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Lecture

解　説

SNPとテーラーメイド医療*

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Key Words: SNR, tailor-made medicine, anti-cancer drug, pharmacokinetics, adverse drug reaction

はじめに

ヒトゲノムプロジェクトが終了し，約30億塩基対からなるヒトゲノム配列が明らかとなった現在では，解明されたゲノム情報をいかに利用するかが最重要課題となっており，医療においては，Single Nucleotide Polymorphism (SNP)をテーラーメイド医療に利用するための研究が進められている．本稿では，遺伝子多型と臨床的事象との関連解析にあまり馴染みのない方を対象に，簡単にSNPについて解説し，抗がん剤治療を中心にSNPの臨床的意義について紹介する．

SNPとは

DNAはヌクレオチド・ポリマーであるが，ヌクレオチドを構成する塩基には，アデニン，グアニン，シトシン，チミンの4種類があり，ゲノム配列を表すときには，それぞれのヌクレオチドをA, G, C, Tと表記する．ゲノム塩基配列中のある部位で，一塩基の置換が起き，そのために特定の集団で遺伝子の塩基配列に多様性がみられるときに，これを一塩基多型，Single Nucleotide Polymorphism (SNP)と呼ぶ．たとえば，ビリルビンのグルクロン酸抱合を触媒する酵素，UDP-グルクロン酸転移酵素1A1をコードする遺伝子*UGT1A1*の翻訳開始点の塩基から数えて211番目の塩基がGからAへ置換されたときには，このSNPを*UGT1A1* 211G>Aと表記する．このとき，UGT1A1は酵素の名前でもあるので，遺伝子を表すときには斜体で表し，酵素などの蛋白と区別をする．AからGへの置換が起きた場合には，置換部位がAの遺伝子とGの遺伝子は互いに対立遺伝子〔アレル(allele)ともいう〕の関係にあるが，集団の中で少数派の対立遺伝子の頻度が1％以上の場合をSNPと呼ぶことが多い．ヒトの30億個の塩基対の中で，SNPは平均して約1000塩基対に1か所あると言われており，約300万個存在することになる．

集団内の塩基配列の多様性のことを広く遺伝子多型という．遺伝子多型には，SNPのほかに，1塩基から数百塩基の挿入や脱落，variable number of tandem repeat (VNTR)またはミニサテライトVNTRと呼ばれる数塩基から数十塩基で構成される配列の繰返し数の変異，short tandem repeat polymorphism (STRP)またはマイクロサテライトVNTRと呼ばれる10個以下の塩基の配列が2から数十回反復するときの反復数の変異などがある．5フルオロウラシルの標的酵素であるthymidylate synthaseをコードする*TYMS*の転写調整領域には，28塩基からなる配列の繰返し数が，日本人では2～5回と変動するVNTRがあることが知られている[1]．

SNPと蛋白の機能変化

医療の場において，SNPが注目を浴びる理由は，特定のSNPが，薬物の代謝能や薬物に対する感受

* SNPs and tailor-made medicine.

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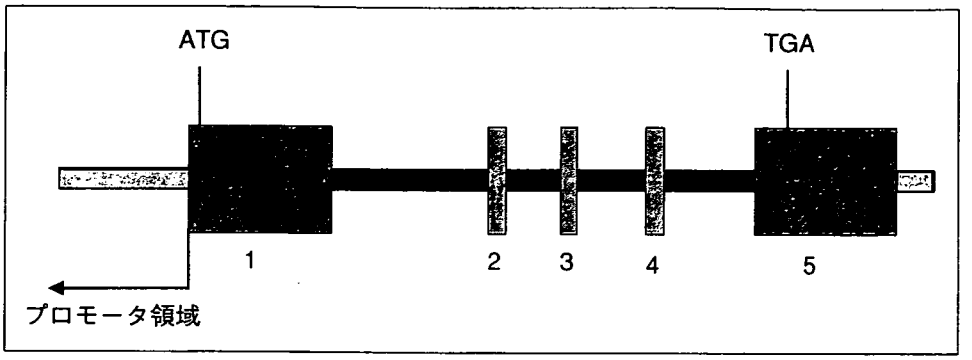

図1　遺伝子*UGT1A1*の構造
番号の振られた長方形はエクソンを表し，エクソンに挟まれた部分がイントロンを表す．エクソン1のATGは翻訳開始コドン，エクソン5のTGAは翻訳終止コドンを表す．エクソン1のATGより上流部分は5'-UTR(非翻訳領域)，エクソン5のTGAより下流部分は3'-UTRである．

性に影響を及ぼすことや，特定の疾患の発症のしやすさなどと関連することが知られるようになったからである．先の，*UGT1A1* 211G>Aの例では，父親と母親からともにAタイプの対立遺伝子を引き継ぎAAという遺伝子タイプをもつヒトは，両親からGとAを1本ずつ引き継ぎGAという遺伝子タイプをもつヒト，および，ともにGタイプの遺伝子を引き継ぎGGの遺伝子タイプをもつヒトに比較して，有意に血液中のビリルビン濃度が高く[2]，*UGT1A1* 211G>Aは高ビリルビン血症を伴ったGilbert症候群の原因SNPの一つである[3]．これは，*UGT1A1*の211番目の塩基がGからAに置換されることによって，翻訳されて生成した酵素の71番目のアミノ酸がグリシンからアルギニンに変わり(Gly71Argと表記する)，その結果，酵素活性が著しく低下したことによる．なお，AAのことをAのホモ接合，AGのことをAのヘテロ接合ともいう．

