgemfibrozilとの併用で2倍程度増加することから，gemfibrozilあるいはそのグルクロン酸抱合体によるOATP-C阻害の重要性が示唆されたと考えられる．

以上の様に，2つの研究で異なる機序が示されたが，少なくともcerivastatinは他のstatinよりも代謝面でgemfibrozilの影響を受け易く，結果として血中濃度がより顕著に増加したものと考えられる．

3. 米国におけるstatinによる横紋筋融解症の発症解析

Statinによる横紋筋融解症の発症解析研究は数多く行われ，それらの結果からcerivastatinの単独服用で横紋筋融解症のリスクが明らかに高く，さらにfibrate，特にgemfibrozilとの併用でリスクがさらに増加することが明らかとなった．以下にそのいくつかを紹介する．

Thompsonらは，1990年1月から2002年3月までのstatinによる横紋筋融解症の症例報告3,339件中，cerivastatinによるものが1,899件（57%）を占めていること[12]，また，Staffaらは同時期の処方を基準とすると，全statin約4億7千4百万処方中cerivastatinの処方が約

Table.1 Reported Cases of Fatal Rhabdomyolysis by Statin, Numbers of Prescriptions, Reporting Rate per Million Prescriptions, and Relative Reporting Rate for Cerivastatin vs Each of the Other Statins*

	Atorvastatin Calcium	Fluvastatin Sodium	Lovastatin	Pravastatin Sodium	Simvastatin	Subtotal of All Statins†	Cerivastatin Sodium
Date approved	12/17/96	12/31/93	8/31/87	10/31/91	12/23/91		6/26/97
Prescriptions, No	140,360,000	37,392,000	99,197,000	81,364,000	116,145,000	474,458,000	9,815,000
No. of cases	6	0	19	3	14	42	31
Rate per million Prescriptions	0.04	0	0.19	0.04	0.12	0.09	3.16
RRR(95% CI)	74(30-217)	..(≥30)	16(9-31)	86(27-438)	26(14-53)	36(22-58)	

Abbreviations: CI, confidence interval; RRR, relative reporting rate for cerivastatin compared with each of the other statins or all other statins combined. Ellipses indicate that 0 events for fluvastatin means that 1 dividing by 0 results in an undefined number; thus, 30 represents the lower 95% CI.

*：Includes US cases reported to the Food and Drug Administration before June 26, 2001. Data are from Staffa et al[13].

†：Subtotal data do not include cerivastatin.

980万件で僅か2.0％であることを示した[13]．従って，relative reporting rate（RRR）は他のstatinの65倍となる．さらにStaffaらは横紋筋融解症による死亡報告を解析した結果，cerivastatinが他のstatinの16-86倍多いことを示した（Table.1：Psatyら[14]のTable.3を引用）．また，gemfibrozilとの併用患者を除いた場合でもcerivastatinでの死亡報告は10-50倍であった．

さらに，1997年から2000年までのFDA副作用症例データの解析では，statinの単独服用により発症した全横紋筋融解症のうち35.7％がcerivastatinによるもので，fibrateとの併用の場合は80.6％がcerivastatinとの併用であった（Table.2：Psatyら[14]のTable.4を引用）．

一方，米国で11の管理された医療計画による1998年1月1日から2001年6月30日までのコホート研究が行われ，その解析結果が報告された（Table.3：Grahamら[16]のTable.1の一部を引用）．脂質低下薬服用者152,460人のうち，24件の入院を要する横紋筋融解症が発生した．10,000人／年当たりの単独服用（atorvastatin，pravastatin及びsimvastatin）の平均発症率は0.44，cerivastatinで5.34，fibratesで2.82であった．また，atorvastatin，pravastatin及びsimvastatinとfibratesとの併用で5.98に，cerivastatinとfibratesとの併用で1,035に増加した．この結果から，治療年当たり横紋筋融解症を1件発症するに要する患者数はstatin単独服用で22,727人，cerivastatinとfibratesの併用では9.7-12.7人となる．

4. 米国におけるcerivastatinの市販後安全対策

Cerivastatinが安全性の問題で訴訟に至った結果，本来公表されない社内資料が公開され，詳細な状況が把握できたため，cerivastatinをモデルとして現在の米国における新医薬品の市販後安全性監視状況の解析・評価が*JAMA*（*Journal of American Medical Association*）に掲載され，さらにそれに対する反論や編集者の意見も同時に掲載されたので，それらの要点をまとめた．

Psatyらは未公表資料に基づいて，社内での対応状況や時期，並びに規制当局の体制等を次のように解析・評価した[14]．Cerivastatinは1998年2月に市販されたが，企業はその後100日以内に横紋筋融解症またはクレアチンキナーゼの顕著な増加例を7件把握しており，そのうちの5件はgemfibrozilとの併用で，この時点でcerivastatinとgemfibrozilとの相互作用が強く疑われていた．その後もgemfibrozilとの併用による横紋筋融解症が高率に発症していたが，1999年12月に至って併用禁忌となり，緊急安全情報（Doctor letter）を出した．しかし，この緊急安全情報はあまり効果がなかったようで，gemfibrozilとの併用は続き，1999年5月から8月ま

Table.2　Cases of Statin-Associated Rhabdomyolysis by Drug Reported to the Food and Drug Administration Adverse Event Reporting System (October 1997 to December 2000) *

	No. (%) of Cases†						
	Atorvastatin Calcium	Fluvastatin Sodium	Lovastatin	Pravastatin Sodium	Simvastatin	Cerivastatin Sodium	Total
Fibrate coprescription							
With	13(5.2)	2(0.8)	2(0.8)	8(3.2)	23(9.3)	200(80.6)	248(100)
Without	73(13.9)	8(1.5)	30(5.7)	62(11.8)	164(31.3)	187(35.7)	524(100)
Total	86(11.1)	10(1.3)	32(4.1)	79(9.1)	187(24.2)	387(50.1)	772(100)

*：Data are from Fisher et al[15].
†：Percentages may not sum to 100 due to rounding.

Table.3　Description of Inception Cohorts for Patients Using Statin and Fibrate Drug Therapy

	Statins						Fibrates	
	Atorvastatin N = 130,865	Cerivastatin N = 12,695	Fluvastatin N = 4,706	Lovastatin N = 1,207	Pravastatin N = 35,713	Simvastatin N = 46,799	Gemfibrozil N = 14,677	Fenofibrate N = 5,808
Person-years, No								
Monotherapy	129,367	7,486	3,292	775	33,149	40,940	8,102	2,529
Co-medication	2,664	89	25	10	543	552	2,512	905
Rhabdomyolysis cases, No								
Monotherapy	7	4	0	0	0	2	3	0
Co-medication	1*	6†	0	0	0	1‡	4†‡	1*

*：One patient was included in both the atorvastatin and fenofibrate combination therapy inception cohorts.
†：Three patients were included in both the cerivastatin and gemfibrozil combination therapy inception cohorts.
‡：One patient was included in both the simvastatin and gemfibrozil combination therapy inception cohorts.

での横紋筋融解症32例中の併用例が20例（63％）に対して，1999年9月から2000年2月が130症例中91例(70％)，2000年3月から7月が55症例中34例（62％）と殆ど減少しなかった．また，cerivastatin単独服用でも横紋筋融解症の発症率が高かった．企業はこれらの情報及び社内研究資料の公表並びに対応を実施する多くの機会を逃した．一方，規制当局の市販後安全性管理は組織としての体制も整っておらず，予算面も不十分で機能しているとはいえない．企業にはリスク・ベネフィットに基づいた安全対策を講じる上で，経済面も含めた利害の対立があり，企業だけでの適切な対応は難しい．今後は，独立した評価機構等を作り，添付文書の改訂から市場撤退までの市販後安全性管理の意志決定をすべきであるとしている．

これに対し，企業としては症例報告等の報告事項をしっかり遂行しており，FDAも提出した資料を適切に評価してきたと反論があった[17]．なお，対策の実施や評価結果の公表に当たって，FDAは症例報告からでは正確な副作用発現率が求められないこと，他剤とのリスクの違いを比較するために，対象とする薬剤を用いて良く管理された2剤比較試験を行わないで結論を出すことは出来ないとしている．したがって，企業の対応は患者の安全性と福祉を考え，責任を持った，適切で一貫したものであったと見解を述べた．

なお，JAMAでの掲載は市販後医薬品の安全性監視活動の将来像を模索するために，cerivastatinをモデルに討議されたものであり，JAMAの編集者も参加して，最終的な結論として独立した市販後安全監視機構等を設立すべきであると勧告している[18～20]．

5. 未公開情報から見えた新事実

Psatyらが紹介した社内資料の中に，1999年7月に得られた臨床試験結果としてcerivastatinを1.6 mg服用することによって，重篤なクレアチンキナーゼの上昇（正常最大値の10倍以上）が12％のヒトに，軽度のクレアチンキナーゼの上昇（正常最大値の3倍以上）が50％のヒトに見られ，さらに0.8 mgを服用した場合に比べて1.6 mgの服用では副作用が指数的に増加したことが述べられている[14]．1999年8月2日の会議メモでは社内の専門家はこの用量を一般に拡大すべきではないと勧告している．この結果は，cerivastatinについては，狭い用量範囲内で用量依存的に横紋筋融解症を発現する可能性が高いこと，cerivastatinの血中濃度を増加させる様な薬剤相互作用は横紋筋融解症のリスクを明確に上げるであろうことを示唆している．他のstatinでは，臨床的に横紋筋融解症の発現が用量依存的であることを示唆する資料は殆どないが，Smithら[21]は雌のSDラットにsimvastatin, lovastatin及びpravastatinを投与すると用量依存的に筋症が発現すること，これらのstatinとcyclosporineとの併用によりこの筋毒性発現が強く増強されることを報告している．また，rosuvastatinでは，臨床試験中に，用量依存的なタンパク尿及び血尿の発生が報告され，また，高用量（80 mg群）で横紋筋融解症の発生が複数報告されたこと[22,23]から，米国での承認用量は他のstatin類と安全性が変わらない40 mgまでとされている．これらのことから，cerivastatin以外のstatinについても血中濃度と横紋筋融解症に相関性があることが示唆される．なお，rosuvastatinについては，主なCYPによる代謝は受けないとされているが，一方ではアジア系の被験者で血中濃度が他人種の2倍となることが開発中及び市販後の試験で報告され[24,25]，人種差が考えられている．現時点ではrosuvastatinの日本の承認審査資料等は公開されておらず，詳細は不明であるが，開発企業のwebsiteでは，日本の承認用量を欧米の半量とすること，また，適切な市販後調査を行うことが承認条件とされたとしている[26]．しかしながら，市販後調査の方法，規模によっては，安全性情報の収集に適切ではない可能性が指摘されていること[27]，cyclosporineは，本剤を含め，全てのstatinの血中濃度を数倍以上に増加させることが知られており，また，cyclosporineは，移植領域だけでなく，皮膚科領域を含む自己免疫疾患へも効能が拡大されていることから，今後，十分な注意が必要と考えられる．従って，statinを服用する際には薬物動態相互作用を示す薬剤との併用[28,29]や食品[30]の摂取には充分注意すべきであると考えられる．

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Is the Monkey an Appropriate Animal Model to Examine Drug-Drug Interactions Involving Renal Clearance? Effect of Probenecid on the Renal Elimination of H_2 Receptor Antagonists

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ABSTRACT

The renal drug-drug interaction between famotidine (an H_2 receptor antagonist) and probenecid has not been reproduced in rats. We have proposed that this is caused by a species difference in the transport activity by human/rat organic anion transporter (OAT) 3 and the expression of organic cation transporter (OCT) 1 in the rodent kidney. Since monkey OATs (mkOATs) exhibit similar transport activities to human orthologs, it is hypothesized that in vivo studies in monkeys will allow a more precise prediction of renal drug-drug interactions in humans. Famotidine and cimetidine were efficiently taken up by mkOAT3-expressing human embryonic kidney cells (K_m, 154 and 71 μM, respectively), and their uptake was strongly inhibited by probenecid (K_i, 3.0–5.7 μM). Quantification of mkOCT1 and mkOCT2 mRNAs in the monkey kidney using real-time reverse transcription-polymerase chain reaction revealed their predominant expression in the liver and kidney, respectively. Crossover studies were conducted in cynomolgus monkeys. Famotidine was given by i.v. administration, with or without probenecid. Probenecid treatment caused a 65% reduction in the renal clearance (0.426 ± 0.079 versus 0.165 ± 0.027 l/h/kg) and a 90% reduction in the tubular secretion clearance (0.275 ± 0.075 versus 0.0230 ± 0.0217 l/h/kg), whereas it had no effect on the renal clearance of cimetidine. In contrast to the species-dependent effect of probenecid, allometric scaling using animal data (rat, dog, and monkey) successfully predicted the renal and tubular secretion clearance of famotidine in humans. These results suggest that monkeys are more appropriate animal species for predicting the renal drug-drug interactions in humans.

The kidney plays an important role in the detoxification of xenobiotics and endogenous waste as well as maintaining the balance of ions and nutrients in the body. Urinary excretion is the major detoxification mechanism of the kidney, and this is governed by glomerular filtration, tubular secretion across the proximal tubules, and reabsorption. Transporters play important roles in the tubular secretion of drugs. Many studies have described the role of multispecific organic anion and cation transporters [organic anion transporter (OAT)/*SLC22* and organic cation transporter (OCT)/*SLC22*] in the renal uptake of drugs (Lee and Kim, 2004; Wright and Dantzler, 2004; Shitara et al., 2005). Both Oct1 (*Slc22a1*) and Oct2 (*Slc22a2*) are involved in the renal uptake of organic cations on the basolateral membrane of the proximal tubules in rodents, whereas OCT2 plays a predominant role in human kidney (Lee and Kim, 2004; Wright and Dantzler, 2004). As renal organic anion transporters, two isoforms (Oat1/*Slc22a6* and Oat3/*Slc22a8*) in rodents and three isoforms (OAT1, OAT2/SLC22A7 and OAT3) in humans, have been identified on the basolateral membrane of the proximal tubules (Lee and Kim, 2004; Miyazaki et al., 2004; Wright and Dantzler, 2004).

Probenecid, an antipodagric drug, is a well known inhibitor of organic anion transporters. Drug interactions with probenecid have been reported involving renal excretion in humans resulting in a prolongation of the plasma elimination half-life (Cunningham et al., 1981). For famotidine, an H_2 receptor antagonist, its effect has been reported to be

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ABBREVIATIONS: OAT, organic anion transporter; OCT, organic cation transporter; h/mk/rOAT, human/monkey/rat OAT; h/mk/rOCT, human/monkey/rat OCT; HEK, human embryonic kidney; LC, liquid chromatography; MS, mass spectrometry; CMD, cimetidine; FMD, famotidine.

species-dependent. The renal secretion clearance of famotidine in humans was significantly reduced by oral coadministration of probenecid (Inotsume et al., 1990), whereas this interaction has not been reproduced in rats, although the plasma concentration of probenecid achieved a similar level to that in clinical studies (Lin et al., 1988). In contrast to famotidine, the renal secretion clearance of cimetidine, another H_2 receptor antagonist, in both humans and rats was only slightly (ca. 20%) reduced by coadministration with probenecid (Lin et al., 1988; Gisclon et al., 1989). These results suggest that the contribution of transporters involved in the tubular secretion of famotidine is different between rodents and humans, and the organic anion transporter plays a more important role in humans. We found that the transport activity of famotidine by hOAT3 is greater than that by rOat3 and that the unbound plasma concentration of probenecid is sufficiently higher than its K_i values for rat and human OAT3 (Tahara et al., 2005a). Therefore, we hypothesized that this increases contribution of OAT3, a probenecid-inhibiting fraction, to the renal uptake of famotidine in humans together with the fact that hOCT1 is not present in the kidney.

To predict the possibility of drug-drug interactions in humans, such species differences have to be overcome. The monkey has been used in pharmacological, toxicological, and pharmacokinetic studies by many pharmaceutical companies, and it is recognized as a suitable animal model for the validation of in vitro scaling methods since it is the second nearest species to humans in the evolutionary tree. Ward and Smith (2004a,b) have reported that the monkey provides the most qualitatively and quantitatively accurate predictions of human pharmacokinetics by retrospectively analyzing the pharmacokinetic parameters of 103 nonpeptide xenobiotics in monkeys and humans. In addition, we have demonstrated that there is a good correlation of the transport activities with respect to that of reference compounds between mk- and hOAT3, whereas the correlation was poor between rat and human OAT3 (Tahara et al., 2005b). According to our mRNA quantification, mkOCT1 and mkOCT2 are predominantly expressed in the liver and kidney, respectively. Therefore, we consider that monkeys will be a better animal model for predicting drug-drug interactions involving multiple transporters.

In the present study, we examined the effect of probenecid on the pharmacokinetics of famotidine and cimetidine in cynomolgus monkeys to investigate whether the drug-drug interaction between probenecid and famotidine can be reproduced in monkeys. In addition, the uptake of H_2 receptor antagonists (cimetidine, famotidine, and ranitidine) by HEK293 cells expressing mkOAT1 and mkOAT3 and the inhibitory effect of probenecid on the uptake of the H_2 receptor antagonists were also examined.

Materials and Methods

Materials. Famotidine and probenecid were purchased from Nacalai Tesque (Kyoto, Japan), and cimetidine and ranitidine were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were of analytical grade and commercially available.

Cells and Reagents for in Vitro Studies. In vitro experiments were carried out using HEK293 cells stably transfected to express functional mkOAT1 or mkOAT3 and corresponding control cells transfected with the pcDNA3.2 expression vector. Generation of both cell lines and their characterization are described elsewhere (Tahara et al., 2005b). The cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 400 μg/ml G418 (Invitrogen) at 37°C with 5% CO_2 and 95% humidity on the bottom of a dish. mkOAT1- and mkOAT3-expressing cells were seeded in polylysine-coated 12-well plates (Becton Dickinson, Franklin Lakes, NJ) at a density of 1.2×10^5 cells/well. Cell culture medium was replaced with culture medium supplemented with 5 mM sodium-butyrate 24 h before transport studies to induce the expression of those proteins.

Transport Assay. Transport studies were carried out as described previously (Tahara et al., 2005a). Uptake was initiated by adding medium containing 10 μM of compounds, in the presence or absence of inhibitor, after cells had been washed twice and preincubated with Krebs-Henseleit buffer at 37°C for 15 min. The Krebs-Henseleit buffer consists of 118 mM NaCl, 23.8 mM $NaHCO_3$, 4.83 mM KCl, 0.96 mM KH_2PO_4, 1.20 mM $MgSO_4$, 12.5 mM HEPES, 5 mM glucose, and 1.53 mM $CaCl_2$ adjusted to pH 7.4. The uptake was terminated at designed times by adding ice-cold Krebs-Henseleit buffer after removal of the incubation buffer. Then, cells were washed twice with 1 ml of ice-cold Krebs-Henseleit buffer. For the determination of the uptake of cimetidine, ranitidine, and famotidine, cells were dissolved in 300 μl of 0.2 N NaOH and kept overnight. Aliquots (150 μl) were transferred to vials after adding 30 μl of 1 N HCl. Aliquots (100 μl) were used for LC-MS quantification as described below. The remaining 10 μl of the aliquots of cell lysate was used to determine the protein concentration by the method of Lowry with bovine serum albumin as a standard. Ligand uptake was given as the cell/medium concentration ratio determined as the amount of ligand associated with cells divided by the medium concentration.