しかし，SNPは，常にこのような蛋白の機能変化を伴うとは限らない．遺伝子は，図1に示すように，mRNAに対応するエクソン(exon)，エクソンに挟まれた転写後スプライシングにより除かれるイントロン領域，および，上流の転写調節領域から構成され，蛋白の機能変化の有無はSNPの生じる位置に依存するからである．翻訳領域は，5'側と3'側の非翻訳領域(untranslated region：UTR)に挟まれており，5'-UTRは翻訳の調節や隣接する5'上流領域とともに転写調節に関わり，3'-UTRはmRNAの安定性[4]に関与していると言われている．アミノ酸置換が起きるのは，SNPが遺伝子中の翻訳領域に位置する場合である．蛋白は，DNAから転写されて生成したmRNAが，3つの塩基配列ごとに1つのアミノ酸に翻訳されて生成されていく．この3つの塩基配列の組合わせがコドンと呼ばれる遺伝子暗号で，コドンには翻訳停止の暗号も含まれる．*UGT1A1*のSNPでは，211番目の塩基を含むコドンGGAがAGAに変化したためにコードされるアミノ酸がグリシンからアルギニンに変わった．アミノ酸の変異の位置が蛋白の機能を発揮するために重要な部位であるときには蛋白機能が変化し，そうでないときには機能に変化がみられない．一方，塩基置換後のコドンが終止コドンとなることもあり，この場合には，正常な長さの蛋白が作られず，機能を有する蛋白の欠損を招く．なお，生成した蛋白の機能変化の有無にかかわらず，アミノ酸の置換を伴うSNPをnon-synonymous SNPという．1つのアミノ酸は2つ以上のコドンと対応していることが多いので，翻訳領域のSNPでもアミノ酸置換を伴わないことがあり，これをサイレントSNPあるいはsynonymous SNPと呼ぶ．

転写調節領域には，RNAの転写調節に関与する部位があり，この領域のSNPは生成した蛋白の機能には影響を与えないが，mRNAの発現量と蛋白の発現量に影響を与えることがある．3'-UTRは，mRNAの安定性に関与していると言われているので，この領域のSNPは蛋白の発現量に影響を及ぼす可能性がある．スプライシングで取り除かれるイントロンに位置するSNPが，スプライシ

表1 主な代謝酵素の機能変化を伴うSNPの頻度の人種差

遺伝子	対立遺伝子	塩基置換	アミノ酸置換または機能変化	対立遺伝子の頻度		
				黒人	白人	アジア人
CYP2C9	*2	430C>T	Arg144Cys	0.02-0.04	0.10-0.15	ND
	*3	1075A>C	Ile359Leu	0.01-0.02	0.05-0.10	0.01-0.04
CYP2C19	*2	681G>A	スプライシング異常	0.11-0.25	0.13-0.19	0.21-0.45
	*3	636G>A	Trp212stop	0-0.02	0-0.003	0.05-0.13
CYP3A4	*16	554C>G	Thr185Ser	−	−	0.01-0.05
CYP3A5	*3	IVS3-237A>G	スプライシング異常	0.06-0.84	0.85-0.95	0.61-0.82
UGT1A1	*6	211G>A	Gly71Arg	ND	ND	0.01-0.24

ND：検出されず （文献[5]より引用）

ングの異常を招き，結果として，機能を有する蛋白が生成されずに活性が損なわれることもある．

人種差

SNPを含む遺伝子多型の種類と頻度には人種差があることが知られている．チトクロームP450(CYP)とUGT1Aは，市販されている薬物の大半の代謝を担っており，これらの酵素をコードする遺伝子の種類と頻度については，Saitoらの総説に詳しく述べられている[5]．この中から，*UGT1A1*と*CYP*のいくつかのサブファミリーで，機能が変化することが知られている代表的なSNPについて，白人，黒人，アジア人における頻度を表1にまとめた．

*CYP2C9*のnon-synonymous SNPである430C>Tおよび1075A>Cは，野生型に比較して著しく機能が低下する[6]．白人においては，*CYP2C9*2*(430C>T) 保有者の頻度が高く，抗血液凝固剤のワーファリンや抗糖尿病薬のグリメピリドなどのクリアランス(薬物の消失速度と体内の薬物濃度を関連づける比例定数)の低下を招くとして問題視されているが，アジア人ではこのSNPはほとんど検出されず，むしろ，*CYP2C9*3*(1075A>C)によるこれら薬物のクリアランス低下が問題になっている．

CYP2C19はプロトンポンプ阻害剤のオメプラゾールを水酸化する酵素である．*CYP2C19*2*(681G>A)および*CYP2C19*3* (636G>A)の産物は，ともに酵素活性をまったく示さず，これらのSNPの保有者はCYP2C19によって代謝される薬物のプア・メタボライザー(PM)である[7]．ピロリ菌除菌治療においては，抗生物質による除菌効果を高めるために胃内のpHを高く保つことを目的としてオメプラゾールが使用されるが，*CYP2C19*2*の保有者では，このSNPをもたない患者よりもオメプラゾールのクリアランスが低いために，高いピロリ除菌効果を得られる[8]．そこで，ピロリ除菌治療においては，*CYP2C19*のタイプに応じてオメプラゾールの投薬量を調節するテーラーメイド医療の試みが始まっている[9]．*CYP2C19*2*および*3*の頻度は白人よりも日本人において高く，ピロリ除菌達成率は日本人の方が高い[10]．

抗うつ剤などの代謝に関与するCYP2D6の遺伝子多型は，全遺伝子の欠損(*5)から遺伝子全体の重複などがあり非常に複雑である．白人のSNPとしては，スプライシングに異常をきたす*CYP2D6*4*(1846G>A)が有名であるが，東アジア人では機能が低下するSNPとして*CYP2D6*10*〔100C>T(Pro34Ser)〕が多く見受けられる[5]．

非常に広範な薬物代謝に関与すると言われているCYP3A4の遺伝子には，機能が変化するSNPで頻度の高いものはないが，日本人では*CYP3A4*16*(554C>G)と呼ばれる低活性をひき起こすSNPが知られている[5]．一方，*CYP3A5*には，イントロン3にSNP(IVS-237A>G)があり，Gタイプはスプライシングに異常をきたす結果CYP3A5の酵素が発現しない．CYP3A5とCYP3A4の基質特異性が類似していることから，薬物によってはCYP3A活性の個人間変動に*CYP3A5*3*(IVS-237A>G)が大きく関与している場合もあり得る[11]．白人とアジア人では，酵素欠損を招く*CYP3A5*3*の頻度は非常に高いが，黒人では民族差が著しい．