Kinetic Analyses of the Transport Study. Kinetic parameters were obtained using the Michaelis-Menten equation:

$$v = V_{max} \times S/(K_m + S) \quad (1)$$

where v is the uptake rate of the substrate (picomoles per minute per milligram of protein), S is the substrate concentration in the medium (micromolar), K_m is the Michaelis-Menten constant (micromolar), and V_{max} is the maximum uptake rate (picomoles per minute per milligram of protein). To obtain the kinetic parameters, the equation was fitted to the uptake velocity using a MULTI program (Yamaoka et al., 1981). The input data were weighted as the reciprocals of the observed values, and the Damping Gauss Newton Method algorithm was used for fitting. Inhibition constants (K_i) of several compounds were calculated assuming competitive inhibition using the following equation since the substrate concentration was sufficiently low compared with the K_m values.

$$CL_{+inh} = CL/(1 + I/K_i) \quad (2)$$

where CL represents the uptake clearance, and the subscript (+inh) represents the value in the presence of inhibitor. I represents the concentration of inhibitor (micromolar). Fitting was performed by an iterative nonlinear least-square method using a MULTI program, and the Damping Gauss Newton Method algorithm was used for fitting (Yamaoka et al., 1981).

Real-Time RT-PCR Analysis. Male cynomolgus monkey liver and kidney was purchased from BOZO Research (Shizuoka, Japan). Total RNA was extracted using the extraction solution of ISOGEN (NIPPON GENE, Tokyo, Japan) according to the manufacturer's protocol. The total RNA was reverse-transcribed using SuperScript First-Strand Synthesis System for RT-PCR kit (Invitrogen) with an oligo$(dT)_{12-18}$ as primer. Fifty nanograms of the RT reaction mixture was taken for a real-time PCR (SYBR, Green I chemistry) (94°C for 5 min, 94°C for 30 s, 65°C for 30 s, 72°C for 30 s, for 45 cycles; Applied Biosystems ABI PRISM 7700 Sequence Detector system; Applied

Biosystems, Foster City, CA) using Ex *Taq* polymerase (Takara Bio, Kyoto, Japan) with specific primers based on human OCTs (hOCT1 sense primer, 5′-TAAAGATAATGGACCACATCGC-3′; antisense primer, 5′-TATGATGTTTAACCAGTGCAGG-3′, accession no. NM_003057-809; hOCT2 sense primer, 5′-AGTTGCCTATACAGTTGGGCTC-3′; antisense primer, 5′-CAGGGCAGAGTAGAAGAAATCC-3′, accession no. NM_003058-77; hOCT3 sense primer, 5′-GACCAAGGATTTGAGAAAGTTG-3′; antisense primer, 5′-AGGGAATCTGTGGCTCTAGG-3′, accession no. NM_021977-172). All values are expressed as relative luminescence units per 50 ng of total RNA normalized with that of β-actin.

In Vivo Study in Monkeys. Four male cynomolgus monkeys were obtained from Siconbrec Inc. (Manila, Philippines). The mean body weight of the monkeys was 5.7 kg, ranging from 4.7 to 6.5 kg. The four cynomolgus monkeys received a single i.v. dose of famotidine and cimetidine, at a dose of 0.3 and 4 mg/kg, after an oral dose of 10 ml of vehicle as a control phase. Thereafter, a study with a randomized crossover design was conducted at intervals of 1 month. The monkeys received the same amount of famotidine, cimetidine, and 22.5 mg/kg probenecid, that is, 15 mg/kg probenecid 2 h before and 7.5 mg/kg simultaneously with an i.v. dose of famotidine and cimetidine. The study protocol for the animal experiment was approved by Animal Care Committee of Kyowa Hakko Kogyo Co., Ltd (Shizuoka, Japan).

Sample Collection. Blood samples (0.5 ml each) for the determination of famotidine and cimetidine were taken with heparinized syringe at 5, 10, and 15 min and 0.5, 1, 2, 4, and 8 h after the administration of famotidine and cimetidine. Urine samples were collected from 0 to 4, 4 to 8, and 8 to 24 h after dosing. Plasma was separated immediately and kept at −40°C until analysis. A part of the urine sample was stored at −40°C until analysis. The remaining urine samples were discarded after the urine volume had been recorded.

Determination of Protein Binding in Plasma. Plasma (150 μl) obtained at 30 min during the i.v. administration was directly applied to an MPS micropartition device (Millipore Corporation, Bedford, MA). The micropartition device was then centrifuged at 1500*g* for 15 min, and the unbound cimetidine, famotidine, and probenecid concentration in the filtrate was determined by LC-MS. The free fraction in plasma (f_p) was determined as the ratio of the unbound concentration in the filtrate to the total concentration.

Determination of Plasma and Cell Lysate Concentrations. The quantification of cimetidine, famotidine, ranitidine, and probenecid was performed by high-performance liquid chromatography (Alliance 2690; Waters, Milford, MA) connected to a mass spectrometer (ZQ; Micromass, Manchester, UK) (Nagata et al., 2004). Aliquots (100 μl) of plasma and urine containing famotidine, cimetidine, and probenecid were precipitated with 200 μl of methanol containing an internal standard (ranitidine), mixed, then centrifuged, and 25 μl of the supernatants was injected into the LC-MS. High-performance liquid chromatography analysis was performed on a Capcell Pak C_{18} MG column (3 μm, 4.6 mm i.d., 75 mm; Shiseido, Tokyo, Japan) at room temperature. Elution was performed with a 0 to 90% linear gradient of 10 mM ammonium acetate-acetonitrile over 4 min at 0.8 ml/min. A portion of eluent (split ratio, 1:3) was introduced to the MS via an electrospray interface. Detection was performed by selected ionization monitoring in positive ion mode (*m/z*, 253; *m/z*, 315; *m/z*, 338; and *m/z*, 286 for cimetidine, ranitidine, famotidine, and probenecid).

The lower limit of quantitation for famotidine and cimetidine was 5 ng/ml in plasma and 10 ng/ml in urine, respectively. The standard concentration of both drugs ranged from 5 to 1000 ng/ml in plasma and 10 to 1000 ng/ml in urine, respectively. The within-day coefficient of variation of both drugs for plasma and urine determinations was less than 10%. Creatinine concentrations in plasma and urine were determined by an enzymatic method (creatinase/sarcosine oxidase/peroxidase) using an AutoAnalyzer 7070 (Hitachi Instruments Service, Tokyo, Japan). Probenecid and H_2 receptor antagonists did not interfere with the quantification of creatinine.

Pharmacokinetic Analysis. Plasma concentration time data (Cp) of famotidine and cimetidine were fitted to a two-compartment model using a MULTI program (Yamaoka et al., 1981).

$$Cp = A \times e^{-\alpha t} + B \times e^{-\beta t} \quad (\alpha > \beta). \tag{3}$$

The following parameters were calculated whenever appropriate: $t_{1/2\alpha}$ (the distribution half-life, calculated as $0.693/\alpha$), $t_{1/2\beta}$ (the elimination half-life, calculated as $0.693/\beta$); $AUC_{0-\infty}$ (the total area under the plasma concentration time curve extrapolated to infinity, calculated as $A/\alpha + B/\beta$); CL_p (the plasma clearance, calculated as dose/$AUC_{0-\infty}$); MRT [the mean residence time, calculated as $(A/\alpha^2 + B/\beta^2)/AUC_{0-\infty}$)]; $V_{d,ss}$ (the steady-state distribution volume, calculated as MRT × CL_p); V_c [the distribution volume of the central compartment, calculated as dose/(A + B)]; and CL_{dis} (the distribution clearance, calculated as $K_{12} \times V_c$). The renal clearance (CL_{renal}) of famotidine and cimetidine was calculated as $CL_{renal} = Ae/AUC_{0-\infty}$, where Ae is the amount of famotidine and cimetidine excreted in the urine within 24 h. The tubular secretion clearance (CL_{sec}) was calculated as $CL_{sec} = CL_{renal} - fp \times GFR$, where fp is the unbound fraction of famotidine and cimetidine in plasma, and GFR is the glomerular filtration rate. The percentage of drug excreted in the urine (fe) was calculated as fe = Ae/dose. The creatinine clearance values were used for the values of GFR in this study. The creatinine clearances in cynomolgus monkeys treated with or without probenecid were determined as 0.241 ± 0.021 (0.217–0.252) and 0.189 ± 0.025 (0.169–0.217) l/h/kg, respectively (mean ± S.D., not significant, $p > 0.05$). The two-tailed paired Student's *t* test was used for a statistical analysis, and value of $p < 0.05$ was considered significant.

Results

Uptake of H_2 Receptor Antagonists by mkOAT1 and OAT3. Figure 1 shows the time profiles and concentration dependence of the uptake of the H_2 receptor antagonists by mkOAT1-, mkOAT-3, and vector-HEK, respectively. As reported previously in hOAT1-HEK (Tahara et al., 2005a), the uptake of cimetidine by mkOAT1-HEK was slightly greater than that by mock cells (7.27 ± 0.20 versus 3.48 ± 0.04 μl/mg protein at 5 min). A slight increase was also observed in the uptake of ranitidine by mkOAT1-HEK (18.8 ± 2.27 versus 11.4 ± 1.11 μl/mg protein at 5 min), but no specific uptake was observed for famotidine. The uptake of cimetidine, famotidine, and ranitidine by mkOAT3-HEK was significantly greater than in vector-HEK (Fig. 1). Since the uptake of cimetidine, ranitidine, and famotidine by mkOAT3-HEK increased linearly up to 3 and 5 min of incubation, the uptake of cimetidine, famotidine, and ranitidine by mkOAT3 at 3 min was used for further characterization.

The concentration dependence of the uptake of the H_2 receptor antagonists by mkOAT3-HEK was examined (Fig. 2). Their specific uptake by mkOAT3 consisted of one saturable component. The kinetic parameters are summarized in Table 1. The K_m values of the H_2 receptor antagonists for mkOAT3 were almost identical; however, the intrinsic transport activity (V_{max}/K_m) of cimetidine was greater than that of famotidine and ranitidine.

Effect of Probenecid on mkOAT3-Mediated Transport of Famotidine and Cimetidine. The inhibitory effect of probenecid on the mkOAT3-mediated transport of cimetidine and famotidine was examined (Fig. 3). Probenecid strongly inhibited the mkOAT3-mediated transport of cimetidine and famotidine in a concentration-dependent manner.

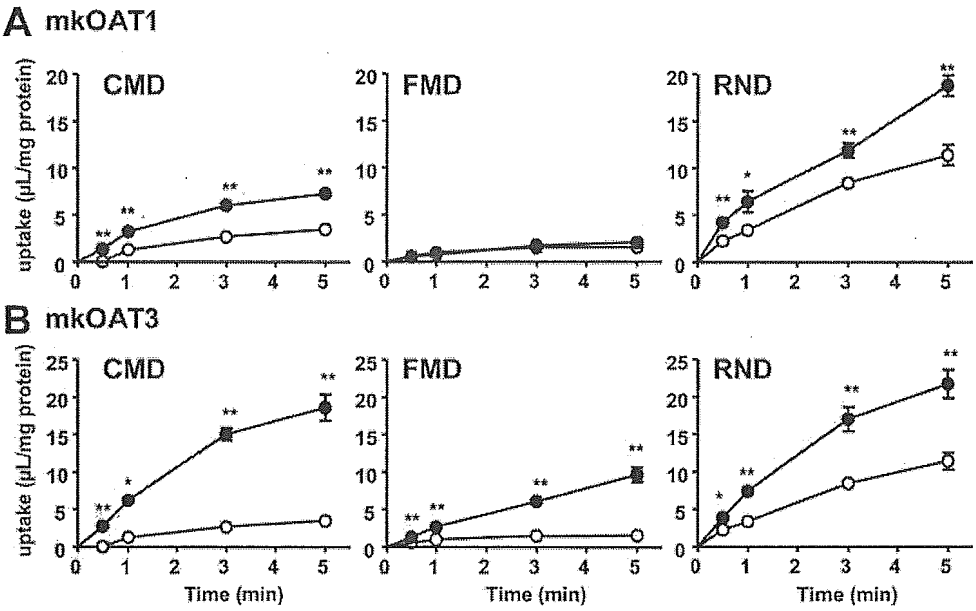

Fig. 1. Time profile of the uptake of H2-receptor antagonists by mkOAT1- and mkOAT3-HEK. The time-dependent uptake of cimetidine (CMD), famotidine (FMD), and ranitidine (RND) (10 μM) by mkOAT1- and mkOAT3-HEK was examined at 37°C. Closed and open circles represent the uptake by mkOATs-HEK and vector-HEK, respectively. Statistical differences in the uptake of mkOATs-HEK were compared with vector-HEK by a two-tailed unpaired Student's t test with $p < 0.05$ as the limit of significance (*, $p < 0.05$; **, $p < 0.01$). Each data point was connected with the rigid line and represents the mean ± S.E. ($n = 3$).

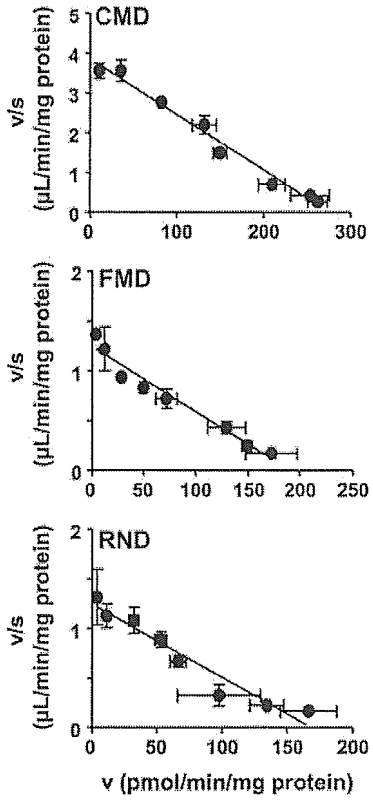

Fig. 2. Concentration dependence of the uptake of H2-receptor antagonists by mkOAT3-HEK. The concentration dependence of mkOAT3-mediated CMD, FMD, and ranitidine (RND) uptake is shown as Eadie-Hofstee plots. The mkOAT3-mediated uptake of cimetidine, famotidine, and ranitidine for 3 min was determined at different concentrations (3–1000 μM, range of concentration used). The mkOAT3-mediated transport was obtained by subtracting the transport velocity in vector-HEK from that in mkOAT3-HEK. The rigid line represents the fitted line obtained by nonlinear regression analysis as described under *Materials and Methods*. Each point represents the mean ± S.E. ($n = 3$).

The K_i values of probenecid for cimetidine and famotidine uptake by mkOAT3-HEK were determined to be 5.68 ± 0.78 and 2.97 ± 1.53 μM.

Semiquantitative Real-Time RT-PCR Analysis of Organic Cation Transporters. A method for the detection of monkey OCTs that combines reverse transcription with real-time RT-PCR was developed using specific primers designed from a nucleotide sequence of hOCT1, hOCT2, and hOCT3. The mRNA expression levels of putative mkOCT1, mkOCT2, and mkOCT3 in the liver were 121, 0.0640, and 0.0846, and those in the kidney were 0.0110, 1400, and 0.0879 (relative luminescence units per 50 ng of total RNA). The relative expression level of putative mkOCT1 was more than 10,000-fold higher in the liver than in the kidney, whereas that of putative mkOCT2 was more than 20,000-fold higher in the kidney than in the liver. In contrast, the expression level of putative mkOCT3 mRNA was comparable in the liver and kidney. Using these primer sets, electrophoretic analysis showed a single band of OCT1 [404 base pairs (bp)], OCT2 (430 bp), and OCT3 (419 bp) in monkey liver and kidney as well as human mixed cDNA (data not shown).

Effect of Probenecid on Pharmacokinetics of Famotidine in the Monkeys. The mean plasma concentration time profile of famotidine in cynomolgus monkeys is shown in Fig. 4A. The mean plasma pharmacokinetic parameters are summarized in Table 2. The urinary recovery over the 0- to 24-h collection period and the renal and tubular secretion clearances are included in Table 2. There was a significant difference between the probenecid-treated and untreated groups with regard to the pharmacokinetic parameters of famotidine, such as $AUC_{0-\infty}$, $V_{d,ss}$, CL_p, CL_{renal}, and CL_{sec}, but no significant difference in the $t_{1/2\alpha}$, $t_{1/2\beta}$, fp, and fe compared with the controls. The fp and fe in cynomolgus monkeys with or without probenecid treatment were determined as 74.4 ± 9.7 versus 70.3 ± 6.0% and 49.3 ± 13.4 versus 38.5 ± 10.9%. The plasma and renal clearances of famotidine was reduced to 50 and 65% of the control values, and the steady-state distribution volume was also reduced by probenecid. Probenecid completely blocked the renal tubular secretion of famotidine reducing it from 0.275 ± 0.075 to 0.0230 ± 0.0217 l/h/kg and concomitantly increased the total exposure (AUC) of famotidine by approximately 2-fold. The

TABLE 1

Kinetic parameters of the uptake of H_2-receptor antagonists by organic anion transporter 3

K_m and V_{max} were determined by nonlinear regression analysis as described under *Materials and Methods*. Data are taken from Fig. 2. Each value represents the mean ± computer-calculated S.D. The value in parentheses represents the relative transport activity with regard to cimetidine transport.

Isoform	Cimetidine			Famotidine			Ranitidine		
	K_m	V_{max}	V_{max}/K_m	K_m	V_{max}	V_{max}/K_m	K_m	V_{max}	V_{max}/K_m
	μM	*pmol/min/mg protein*	*μl/min/mg protein*	*μM*	*pmol/min/mg protein*	*μl/min/mg protein*	*μM*	*pmol/min/mg protein*	*μl/min/mg protein*
mkOAT3	70.9 ± 4.1	274 ± 9	3.86 (1)	154 ± 14	190 ± 11	1.23 (0.32)	125 ± 14	154 ± 12	1.27 (0.33)
hOAT3[a]	149 ± 35	1470 ± 230	9.86 (1)	124 ± 4	448 ± 10	3.61 (0.37)	234 ± 49	551 ± 49	2.35 (0.24)
rOat3[a]	90.7 ± 4.8	512 ± 17	5.64 (1)	345 ± 22	252 ± 12	0.73 (0.13)	155 ± 9	1660 ± 63	10.7 (1.90)

[a] Tahata et al., 2005a.

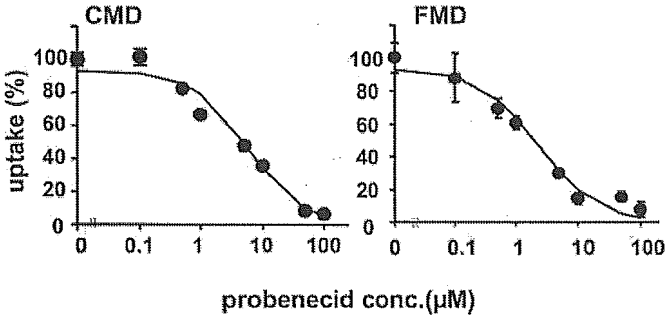

Fig. 3. Inhibitory effect of probenecid on the uptake of cimetidine and famotidine by mkOAT3-HEK. The uptake of CMD and FMD (10 μM) by mkOAT3 for 3 min was determined in the absence or presence of probenecid at the designated concentrations. The values were expressed as a percentage of cimetidine or famotidine transport by mkOAT3-HEK in the presence of probenecid versus that in the absence of probenecid. The rigid line represents the fitted line obtained by nonlinear regression analysis as described under *Materials and Methods*. Each point represents the mean ± S.E. (n = 3).

nonrenal clearance of famotidine was reduced from 0.445 ± 0.168 to 0.244 ± 0.029 l/h/kg, although this was not statistically significant ($p > 0.05$).

Effect of Probenecid on the Pharmacokinetics of Cimetidine in the Monkeys. The mean plasma concentration time profiles of cimetidine in the cynomolgus monkey are shown in Fig. 4B. The mean plasma pharmacokinetic parameters are summarized in Table 3. The urinary recovery over the 0- to 24-h collection period and the renal and tubular secretion clearances are included in Table 3. As observed in Fig. 4B and Table 3, probenecid had very little effect on the pharmacokinetics of cimetidine in the cynomolgus monkeys. Probenecid treatment produced no significant difference in any of the pharmacokinetic parameters of cimetidine. The fp and fe in cynomolgus monkeys with or without probenecid treatment were determined as 79.1 ± 4.8 versus 81.7 ± 2.0% and 37.0 ± 3.9 versus 36.3 ± 3.9%.