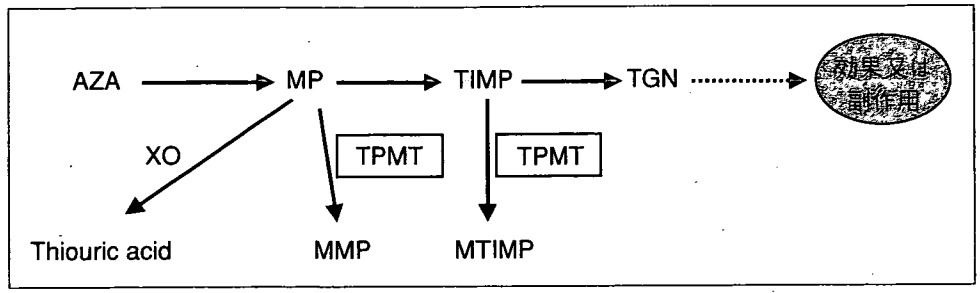

図2　アザチオプリンおよびメルカプトプリンの代謝経路
AZA：アザチオプリン，MP：メルカプトプリン，TIMP：thioinosine monophosphate, TGN：thioguanine nucleotide, MMP：メチルメルカプトプリン，MTIMP：methyl-TIMP, XO：xanthine oxidase, TPMT：thiopurine S-methyltransferase
（文献[12]より引用改変）

代謝酵素に限らず，トランスポータや薬物の薬理作用に関連する遺伝子についても，人種が異なればSNPの位置も頻度も違ってくるために，テーラーメイド医療においてSNPのタイプ診断を行う場合には，人種ごとの対応が迫られる．

抗がん剤による薬物治療とSNP

Peutz-Jeghers症候群，Cowden症候群，Li-Fraumeni症候群のように，一部の乳がんや大腸がんでは，がん易罹患性遺伝子との関連がつけられており，また，現在もさまざまな遺伝子の多型とがんの発症のしやすさと関連が研究されているが，本稿では紙面の関係でこれらを割愛し，次に，抗がん剤治療とSNPとの関連について，簡単に紹介する．

小児急性リンパ性白血病の治療に使用されるメルカプトプリン，および，クローン病などの治療に用いられる免疫抑制剤アザチオプリンは，図2に示すような代謝経路で活性化を受け，また，解毒化される[12]．チオプリンS-メチルトランスフェラーゼ(TPMT)はメルカプトプリンの解毒化を担っているが，TPMTの活性が低下すると，造血系にthioguanine nucleotide(TGN)が蓄積し白血球数減少症など重篤な副作用が生じる[13]．*TPMT*にはいくつかのSNPが知られているが[14]，日本人におけるTPMT活性の低下の主たる原因は，エクソン10のSNP, *TPMT*3C*(719A>G, Tyr240Cys)であり，その頻度は0.8%である[15]．一方，白人では，*TPMT*3A*(460G>A, Ala154Thr)が主流であり，その頻度は約7.5%である[14]．米国におけるメルカプトプリンの添付文書には，メルカプトプリンの薬物動態および副作用が*TPMT*の遺伝子多型と関連があること，活性欠損型*TPMT*をホモ接合で有する患者では通常の投与量では毒性が強く現れ投与量を減らすことが必要であることが記されているが，このような患者への初期投与量は確立されていないとも記述されている．活性欠損型*TPMT*をヘテロ接合で有する患者は，通常の投与量にほぼ耐えられるが，時に減量が求められることもある，としている．アザチオプリンの添付文書もFDAのホームページ上で見ることができるが，そこには活性欠損型*TPMT*をホモ接合で有する患者ではアザチオプリン以外の薬物による治療を考えた方がよいと記されており，メルカプトプリンの添付文書に比べ，*TPMT*の遺伝子多型について一歩踏み込んだ表現となっている．日本では，アザチオプリンの添付文書ではTPMT活性欠損への注意喚起がなされているが，メルカプトプリンの添付文書ではTPMT活性欠損への言及がなされていない．

次に著者らが国立がんセンターと共同で行ったイリノテカン[16]およびゲムシタビン[17]の薬物動態および副作用とSNPとの関連解析の結果を紹介する．

消化器がんや肺がんの治療に用いられるイリノテカン(CPT-11)はプロドラッグで，体内で加水分解されて生成するSN-38がトポイソメラーゼI阻害作用を有する．SN-38はUGT1A1によってグルクロン酸抱合を受け解毒化されるので，UGT1A1の活性はCPT-11の重篤な副作用の発生と強い相関を示す．*UGT1A1*の遺伝子多型では，転写調節領域のTATAボックス内の変異*UGT1A1*28*が有名であるが，白人および黒人に比較して，日本

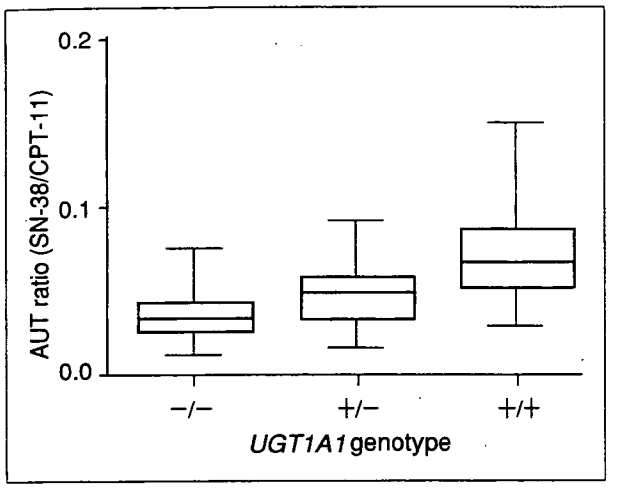

図3 *UGT1A1*のTATAボックスおよび211番目の塩基に基づく遺伝子タイプとイリノテカンの薬物動態，好中球減少症発現との関連

TATAボックスにおける多型をTAの繰返し数に基づきTA6およびTA7で，211番目の塩基の多型を211Gおよび211Aで表す（下線を施された方が頻度が低い対立遺伝子である）．詳細に検討したところ，同一染色体上での2箇所の多型の組合わせは，(TA7-211G)，(TA6-211A)または(TA6-211G)のいずれかであった．(TA7-211G)および(TA6-211A)の組合わせを「＋」で，(TA6-211G)の組合わせを「−」で表すと，ヒトは両親より1本ずつの染色体を引き継いでいるので，2か所の多型の組合わせに基づく遺伝子タイプにより，患者を＋/＋グループ，＋/−グループおよび−/−グループに分けることができる．（文献[16]より引用改変）

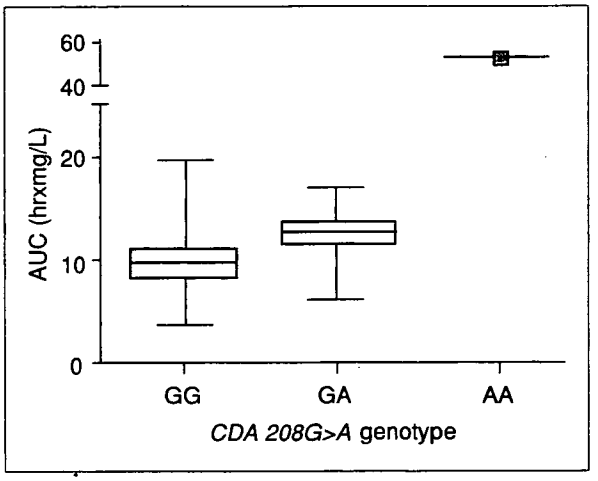

図4 *CDA 208G>A*に基づく遺伝子タイプとゲムシタビンの薬物動態との関連

人における*UGT1A1*28*の頻度は低いことが報告されている．これに代わって，冒頭に紹介した*UGT1A1*6*と命名されている*UGT1A1* 211G>Aは，アジア人にほぼ固有のSNPで(表2)，日本人における頻度は*UGT1A1*28*の頻度に匹敵する．日本人のがん患者177人を対象にした著者らの研究[16]では，TATAボックスの多型と211番目の塩基の多型の組合わせに基づいて患者を3つのグループに分け，CPT-11の薬物動態と血液毒性に及ぼす影響を検討した．**6*または**28*を有する遺伝子タイプを「＋」で表すと，図3に示す通り，＋/＋のグループでは，それ以外のグループよりも，活性代謝物のSN-38のCPT-11に対するAUC(薬物血中濃度－時間曲線下面積)比が高かった．また，CPT-11の単剤投与を受けた患者では，グレード3以上の好中球減少症の発生率が，−/−と＋/−を合わせた群では20%(総数50)であるのに対し，＋/＋群では80%(総数5)と，後者で有意に上昇した($P<0.05$)．*UGT1A1*6*の薬物動態や副作用に対する同様の影響については，韓国人を対象とした試験でも報告されている[18]．米国におけるイリノテカンの添付文書には，「*UGT1A1*28*をホモ接合で有する患者では好中球減少のリスクが高まるために投与量を減らすことが必要」と記述されている．日本においても近い将来にイリノテカンの添付文書に遺伝子多型に関する記載が追加されると予想されるが，日本においては，欧米人と異なり，*UGT1A1*6*に対する記述も必要である．

膵がん治療の第一選択薬であるゲムシタビンは，基本的に外来での投与が可能で，比較的副作用が少ない抗がん剤であるが，時として重篤な副作用を招くことがある．ゲムシタビンはシチジンデアミナーゼ(CDA)によって脱アミノ化を受け解毒される．著者らが行ったがん患者256人を対象にした研究結果から[17]，*CDA*のSNP，208G>A(Ala70Thr)が検出され，このSNPは脱アミノ活性が低下しており，ゲムシタビンのAUCの上昇を招くことが判明した(図4)．ヘテロ接合で*CDA* 208G>Aを有する患者では，ゲムシタビンと他の抗がん剤との併用療法時にグレード3以上の白血球数減少症の発生頻度が有意に上昇し，ホモ接合の患者ではAUCがこのSNPを有しない患者の平均値の5倍に達し，重篤な副作用を発現した．なお，*CDA* 208G>Aの日本人における頻度は4%で，白人では検出されず，一部地域の黒人では日本人よりも高い頻度で検出される．

分子標的治療薬として開発されたゲフィチニ

ブの有効性に，非小細胞肺がんの細胞中の上皮成長因子受容体(EGFR)の変異が強く関連していることが2004年に3つのグループから独立して報告された[19)~21)]．彼らは*EGFR*中の3箇所の遺伝子多型がゲフィチニブの有効性と関連すると報告したが，そのうちの2つは1塩基が置き換えられたアミノ酸変異を伴う体細胞変異である(G719S, L858R)．これらはがん細胞における変異であるが，アジア人ではこれらの変異を有する患者の割合が白人よりも高く，ゲフィチニブの効果が得られやすいと報告されている[20)]．

おわりに

このように，遺伝子のタイピングによって，抗がん剤の副作用を発生しやすいリスクが高い集団を識別する方法や，奏効群を識別するデータが蓄積されつつある．今後は，ハイリスク集団に対する適切な投与量の設定や，遺伝子タイプに基づく適切な抗がん剤の選択法の確立に向けて，前向きの臨床試験を実施していかなければならないと考える．

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原著

Effect of low-dose macrolide antibiotics on theophylline disposition in pediatric patients