Plasma Concentration of Probenecid in the Monkey. The mean plasma concentration time profiles of probenecid in cynomolgus monkeys are shown in Fig. 4C. The maximum (at 1 h) and minimum (at 8 h) plasma concentrations of probenecid were 76.8 ± 10.1 μg/ml (269 μM) and 23.3 ± 2.9 μg/ml (81.8 μM), respectively. Taking the unbound fraction in the plasma (13.1 ± 0.3%) into consideration, the maximum unbound concentration of probenecid (35 μM) in the monkey study was comparable with that observed in the human study (ca. 46 μM) (Inotsume et al., 1990).

Prediction of Renal Clearance of Famotidine by Allometric Scaling. The renal clearance and renal tubular secretion clearance of famotidine in rats (Lin et al., 1987),

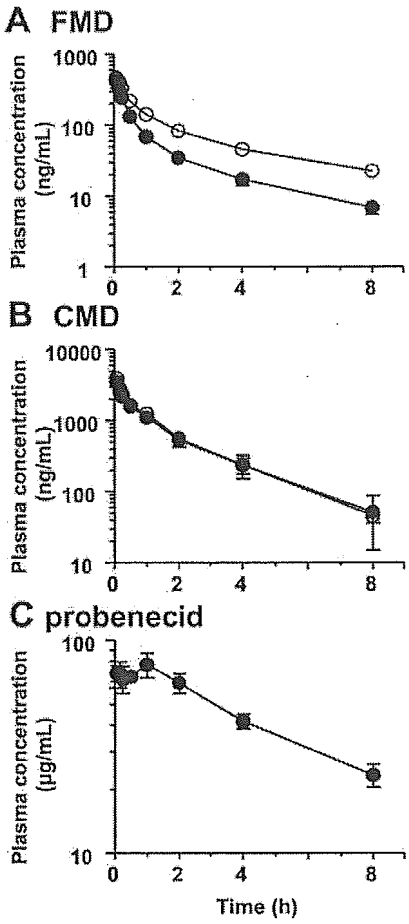

Fig. 4. Plasma concentrations of famotidine, cimetidine, and probenecid in the cynomolgus monkeys. Plasma concentrations of famotidine (A), cimetidine (B), and probenecid (C) in the cynomolgus monkeys after i.v. administration of famotidine and cimetidine at a dose of 0.3 and 4 mg/kg, respectively, following pretreatment with 22.5 mg/kg probenecid (open circles) or control (closed circles). Each data point was connected with the rigid line and represents the mean ± S.E. (n = 4).

dogs (Boom et al., 1997), and monkeys were analyzed as a function of species body weight (W) using the simple allometric equation for interspecies scaling and used to predict these parameters in humans. The corresponding allometric equations based on three species data were $CL_{renal} = 0.957 \times W^{0.710}$ and $CL_{sec} = 0.609 \times W^{0.679}$, respectively. The predicted values of CL_{renal} and CL_{sec} based on a 70-kg body weight in humans were 19.5 and 10.9 l/h, respectively, which were very similar to the observed values (13.3–18.2 and 8.31–13.2 l/h) (Inotsume et al., 1990; Gladziwa and Klotz, 1993).

TABLE 2

Pharmacokinetic parameters of famotidine in the cynomolgus monkeys

Pharmacokinetic parameters were determined by two-compartment model with nonlinear regression analysis as described under *Materials and Methods*. Each value represents the mean ± S.D. (n = 4). Data are taken from Fig. 4.

Probenecid	$t_{1/2\alpha}$	$t_{1/2\beta}$	$AUC_{0-\infty}$	CL_p	CL_{dis}	V_c	$V_{d,ss}$	CL_{renal}	CL_{sec}	$CL_{non\ renal}$
	h		*ng·h/ml*	*l/h/kg*		*l/kg*			*l/h/kg*	
Without	0.234 ± 0.058	2.69 ± 0.38	350 ± 48	0.870 ± 0.120	0.816 ± 0.116	0.573 ± 0.062	2.08 ± 0.28	0.426 ± 0.079	0.275 ± 0.075	0.445 ± 0.168
With	0.314 ± 0.087	3.58 ± 0.87	740 ± 80**	0.409 ± 0.044**	0.641 ± 0.100*	0.626 ± 0.141	1.60 ± 0.25*	0.165 ± 0.027**	0.0230 ± 0.0217*	0.244 ± 0.029
Ratio to control	1.4 (0.98–2.0)	1.3 (1.1–1.8)	2.1 (1.9–2.4)	0.47 (0.42–0.53)	0.79 (0.68–0.95)	0.96 (0.76–1.3)	0.77 (0.69–0.91)	0.36 (0.30–0.51)	0.078 (0.031–0.14)	0.61 (0.41–0.88)

Statistical differences were calculated between the probenecid-treated and untreated groups by two-tailed paired Student's t test with $p < 0.05$ as limit of significance (*, $p < 0.05$; **, $p < 0.01$).

TABLE 3

Pharmacokinetic parameters of cimetidine in the cynomolgus monkeys

Pharmacokinetic parameters were determined by two-compartment model with nonlinear regression analysis as described under *Materials and Methods*. Each value represents the mean ± S.D. (n = 4). Data are taken from Fig. 4. Statistical differences were calculated between the probenecid-treated and untreated groups by two-tailed paired Student's t test with $p < 0.05$ as limit of significance (*, $p < 0.05$; **, $p < 0.01$).

Probenecid	$t_{1/2\alpha}$	$t_{1/2\beta}$	$AUC_{0-\infty}$	CL_p	CL_{dis}	V_c	$V_{d,ss}$	CL_{renal}	CL_{sec}	$CL_{nonrenal}$
	h		*ng·h/ml*	*l/h/kg*		*l/kg*			*l/h/kg*	
Without	0.257 ± 0.122	1.70 ± 0.32	3970 ± 708	1.03 ± 0.17	1.27 ± 0.74	1.03 ± 0.19	1.97 ± 0.13	0.385 ± 0.061	0.222 ± 0.028	0.647±0.185
With	0.261 ± 0.117	1.64 ± 0.10	4140 ± 507	0.978 ± 0.123	1.13 ± 0.62	0.983 ± 0.187	1.82 ± 0.09	0.354 ± 0.066	0.216 ± 0.054	0.624 ± 0.148
Ratio to control	1.1 (0.80–1.8)	0.99 (0.75–1.2)	1.1 (0.99–1.1)	0.95 (0.91–1.0)	1.0 (0.49–1.6)	0.97 (0.78–1.2)	0.93 (0.79–1.0)	0.91 (0.84–1.0)	0.92 (0.86–1.0)	0.98 (0.82–1.2)

Discussion

In the present study, we examined whether the drug-drug interaction between famotidine and probenecid could be reproduced in monkeys. The transport activities of the H_2 receptor antagonists by mkOAT3 were compared with those by hOAT3, and the mRNA expression of the hOCT isoforms in the monkey kidney was quantified. To draw a definite conclusion, the effect of probenecid on the renal clearance of famotidine and cimetidine was examined in cynomolgus monkeys.

Famotidine was transported only by mkOAT3, whereas cimetidine and ranitidine are substrates of mkOAT1 and mkOAT3 (Fig. 1). The K_m values for mkOAT3-mediated uptake were similar to those for hOAT3 (Table 1). Previously, it had been shown that the uptake of cimetidine relative to the uptake of benzylpenicillin was similar for mk- and hOAT3 (Tahara et al., 2005b). This also holds true for famotidine uptake. In addition, the relative activity of famotidine exhibited by mkOAT3 was greater than that by rOat3, consistent with previous results in humans (Tahara et al., 2005a). These results support the hypothesis that the transport of the H_2 receptor antagonists by mkOAT3 is similar to that by hOAT3, rather than the rodent isoforms (Table 1).

OCT1 is the liver-specific isoform in humans, whereas it is expressed both in rodent liver and kidney (Motohashi et al., 2002; Slitt et al., 2002). This makes the contribution of organic cation transporters to renal uptake greater in rodents than in humans. Quantification of mRNA expression revealed that mkOCT1 and mkOCT2 are predominantly expressed in the liver and kidney, respectively, whereas mkOCT3 is expressed at considerably lower levels in these tissues. This expression patterns are similar to those in humans (Motohashi et al., 2002). Consequently, like human kidney, putative mkOCT2 plays a predominant role in the monkey kidney.

These results suggest that, as far as basolateral transporters are concerned, the monkey OATs and OCTs have similar characteristics to the human orthologs, and this prompted us to perform an in vivo study to obtain conclusive evidence. In monkeys, both famotidine and cimetidine are predominantly excreted into the urine, and the tubular secretion and glomerular filtrate rates that account for their renal clearance, are almost identical (Tables 2 and 3). When probenecid was given orally, the renal and renal tubular secretion clearance were reduced by 65 and 90%, respectively, resulting in a 2.0-fold increase in the AUC (Fig. 4A; Table 2). In addition, the steady-state distribution volume was reduced by 23% by probenecid. This is consistent with the previous findings in humans (Inotsume et al., 1990). It seems that the inhibition of the uptake by tissues, including the kidney, accounts for this effect. On the other hand, the plasma concentration and renal clearance or distribution clearance of cimetidine were not affected by probenecid (Fig. 4B; Table 3). Probenecid achieves a clinically relevant unbound concentration in monkey plasma (11–35 μM), which is sufficient to markedly inhibit mkOAT3, suggesting that the interaction could involve mkOAT3-mediated uptake, at least in part. Taking into account the degree of inhibition of tubular secretion clearance by probenecid, the probenecid-sensitive transporter, mkOAT3, plays a major role in the renal tubular secretion of famotidine, but not cimetidine, in monkeys. These results are consistent with the clinical studies (Gisclon et al., 1989; Inotsume et al., 1990). Consequently, monkeys, rather than rodents, can be used to predict drug-drug interactions involving tubular secretion, particularly when multiple transporters are involved.