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Summary: Low-dose macrolide antibiotics are administered for the treatment of certain chronic inflammatory sinopulmonary diseases (e.g., sinusitis and panbronchiolitis). Because 14-membered ring macrolide antibiotics administered at antimicrobial doses have been suggested to elicit a significant inhibitory effect on the hepatic metabolism of theophylline, we studied whether low-dose macrolide antibiotic therapy also evokes a clinically relevant alteration in the disposition of theophylline. The steady-state serum theophylline concentration and the urinary concentrations of theophylline and its metabolites [i.e., 1-methyluric acid (1MU), 3-methylxanthine (3MX), 1,3-dimethyluric acid (DMU), and caffeine] were examined in nine stable asthmatic children who received both theophylline and low-dose erythromycin or clarithromycin (12.3 ± 4.5 mg/kg body weight/day) and in ten children who received theophylline alone. An immunoassay and high-performance liquid chromatography with ultraviolet detection (HPLC-UV) were used for serum and urinary drug assay, respectively. Results demonstrated no significant differences in serum theophylline concentration, the metabolic clearance of theophylline to its metabolites, and the renal clearance of theophylline between the two groups. In conclusion, the administration of low-dose macrolide antibiotics for the treatment of sinopulmonary diseases would not stipulate a dosage reduction of concomitantly administered theophylline in pediatric patients

小児患者のテオフィリン体内動態におよぼす少量マクロライド療法の影響

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要旨：少量マクロライド療法は副鼻腔炎や汎細気管支炎のような慢性気道炎症に対して行われる。14員環マクロライド抗菌薬を治療量で投与するとテオフィリンの肝代謝が有意に阻害されるが，我々は少量マクロライド療法もテオフィリン代謝に臨床的に影響のある変化を及ぼすのかどうかを検討した。テオフィリンと少量マクロライド抗菌薬（12.3 ± 4.5 mg/kg/日）を併用した非発作時の気管支喘息患児９名とテオフィリン単独投与の患児10名を対象として，定常状態でのテオフィリン濃度

とテオフィリンおよびその代謝産物尿中濃度を測定した。血中および尿中濃度はそれぞれ免疫測定法，高速液体クロマトグラフ法で測定した。両群間でテオフィリン血中濃度，テオフィリンの代謝クリアランス，腎クリアランスに有意差はなかった。よって，小児の呼吸器疾患に対する少量マクロライド療法を行う際，併用するテオフィリン投与量の減量は必要ないと判断された。

Key word : theophylline, urinary theophylline metabolites, low-dose macrolide antibiotics, drug interaction, asthma

Introduction

Macrolide antibiotics are commonly prescribed for the treatment of upper and lower respiratory tract infections caused by various pathogenic organisms at oral doses of 30-50 mg/kg body weight/day in children or 1-2 g/day in adults[1]. In addition, a long-term low-dose (e.g., approximately 50% less than the standard anti-infective dose) administration of these drugs is considered an important adjunct in the treatment of various upper and lower respiratory tract diseases (e.g., chronic sinusitis, diffuse panbronchiolitis, bronchiectasis, and cystic fibrosis) owing to their remarkable effects of promoting and sustaining the tissue reparative process in inflammation[2]. Possible mechanisms underlying these distinct actions of macrolide antibiotics may be associated with the down-regulation of the host inflammatory response to tissue injuries[3,4].

There is concern about possible drug interactions in low-dose macrolide antibiotic therapy, because 14-membered ring macrolide antibiotics (e.g., erythromycin, and clarithromycin) at standard doses interfere with the hepatic metabolism of many drugs[5]. In particular, their interaction with theophylline is of clinical interest, because theophylline has a rather narrow therapeutic range (10-20 μg/mL) and its toxicity could be potentially fatal[6]. To our knowledge, however, there is a paucity of information on whether low-dose macrolide antibiotic therapy is associated with a clinically relevant drug interaction with theophylline in pediatric patients.

Theophylline undergoes extensive hepatic metabolism; it is converted to 1, 3-dimethyluric acid (DMU) by cytochrome P450 1A2 (CYP1A2), 2E1, and 3A4, and to 3-methylxanthine (3MX) and 1-methylxanthine by CYP1A2[7,8]. 1-Methylxanthine is subsequently oxidized to 1-methyluric acid (1MU) by xanthine oxidase[7]. Because these metabolites are recovered in urine, the metabolic clearance of the metabolites of theophylline serve as useful indices of the in vivo activity of distinct CYP isoforms involved in the formation of respective metabolites[9]. In this context, we determined whether low-dose macrolide antibiotics alter the systemic disposition of theophylline and the urinary indices of CYP isoform activity in pediatric patients. Here, we present that low-dose macrolide antibiotics can be safely administered without clinically relevant interference with theophylline disposition.

Patients and study design

Eighteen pediatric patients (14 males and 4 females) who were admitted to Showa University Hospital owing to an acute exacerbation of asthma or bronchitis participated in the study. After their asthmatic symptoms had subsided, they received a sustained-release formula of theophylline (Theodur®) for a round-the-clock theophylline therapy. When the study was performed, none of the patients had a body temperature of 37.5℃ or higher, or crackles or wheezing upon auscultation. None exhibited any signs or symptoms indicative of congestive heart failure. The patients were divided into

two groups according to their clinical conditions: nine of the 18 patients who exhibited persistent signs and symptoms of sinusitis received low-dose macrolide therapy (the macrolide-antibiotic-treated group) and the remaining patients were administered theophylline alone (the control group). One patient (No. 5) initially assigned to the control group was subsequently placed into the macrolide-antibiotic-treated group when she was admitted to the hospital owing to asthma attacks with signs of sinusitis. Another patient (No. 7) was considered to have chromosomal anomaly of trisomy 21. The macrolide antibiotics (i.e., erythromycin for 8 patients and clarithromycin for one patient) were administered orally twice daily. Clarithromycin and erythromycin were in dry syrup formulation (Clarith®) and dry syrup as ethylsuccinate (Erythrocin®), respectively. During the study period, the patients received no other medications that might have affected the disposition of theophylline (e.g., anticonvulsants, rifampicin, and quinolone antibiotics). None of the patients exhibited abnormal liver or renal function as determined by routine blood biochemistry (Table 1). None showed complications of clinical conditions that may have altered theophylline clearance (e.g., dehydration, hypoxia, acidosis, viral infections, and acute febrile illness)[6) 10) 11)]. Informed consent was obtained from either one or both parents of each child before enrollment in the study. The study was approved by the ethics committee of Showa University School of Medicine.

Blood and urine samples for the measurement of theophylline or its metabolites were obtained from the children at least 3 days after the initiation of theophylline therapy to ensure the attainment of the steady-state. Blood samples were drawn 3.5 - 6 hours after the oral administration of theophylline. Serum theophylline concentration was measured by fluorescence polarization immunoassay (TDX®, Abbott Laboratories, Chicago) in the Clinical Biochemistry Laboratory, Showa University Hospital. Because of difficulties in collecting urine samples from children, the collection periods of urine samples varied among the patients. Nonetheless, urine volume was measured and a portion of each urine sample was stored at −30℃ before performing the assay. The urinary concentrations of theophylline, 1-methyluric acid (1MU), 3-methylxanthine (3MX), 1,3-dimethyluric acid (DMU), and caffeine were measured as follows. To a 50-μL urine sample, 300 μL of 0.01 M acetate buffer (pH 4.0) and 50 μL of 1 mg/mL β-hydroxyethyltheophylline (IS) were added. Then, the sample was extracted with a 5-mL mixture of ethylacetate/2-propanol (93/7, vol/vol). The organic layer was transferred to another glass tube, and then evaporated to dryness under vacuum. The residue was reconstituted with a 200-μL mixture of 0.01 M acetate buffer (pH 4.0)/methanol (92/8, vol/vol), and a portion (20 μl) of the reconstituted solution was injected onto an HPLC (high-performance liquid chromatography) column. The HPLC system constituted of a pump (L-7100, Hitachi, Tokyo, Japan), an automatic sample injector (L-7200, Hitachi), a C18 reversed-phase column (5 μm, Capcell Pak UG120, 150 x 4.8, i. d., Shiseido, Tokyo, Japan), a UV detector (L-7400, Hitachi) set at 273 nm, and a chromato-integrator (L-7500, Hitachi). The mobile phase was a mixture of 0.01 M acetate buffer (pH 4)/methanol (92/8, vol/vol) and was delivered at a flow rate of 1 mL/min. The column temperature was maintained at 30 oC using a temperature-controlled water bath. The recoveries of theophylline and its metabolites from urine were > 92%, and within- and between-day coefficients of variation (CV) were < 5% and < 7%, respectively. The partial (fractional) metabolic clearance of theophylline to the respective metabolites was calculated by dividing the urinary excretion rates of the metabolites during the respective urine collection period by the serum theophylline concentration

Table 1. Demographic data and serum data of pediatric patients who received theophylline with and without low-dose macrolide antibiotics

Patient	Gender	Age (years)	Weight (kg)	AST (IU/L)	ALT (IU/L)	Cr (mg/dL)	Theophylline Dose (mg/kg/day)	Theophylline Serum conc. (μg/mL)	Theophylline Sampling time (hr postadministration)
Those who received theophylline alone									
1	female	0	5.9	38	27	0.2	13.6	13.8	4.5
2	male	1	12.4	30	14	0.3	11.3	7.2	4.3
3	male	1	9.4	25	11	0.2	10.6	3.7	4.1
4	male	2	9.1	46	32	0.2	13.2	11.3	4.1
5-1	female	2	12.8	25	13	0.2	12.5	13.5	4.2
6	male	4	19.6	20	9	0.4	7.6	9.4	4.5
7	male	5	13.1	24	14	0.4	9.2	7.4	4.5
8	male	6	25.6	22	13	0.3	9.8	8.3	5.0
9	male	6	19.2	14	7	0.3	12.5	10.1	5.3
10	female	7	27.6	17	7	0.4	10.9	11.9	5.5
	M/F = 7/3								
mean ± S.D.		3.4 ± 2.5	15.5 ± 7.2	26.1 ± 9.7	14.7 ± 8.3	0.3 ± 0.1	11.1 ± 1.9	9.7 ± 3.1	4.6 ± 0.5
Those who received theophylline and low-dose macrolide antibiotics									
11	male	1	11.0	38	12	0.3	10.9	5.8	3.8
12	male	1	10.2	28	12	0.2	11.8	10.9	3.9
13	male	1	11.4	28	10	0.2	10.5	10.6	4.7
14	male	2	14.0	35	16	0.3	10.7	8.9	4.8
5-2	female	2	12.8	30	10	0.2	9.4	11.2	4.0
15	male	3	15.0	26	16	0.3	10.0	9.0	3.7
16	male	4	16.2	19	9	0.3	12.4	11.0	4.3
17	female	4	15.2	25	13	0.3	13.2	10.7	4.0
18	male	7	27.0	19	8	0.4	9.3	9.2	4.8
	M/F = 7/2								
mean ± S.D.		2.8 ± 2.0	14.8 ± 11.2	27.6 ± 6.4	11.8 ± 2.9	0.3 ± 0.1	10.9 ± 1.3	9.7 ± 1.7	4.2 ± 0.4
Mann-Whitney *U* test		$p = 0.648$	$p = 0.902$	$p = 0.413$	$p = 0.652$	$p = 0.793$	$p = 0.624$	$p = 0.87$	$p = 0.093$

AST= asparate aminotransferase, ALT = alanine aminotransferase, Cr= creatinine

of each child. We assumed that the rate of metabolite formation from theophylline equals the rates of the appearance of metabolites in urine and that the metabolites are eliminated into urine without further metabolism. The renal clearance of theophylline was calculated by dividing the urinary excretion rate of theophylline by serum concentrations during the sampling period. Because the molecular weights of the respective theophylline metabolites and theophylline differ, the concentrations of the urinary metabolites were adjusted to the molar equivalent of theophylline.