The nonrenal clearance of famotidine was smaller in monkeys treated with probenecid than in the control group (Table 2). Although the difference was not statistically significant, it is likely that increasing the number of animals will make this difference statically significant. The presence of the oxidized metabolite of famotidine in human urine suggests that famotidine undergoes hepatic metabolism (Kroemer and Klotz, 1987). Because probenecid had no effect on OCT1, a candidate transporter responsible for the hepatic uptake of famotidine (Tahara eta al., 2005a), the reduction in the nonrenal clearance of famotidine by probenecid may be caused by inhibition of this metabolism. Currently, there is no report showing that probenecid has an inhibitory effect on metabolism, and this should be examined in future studies.

Interspecies scaling has been successfully used to predict human pharmacokinetic parameters from animals based on the concept of allometry (Lin, 1995; Reigner et al., 1997). It has been shown that simple allometric scaling of the renal clearance is a good predictor for drugs, such as methotrexate and several β-lactam antibiotics (Dedrick et al., 1970; Matsushita et al., 1990), although this is not the case for betamipron and enprofylline (Mahmood, 1998). The renal and renal tubular secretion clearances of famotidine in humans were estimated by simple allometric scaling using data from rats, dogs, and monkeys. A good predictability of the absolute values of the renal and renal tubular clearances from animal experiments (rat, dog and monkey) was observed, although the contribution of the renal transporters differs depending on the species. Therefore, good predictability by the allometric scaling cannot exclude the possibility of a species-dependent contribution by basolateral transporters. In particular, for the quantitative prediction of drug-drug interactions in humans, the contribution of transporters should be estimated using human materials. Alternatively, the relative activity factor method can be applied to predict the in vivo contribution of basolateral organic anion transporters using cDNA transfected cells (Hasegawa et al., 2003). In vivo studies in monkeys will further support the prediction of the occurrence of drug-drug interactions in humans. Nagata et al. (2004) found a drug-drug interaction involving rOat3 in the central nervous system (Nagata et al., 2004). Probenecid given as an i.v. constant infusion increased the concentrations of H_2 receptor antagonists (also given as an i.v. constant infusion) in the cerebrospinal fluid by inhibiting OAT3-mediated efflux at the choroid plexus. The possibility of drug-drug interactions with probenecid involving efflux transport across the barriers of the central nervous system can be also examined in monkeys in future studies.

In conclusion, the drug-drug interactions between the H_2 receptor antagonists (famotidine and cimetidine) and probenecid can be reproduced in monkeys. Hence, a combination of in vitro and in vivo studies could be a useful screening system for evaluating drug-drug interactions involving renal tubular transport in humans.

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INHIBITION OF OAT3-MEDIATED RENAL UPTAKE AS A MECHANISM FOR DRUG-DRUG INTERACTION BETWEEN FEXOFENADINE AND PROBENECID

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ABSTRACT:

AQ: A **Fexofenadine, a nonsedating antihistamine drug, is effective for the treatment of seasonal allergic rhinitis and chronic urticaria. Simultaneous administration of probenecid increases the plasma concentration of fexofenadine due to an inhibition of its renal elimination in healthy volunteers (*Clin Pharmacol Ther* 77:17–23, 2005). The purpose of the present study is to investigate the possibility that the drug-drug interaction between fexofenadine and probenecid involves the renal basolateral uptake process. The uptake of fexofenadine was determined in HEK293 cells expressing human organic anion transporter 1 (OAT1/*SLC22A6*), OAT2 (*SLC22A7*), OAT3 (*SLC22A8*), and organic cation transporter 2 (OCT2/*SLC22A2*). Only hOAT3-HEK showed a significantly greater accumulation of fexofenadine than that in vector-HEK, which was saturable with K_m and V_{max} values of 70.2 μM and 120 pmol/min/mg protein, respectively. Inhibition potency of probenecid for the uptake of fexofenadine was compared between hOAT3 and organic anion-transporting peptide 1B3 (hOATP1B3), a transporter responsible for the hepatic uptake of fexofenadine (*Drug Metab Dispos* 33:1477–1481, 2005). The K_i values were determined to be 1.86 and 282 μM for hOAT3 and hOATP1B3, respectively, with Hill coefficients of 0.76 and 0.64, respectively. The K_i value of probenecid for hOAT3, but not for hOATP1B3, was significantly lower than the maximum unbound plasma concentration of probenecid at clinical dosages. These results suggest that the renal drug-drug interaction between fexofenadine and probenecid is probably explained by an inhibition of the renal uptake of fexofenadine via hOAT3, at least in part.**

Fabb The kidney plays important roles in the detoxification of xenobiotics and endogenous wastes as well as maintaining stable levels of electrolytes and nutrients in the body. Urinary excretion consists of glomerular filtration in the glomeruli, tubular secretion across the proximal tubules, and reabsorption. Many studies have shown the importance of transporters in the tubular secretion of a large number of organic compounds, and a number of studies have described the role of multispecific organic anion and cation transporters (OAT/*SLC22* and OCT/*SLC22*) in the renal uptake of drugs. OCT2 (*SLC22A2*) plays a predominant role in the renal uptake of organic cations in the human kidney, whereas OCT1 plays a predominant role in the hepatic uptake of organic cations in the human liver (Koepsell, 2004; Lee and Kim, 2004; Wright and Dantzler, 2004). Three isoforms of the organic anion transporter family (OAT1/*SLC22A6*, OAT2/*SLC22A7*, and OAT3/*SLC22A8*) have been identified on the basolateral membrane of the human proximal tubules (Lee and Kim, 2004; Miyazaki et al., 2004; Wright and Dantzler, 2004). It has been suggested that hOAT1 plays an important role in the renal uptake of hydrophilic organic anions with a low molecular weight, whereas hOAT3 plays an important role in the renal uptake of amphipathic organic anions as well as a basic drug, famotidine (Hasegawa et al., 2003; Tahara et al., 2005a). The mRNA of hOAT2 in the kidney is markedly lower than that of hOAT1 and hOAT3 (Motohashi et al., 2002), and its role in drug transport in the kidney remains unknown. Identification of the basolateral transporters provides a clue to understanding the molecular mechanisms of drug-drug interactions involving tubular secretion. Takeda et al. (2002) and Nozaki et al. (2004) have shown that rOat3/hOAT3-mediated renal uptake can be a potential drug-drug interaction site with some nonsteroidal anti-inflammatory drugs at clinical dosages by comparing their K_i values for rOat3/hOAT3 with the unbound plasma concentration at their clinical dosages. In addition, we have reported that OAT3 could be the site of an interaction between famotidine and probenecid in humans (Tahara et al., 2005a).

Fexofenadine, an active metabolite of terfenadine, is a nonsedating histamine H_1 receptor antagonist that is prescribed for the oral treatment of allergic rhinitis and chronic idiopathic urticaria. After oral administration of [^{14}C]fexofenadine to healthy volunteers, 92% of the

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ABBREVIATIONS: OAT, human organic anion transporter; hOAT, human OAT; OCT, organic cation transporter; hOCT, human OCT; r, rat; hOATP, human organic anion-transporting peptide; AUC, area under the plasma concentration-time curve; HEK, human embryonic kidney; LC-MS, liquid chromatography-mass spectrometry.

total dose was recovered, 12% in urine and 80% in feces, as the unchanged form (Lippert et al., 1995). Since the average absolute oral bioavailability of fexofenadine was reported to be 33% (Dresser et al., 2005), about 36% of the bioavailable fexofenadine can be excreted into the urine during a 24-h period, and renal elimination makes a significant contribution to the total body clearance in addition to biliary excretion. Interactions of fexofenadine with drugs and food have been reported. The interactions with rifampicin (Hamman et al., 2001), St John's wort (Wang et al., 2002), and fruit juice (Dresser et al., 2002) caused a reduction in the AUC of fexofenadine after oral administration, and these are hypothesized to include modulation of P-glycoprotein or inhibition of OATP2B1 in the small intestine (Cvetkovic et al., 1999; Nozawa et al., 2004). The interactions with verapamil (Yasui-Furukori et al., 2005) and ketoconazole (Simpson and Jarvis, 2000) increased the AUC of fexofenadine, probably because of an increase in the oral absorption produced by inhibition of intestinal P-glycoprotein. Probenecid treatment caused a significant reduction in the unbound renal clearance of fexofenadine (Yasui-Furukori et al., 2005). Because probenecid is a potent inhibitor of OATs (Tahara et al., 2005a), it is possible that this interaction involves renal transporters, such as OAT1, OAT2, and OAT3.

In the present study, to obtain an insight into the basolateral uptake mechanism of fexofenadine, the uptake was determined in cDNA-transfected cells expressing hOAT1, hOAT2, hOAT3, and hOCT2, and the effect of probenecid on the uptake was determined to examine whether it is inhibited by a clinically relevant concentration of probenecid.

Materials and Methods

Fexofenadine hydrochloride was purchased from Toronto Research Chemicals (North York, ON, Canada). Ranitidine was purchased from Sigma-Aldrich (St. Louis, MO). [^{3}H] *p*-aminohippurate (151 GBq/mmol) was purchased from PerkinElmer Life Sciences (Boston, MA). [^{3}H]Benzylpenicillin (740 GBq/mmol) was purchased from GE Healthcare UK (Little Chalfont, Buckinghamshire, UK). All other chemicals and reagents were obtained from Kanto Kagaku (Tokyo, Japan) or Wako Pure Chemicals (Osaka, Japan) and were of the highest grade available.

The stable transfectants expressing hOAT1-, hOAT2-, hOAT3- (Tahara et al., 2005a), hOCT2- (Schlatter et al., 2002), and hOATP1B3-HEK (Shimizu et al., 2005) were established as described previously. These cells were grown in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and G418 sulfate (400 μg/ml) at 37°C with 5% CO_2 and 95% humidity on the bottom of a dish. hOAT1-, hOAT2-, hOAT3-, hOCT2-, and hOATP1B3-HEK were seeded in polylysine-coated 12-well plates at a density of $1.2 \times \sim 10^5$ cells/well. The transport activity by each cell line was confirmed by examining the uptake of ranitidine by hOAT1, hOAT2, hOAT3, and hOCT2.

Transport Studies. Transport studies were carried out as described previously (Tahara et al., 2005a). Uptake was initiated by adding medium containing a 10 μM concentration of the compounds after the cells had been washed twice and preincubated with Krebs-Henseleit buffer at 37°C for 15 min. The Krebs-Henseleit buffer consisted of 142 mM NaCl, 23.8 mM $NaHCO_3$, 4.83 mM KCl, 0.96 mM KH_2PO_4, 1.20 mM $MgSO_4$, 12.5 mM HEPES, 5 mM glucose, and 1.53 mM $CaCl_2$ adjusted to pH 7.4. The uptake was terminated at a designed time by adding ice-cold Krebs-Henseleit buffer after removal of the incubation buffer. Then, cells were washed twice with 1 ml of ice-cold Krebs-Henseleit buffer. For the determination of the uptake of fexofenadine, cells were dissolved in 300 μl of 0.2 N NaOH and kept overnight. Aliquots (150 μl) were transferred to vials after adding 30 μl of 1 N HCl. Aliquots (100 μl) were used for LC-MS quantification as described below. The remaining 10 μl of the aliquots of cell lysate were used to determine the protein concentration by the method of Lowry et al. (1951) with bovine serum albumin as a standard. Ligand uptake was given as the cell-to-medium concentration ratio determined as the amount of ligand associated with cells divided by the medium concentration.

Quantification of Fexofenadine by LC-MS. A sensitive method was developed to determine fexofenadine by high-performance liquid chromatography-electrospray ionization-mass spectrometry with midazolam as the internal standard (Tahara et al., 2005b). The LC-MS consisted of an Alliance HT 2795 separation module with an autosampler (Waters, Milford, MA) and a Micromass ZQ mass spectrometer with an electro ion spray interface (Waters). The optimum operating conditions used were as follows: electrospray probe (capillary) voltage 2.7 kV, sample cone voltage 35 V, and source temperature 100°C. The spectrometer was operated at a drying desolvation gas flow rate of 300 l/h. The mass spectrometer was operated in the selected ion monitoring mode using the respective MH^+ ions, *m/z* 502.3 for fexofenadine and *m/z* 326.3 for the internal standard. The mobile phase used for high-performance liquid chromatography was: methanol (A) and 0.05% formic acid (B). Chromatographic separation was achieved on a C_{18} column (Capcell pak C_{18}, MG, 4.6 mm i.d. × 75 mm, particle size 3 μm; Shiseido, Tokyo, Japan), using a linear gradient from 55% A to 70% A over 5 min and returning to 55% A within 2 min. The quantification limit of this method was 5 nM in the cell lysate. Instrument control and data analysis were performed using MassLynx application software from Waters.

Kinetic Analyses. Kinetic parameters were obtained using the Michaelis-Menten equation: $v = V_{max} \times S/(K_m + S)$, where v is the uptake rate of the substrate (pmol/min/mg protein), S is the substrate concentration in the medium (μM), K_m is the Michaelis-Menten constant (μM), and V_{max} is the maximum uptake rate (pmol/min/mg protein). To obtain the kinetic parameters, the equation was fitted to the uptake velocity using a MULTI program (Yamaoka et al., 1981). The input data were weighted as the reciprocals of the squares of the observed values. Inhibition constants (K_i) of several compounds were calculated assuming competitive inhibition using the following equation: $CL_{+Inh} = CL/(1 + (I/K_i)^S)$, where CL is the uptake clearance, I is the concentration of inhibitor (μM), and S is the Hill coefficient. The subscript (+inh) represents the value in the presence of inhibitor. The substrate concentration was low compared with its K_m value in the inhibition study. The two-tailed unpaired *t* test was used for a statistical analysis and a value of p less than 0.05 was considered significant.

Results

Time Profile of the Uptake of Fexofenadine by hOAT1-, hOAT2-, hOAT3-, and hOCT2-HEK. Figure 1 shows the time profiles of the uptake of the typical substrates and fexofenadine by hOAT1-, hOAT2-, hOAT3-, hOCT2-, and vector-HEK cells. Consistent with our previous report (Tahara et al., 2005a,c), the uptake of the typical substrates by the cDNA transfectants was significantly greater than that in vector-HEK. The uptake of fexofenadine by hOAT3-HEK was significantly greater than that in vector-HEK at all time points, whereas the uptake by hOAT1-, hOAT2-, and hOCT2-HEK was very similar to that of vector-HEK (Fig. 1). Since the uptake of fexofenadine by hOAT3-HEK increased linearly up to 5 min of incubation, the uptake of fexofenadine for 5 min was used for further characterization.

Concentration Dependence of the Uptake of Fexofenadine by hOAT3-HEK, and the Effect of Probenecid. The concentration dependence of the uptake of fexofenadine by hOAT3-HEK was examined (Fig. 2). The uptake was saturable, and the K_m and V_{max} values, determined by nonlinear regression analysis, were 70.2 ± 2.7 μM and 120 ± 3 pmol/min/mg protein, respectively. The inhibitory effect of probenecid on hOAT3-mediated uptake of fexofenadine was examined (Fig. 3). The K_i value of probenecid for the uptake of fexofenadine by hOAT3-HEK was determined to be 1.30 ± 0.30 μM with a Hill coefficient of 0.76.

Time Profile of the Uptake of Fexofenadine by hOATP1B3-HEK, and the Effect of Probenecid. As reported previously by Shimizu et al. (2005), the uptake of fexofenadine using the same hOATP1B3-HEK was greater than that by mock cells (7.59 ± 0.26 versus 3.97 ± 0.22 μl/mg protein at 5 min) (Fig. 4A). The K_i value of probenecid for the uptake of fexofenadine by hOATP1B3-HEK was determined to be 130 ± 40 μM with a Hill coefficient of 0.64 (Fig.

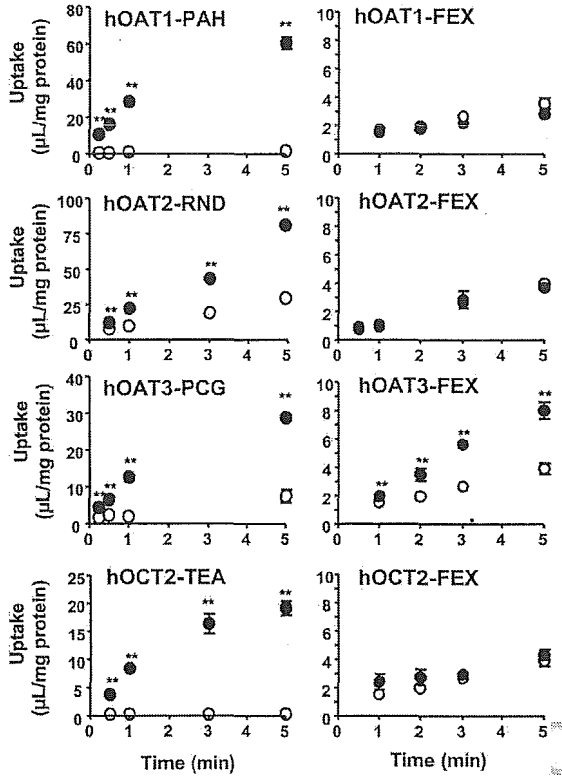

FIG. 1. Time profile of the uptake of typical substrates and fexofenadine by hOAT1-, hOAT2-, hOAT3-, and hOCT2-HEK. The time-dependent uptake of the typical substrates and fexofenadine (10 μM) by hOAT1-, hOAT2-, hOAT3-, and hOCT2-HEK was examined at 37°C. Closed and open circles represent the uptake by OATs/OCTs-HEK and vector-HEK, respectively. Statistical differences in the uptake of OATs/OCTs-HEK were compared with vector-HEK by a two-tailed unpaired t test with $p < 0.05$ as the limit of significance (*, $p < 0.05$; **, $p < 0.01$). Each point represents the mean ± S.E. ($n = 3$).

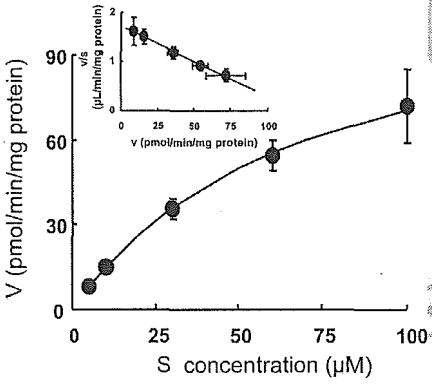

FIG. 2. Concentration dependence of the uptake of fexofenadine by hOAT3-HEK. The time-dependent uptake of fexofenadine (10 μM) by hOAT3-HEK was examined at 37°C. The concentration dependence of hOAT3-mediated fexofenadine uptake is shown as Eadie-Hofstee plots. The hOAT3-mediated uptake of fexofenadine for 5 min was determined at various concentrations (5–100 μM, range of concentrations used). The hOAT3-mediated transport was obtained by subtracting the transport velocity in vector-HEK from that in rOat3-HEK. Each point represents the mean ± S.E. ($n = 3$). Where bars are not shown, the S.E. is contained within the limits of the symbol.

4B). Probenecid is a 100-fold more potent inhibitor of hOAT3 than hOATP1B3.

Discussion

Fexofenadine is an orally active nonsedative histamine H1 receptor antagonist. Only a small amount of the orally administered [^{14}C]fexofenadine was recovered in the urine of healthy volunteers (12%), and urinary excretion has been considered to be a minor elimination pathway. However, the fact that the absolute oral bioavailability of fexofenadine is, on average, 33% means that a considerable amount of fexofenadine is excreted into the urine over a 24-h period (36% of the amount absorbed into the circulating blood) and suggests that renal elimination makes a significant contribution to the total clearance. The renal clearance of fexofenadine is greater than the glomerular filtration rate, indicating that tubular secretion accounts for the major part of the renal clearance (Table 1). Simultaneously administered probenecid caused an approximately 50% increase in the AUC of fexofenadine in healthy subjects, and this is largely explained by a 73% inhibition of the renal clearance of fexofenadine (Table 1) (Yasui-Furukori et al., 2005). In the present study, we examined the possible role of renal organic anion and cation transporters in the drug-drug interaction between fexofenadine and probenecid.