Statistical analysis

All data are presented as mean ± standard deviation (SD). Statistical differences in the means of the clinical variables and the parameters of theophylline disposition between the macrolide-antibiotic-treated and control groups were examined by the Mann-Whitney U test. A p value of < 0.05 was considered to indicate statistically significant difference.

Results

The demographic data of the children who participated in this study are shown in Table 1. No significant differences were observed in any demographic parameters (e.g., age and body weight) between the macrolide-antibiotic-treated and control groups. Among the nine children who underwent low-dose macrolide therapy, eight received erythromycin (i.e., 13.2 ± 3.8 mg/kg body weight/day) and one received clarithromycin (i.e., 4.9 mg/kg body weight/day) at the doses shown in Table 2.

No significant difference in the oral dose of theophylline between the macrolide-antibiotic-treated and control groups was observed (10.9 ± 1.3 and 11.1 ± 1.9 mg/kg body weight/day, respectively). The serum theophylline concentrations of the macrolide-antibiotic-treated and

Table 2. Urinary data of theophylline and its metabolites with and without low-dose macrolide administration in pediatric patients

Patient	Dose of macrolides (mg/kg/day)	Collection periods of urine samples (hours)	CLr of theophylline (mL/min/kg)	CLm of theophylline to metabolites (mL/min/kg) 1MU	3MX	DMU	Caffeine	% of dose recovered
Those who received theophylline alone								
1	-	2.1	0.028	0.041	0.023	0.087	0.000	2.3
2	-	2.3	0.021	0.030	0.022	0.082	0.000	1.3
3	-	2.3	0.040	0.054	0.041	0.112	0.001	1.2
4	-	4.0	0.031	0.052	0.039	0.095	0.000	4.5
5-1	-	0.8	0.233	0.142	0.133	0.384	0.011	4.7
6	-	3.0	0.066	0.061	0.054	0.177	0.002	6.6
7	-	1.5	0.361	0.192	0.167	0.632	0.007	9.9
8	-	2.4	0.103	0.133	0.100	0.226	0.000	6.9
9	-	12.0	0.132	0.136	0.085	0.235	0.007	34.6
10	-	12.3	0.023	0.043	0.033	0.100	0.000	16.1
mean ± S.D.	-	4.2 ± 4.2	0.104 ± 0.112	0.088 ± 0.094	0.070 ± 0.075	0.207 ± 0.220	0.003 ± 0.003	8.8 ± 10.1
Those who received theophylline and low-dose macrolide antibiotics								
11	erythromycin 9.1	1.9	0.053	0.158	0.115	0.218	0.000	3.3
12	clarithromycin 4.9	4.2	0.029	0.013	0.006	0.086	0.003	3.2
13	erythromycin 17.5	3.3	0.087	0.028	0.021	0.062	0.000	4.0
14	erythromycin 10.7	2.5	0.079	0.136	0.054	0.185	0.005	5.7
5-2	erythromycin 10.2	0.7	0.388	0.210	0.152	0.833	0.009	7.4
15	erythromycin 10.0	9.8	0.053	0.064	0.048	0.150	0.001	16.7
16	erythromycin 18.5	5.9	0.071	0.039	0.030	0.117	0.001	8.1
17	erythromycin 16.4	11.9	0.080	0.067	0.042	0.128	0.001	18.5
18	erythromycin 13.0	6.0	0.057	0.061	0.045	0.135	0.001	10.7
mean ± S.D.	12.3 ± 4.5	5.1 ± 3.7	0.100 ± 0.110	0.086 ± 0.067	0.057 ± 0.047	0.213 ± 0.237	0.002 ± 0.003	8.6 ± 5.7
Mann-Whitney *U* test		$p = 0.414$	$p = 0.567$	$p = 1.000$	$p = 0.653$	$p = 0.87$	$p = 0.672$	$p = 0.462$

1MU= 1-methyluric acid, 3MX= 3-methylxanthine, DMU = 1,3-dimethyluric acid
CLm = metabolic clearance of theophylline to its respective metabolites, CLr = renal clearance of theophylline

control groups were comparable and were in the lower therapeutic range (9.7 ± 1.7 and 9.7 ± 3.1 μg/mL, respectively) at comparable blood sampling times (Table 1). No appreciable clinical signs or symptoms possibly attributable to theophylline toxicity were observed in the children during the study period.

No significant differences were observed in the partial metabolic clearance of theophylline to its respective metabolites or in the renal clearance of theophylline between the two groups (Table 2).

Discussion

This study is the first to demonstrate that the oral administration of low-dose (i.e., 12.3 ± 4.5 mg/kg body weight/day) 14-membered ring macrolide antibiotics (i. e., erythromycin and clarithromycin) to pediatric patients unlikely elicits clinically relevant alterations in the systemic disposition of a slow-release formula of theophylline. Recently, these macrolide antibiotics have been shown to possess tissue reparative effects, independent of their antimicrobial activity, in chronic inflammatory sinopulmonary diseases (i.e., chronic sinusitis, asthma, panbronchiolitis, and cystic fibrosis) [2]. Because certain 14-membered ring macrolide antibiotics (e.g., erythromycin and clarithromycin) have been implicated to produce clinically significant drug interactions with theophylline[5] [6] when they are administered at rather higher doses, there is great concern as to whether low-dose macrolide antibiotic therapy evokes a clinically relevant drug interaction with theophylline. Our data clearly indicate that this possibility in pediatric patients is remote.