In cDNA-transfected cells, fexofenadine is efficiently transported only by hOAT3, whereas specific uptake by hOAT1, hOAT2, and hOCT2 was below the limit of detection, suggesting that hOAT3 plays a major role in the renal uptake of fexofenadine (Fig. 1). The transport activity of fexofenadine by hOAT3 was much lower than that of benzylpenicillin (1.71 versus 10.7 μl/min/mg protein). This was in good agreement with clinical data showing that the renal tubular secretion clearance of benzylpenicillin in healthy volunteers was 983 ml/min (Bins and Mattie, 1988), at least 9-fold higher than that of fexofenadine (113 ml/min, Table 1). Probenecid is a potent inhibitor

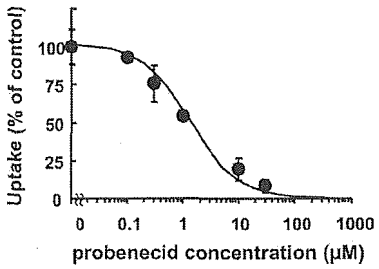

FIG. 3. Inhibitory effect of probenecid on the uptake of fexofenadine by hOAT3-HEK. The uptake of fexofenadine (10 μM) by hOAT3- and hOATP1B3-HEK for 5 min was determined in the absence or presence of probenecid at the designated concentrations. The values are expressed as a percentage of fexofenadine transport by hOAT3- or hOATP1B3-HEK in the presence of inhibitors versus that in the absence of inhibitors. The Hill coefficient value was 0.763 ± 0.047. Each point represents the mean ± S.E. ($n = 3$).

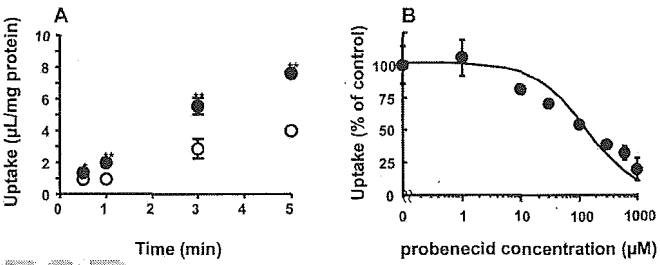

FIG. 4. Time profile of the uptake of fexofenadine by hOATP1B3 and the inhibitory effect of probenecid on the uptake of fexofenadine by hOATP1B3-HEK. The time-dependent uptake of fexofenadine (10 μM) by hOATP1B3-HEK was examined at 37°C. Closed and open circles represent the uptake by hOATO1B3-HEK and vector-HEK, respectively (A). The uptake of fexofenadine (10 μM) by hOATP1B3-HEK for 5 min was determined in the absence or presence of probenecid at the designated concentrations (B). The values are expressed as a percentage of fexofenadine transport by hOATP1B3-HEK in the presence of inhibitors versus that in the absence of inhibitors. The Hill coefficient value was 0.642 ± 0.079. Statistical differences in the uptake of hOATP1B3-HEK were compared with that by vector-HEK using a two-tailed unpaired t test with $p < 0.05$ as the limit of significance (*, $p < 0.05$; **, $p < 0.01$). Each point represents the mean ± S.E. ($n = 3$).

TABLE 1

Effects of cimetidine and probenecid treatments on pharmacokinetic parameters of fexofenadine

Parameter	Control	Cimetidine	Probenecid
$AUC_{(0-\infty)}$ (ng · h/ml)[a]	3637 ± 1199	4124 ± 2019	6150 ± 3972
Ratio to control	1	1.08	1.53
$CL_{renal,\ u}$ (ml/min)[a]	230 ± 78	152 ± 70	74 ± 52
Ratio to control	1	0.610	0.270
CL_{sc} (ml/min)[b]	133	55.0	0 (almost)
Ratio to control	1	0.414	0 (almost)
$I_{u,\ max}$[c]		5.20	24.0
K_i (K_m) for hOAT3		(113)	1.30
R[d]		0.956	0.0514

[a] $CL_{renal,\ u}$, unbound renal clearance (Yasui-Furukori et al., 2005).
[b] CL_{sc}, tubular secretion clearance ($CL_{renal,\ u} - CL_{creatinine}$). Creatinine clearance value was used for the value of glomerular filtration rate (97 ml/min; van Crugten et al., 1985).
[c] $I_{u,\ max}$, maximum unbound plasma concentration of inhibitor (Selen et al., 1982; van Crugten et al., 1985).
[d] R value was calculated according to the following equation: $R = 1/(1 + I_{u,\ max}/K_i)$.

of hOAT3, and the unbound plasma concentration of probenecid at clinical doses (0.5–2.0 g), ranging from 12 to 52 μM (Selen et al., 1982), is greater than its K_i value for hOAT3 (Table 1; Fig. 3). Therefore, probenecid will produce almost complete inhibition of hOAT3 in clinical situations, consistent with clinical reports, 73% inhibition of the renal clearance of fexofenadine by probenecid (Yasui-Furukori et al., 2005). Therefore, inhibition of basolateral uptake can be one of the sites of interaction between fexofenadine and probenecid. Cimetidine inhibits the renal clearance of fexofenadine by 39% on average in healthy subjects (Table 1). Since the clinical plasma concentration of unbound cimetidine at a dose of 400 mg was reported to be 5.2 μM (van Crugten et al., 1986), far below its K_m and IC_{50} values for hOAT3 [113 μM (Tahara et al., 2005c) and 92.4 μM (Khamdang et al., 2004), respectively], it is unlikely that the interaction involves hOAT3. Cimetidine may inhibit efflux process across the brush-border membrane of the proximal tubules. Although fexofenadine has been shown to be a substrate of P-glycoprotein (Cvetkovic et al., 1999; Tahara et al., 2005b), the steady-state plasma concentration was unchanged in Mdr1a/1b knockout mice (Tahara et al., 2005b), suggesting its limited role in the urinary and biliary excretion, and the transporter responsible for the luminal efflux remains unknown. Further studies are necessary to investigate whether the transporter responsible for the luminal efflux is another site of drug-drug interaction with probenecid and cimetidine.

The nonrenal clearance of fexofenadine is explained by biliary excretion. It was found that fexofenadine is a substrate of hOATP1B3, whereas the specific uptake of fexofenadine by OATP1B1 and OATP2B1 is very low (Shimizu et al., 2005). Based on quantitative prediction using the concept of a relative activity factor, hOATP1B3 has been suggested to play a major role in the hepatic uptake of fexofenadine (Shimizu et al., 2005). Our inhibition study revealed that probenecid is a weak inhibitor of hOATP1B3, with a K_i value greater than the unbound concentration achieved by a clinical dose (1 g) of probenecid (24 μM; Selen et al., 1982). Therefore, probenecid probably exhibits only a minimal inhibitory effect on the hepatic uptake of fexofenadine via hOATP1B3. This is consistent with the kinetic consideration that the drug-drug interaction is largely explained by a 73% inhibition of the renal clearance of fexofenadine.

The effect of probenecid on the total body clearance will be less potent since the contribution of the renal clearance of fexofenadine to the total clearance was smaller in rats (15–20%) (Kamath et al., 2005). There are two possibilities to account for this. One is the species difference in OAT3-mediated transport, i.e., basolateral uptake process since OAT3-mediated transport shows poor correlation between rat and human (Tahara at al., 2005c). The other is reabsorption mediated by Oatp1a1 in rats. Oatp1a1 is localized on the brush-border membrane of the kidney (Bergwerk et al., 1996), whereas its human homolog, OATP1A2, exhibits brain-specific distribution (Abe et al., 1999). Oatp1a1 has been suggested to be involved in the reabsorption of organic anions (Gotoh et al., 2002). Since fexofenadine is a substrate of Oatp1a1 (Cvetkovic et al., 1999), it is likely that it undergoes reabsorption from the lumen by Oatp1a1 in the kidney. Oatp1a1 expression exhibits gender difference, leading to the gender difference in the renal clearance of amphipathic organic anions (Gotoh et al., 2002). Female rats may be a better animal model to investigate the pharmacokinetics in humans.

The present study highlights the underlying mechanism of the drug-drug interaction with probenecid focusing on OAT3. Probenecid is also a potent inhibitor of OAT1, and its K_i value is smaller than the clinical unbound plasma concentration of probenecid. Therefore, both OAT1 and OAT3 can be a site of drug-drug interaction with probenecid. This is why probenecid causes a drug-drug interaction with a number of drugs in terms of renal elimination (Cunningham et al., 1981). Adefovir and cidofovir have been suggested to be taken up by the kidney via human OAT1(Ho et al., 2000; Mulato et al., 2000). They are nucleoside phosphonate analogs, a class of novel antivirals structurally related to natural nucleotides, and nephrotoxicity is their main dose-limiting toxic effect. Ho et al. (2000) and Mulato et al. (2000) have demonstrated that hOAT1 is directly involved in the induction of nephrotoxicity since the expression of hOAT1 sensitized a mammary cell line to adefovir and cidofovir, and probenecid reduced the cytotoxicity (Ho et al., 2000; Mulato et al., 2000). In such circumstances, combination with probenecid will have a beneficial effect in suppressing the nephrotoxicity as well as prolonging their plasma retention time, leading to an increase in the concentration in the liver, the target organ for the treatment of hepatitis B.

In conclusion, hOAT3 shows specific uptake of fexofenadine among basolateral transporters and accounts for its renal uptake. Probenecid is a potent inhibitor of hOAT3, and inhibition of hOAT3 is a likely mechanism to account for the increase in the AUC of fexofenadine caused by probenecid treatment in healthy subjects.

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