Regarding serum theophylline concentrations, we measured them at 3.5 - 6 hours after the oral administration when their peaks are

assumed to occur to clarify the inhibitory effect of erythromycin and clarithromycin. It is of interest to explain the apparent contradiction between previous studies[5) 6)] and ours regarding the inhibitory effect of erythromycin and clarithromycin on the systemic clearance of theophylline. Prince et al.[12)] demonstrated that the administration of erythromycin at 1 g/day for 7 days decreased the systemic clearance of theophylline by 25%. They recommended that theophylline dose be decreased by at least 25% in patients whose serum theophylline concentrations are maintained in the middle value or upper portion of the 10 - 20 μg/mL therapeutic range, when erythromycin is added to the therapeutic regimen. However, the doses of the macrolide antibiotics employed in previous studies [5, 6] including that of Prince et al. [12] (e.g., 1-2 g/day for adults and 20 mg/kg body weight/day for children) were approximately twofold greater than those employed in this study (13 mg/kg body weight/day for children). Because the inhibitory effect of macrolide antibiotics on CYP3A4 is dose—dependent [13], the low-dose macrolide antibiotic therapy employed in this study was not associated with appreciable changes in the disposition of theophylline.

Multiple CYP isoforms are involved in the hepatic metabolism of theophylline[7) 8)]. Previous studies demonstrated that CYP3A4, CYP2E1, and CYP1A2 are involved in DMU formation (i.e., 8-oxidation of theophylline). In addition, CYP1A2 is involved in the formation of 1- and 3-MX (1- and 3-demethylations of theophylline, respectively). Because erythromycin and clarithromycin are specific inhibitors of CYP3A4 [5], the metabolic clearance of theophylline to DMU in the macrolide-antibiotic-treated group should have been reduced compared with in the control group, provided that CYP3A4 had been significantly inhibited by low-dose macrolide antibiotic therapy. The observation that there was no significant difference in this parameter between the two groups (Tables 1 and 2) indicates that the inhibitory effect of low-dose macrolide antibiotic therapy on in vivo CYP3A4 activity is limited, if indeed present at all. The observation that multiple CYP isoforms other than CYP3A4 are also involved in DMU formation from theophylline is another possible explanation. In addition, no significant difference in the renal clearance of theophylline was observed between the two groups, indicating that low-dose macrolide antibiotic therapy does not alter the renal excretion of the drug significantly.

Because only a small number of patients were studied and a matched-pair case-control design was not adopted in the present study, we cannot exclude a possibility that the administration of 14-membered macrolide antibiotics at lower doses may elicit a small inhibitory effect on the hepatic metabolism of theophylline. Strictly speaking, a matched-pair case control study is required to confirm the inhibitory effect of low-dose macrolide antibiotics. Nevertheless, our data suggest that a categorical recommendation for a dosage reduction of theophylline should not be required for most patients.

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Reduced Vancomycin Clearance Despite Unchanged Creatinine Clearance in Patients Treated With Vancomycin For Longer Than 4 Weeks

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Abstract: Creatinine clearance-based nomograms are used routinely during the early phase of vancomycin therapy for individualizing doses. The authors studied whether such nomograms are also valid for patients receiving the drug for an extended period of longer than 4 weeks. A retrospective analysis was conducted on the therapeutic drug monitoring data obtained from 85 patients who received an intermittent intravenous infusion of vancomycin. The patients were allocated to one of five groups according to the length of drug exposure: Group 1 (4–7 days; n = 31), Group 2 (8–14 days; n = 22), Group 3 (15–21 days; n = 13), Group 4 (22–28 days; n = 8), and Group 5 (longer than 29 days; n = 11). Systemic clearance of vancomycin and estimated creatinine clearance calculated by Cockcroft & Gault's formula obtained from Groups 2 through 5 were compared with those from Group 1. Patients who had received vancomycin for longer than 4 weeks (Group 5) showed a significant ($P < 0.05$) reduction in systemic clearance of vancomycin by 50% compared with Group 1, whereas creatinine clearance remained unchanged. This study demonstrated that prolonged administration of vancomycin for over 4 weeks may result in a more pronounced reduction in systematic clearance of vancomycin than creatinine clearance. Our data suggest that creatinine clearance-based nomograms for individualizing vancomycin doses should be used with caution in patients who require substantially prolonged drug exposure such as those with infective endocarditis.

Key Words: vancomycin, clearance, pharmacokinetics, therapeutic drug monitoring

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INTRODUCTION

Vancomycin is a bactericidal glycopeptide antibiotic-widely used for the treatment of infections caused by methicillin-resistant *Staphylococcus aureus* and multiresistant coagulase-negative *Staphylococcus* species.[1–4] Some adverse reactions of vancomycin, including nephrotoxicity and ototoxicity, have been shown to be associated with elevated plasma concentrations above the therapeutic range. Therefore, therapeutic drug monitoring of vancomycin is considered advisable, particularly in patients with impaired renal function, when the drug is given over an extended period.[5,6]

For most patients with nosocomial infections (such as pneumonia in ventilator-assisted patients), vancomycin is administered for 1 to 2 weeks.[7] Because vancomycin is a potentially nephrotoxic agent, there is a concern over whether long-term administration of the drug would cause insidious renal damage. Pou et al[2] compared the pharmacokinetics of vancomycin between patients given the drug for less than 10 days and those given for more than 10 days. The mean elimination half-life obtained from the latter was 25% longer than the former and the mean systemic clearance of vancomycin (CL_{VCM}) obtained from the latter was 15% lower than the former, whereas the differences between the groups appeared clinically insignificant. However, for patients with certain types of infection such as osteomyelitis and endocarditis, vancomycin may be given for a far more extended period of longer than 4 weeks to eradicate the causative organisms growing in tissues with poor vascularity, including necrotic bone, sequestrum, and vegetations.[8–10] However, to our knowledge, it remains unknown whether more drastic changes in the disposition and elimination of the drug may occur during long-term administration of vancomycin beyond 4 weeks. In addition, we are unaware of any attempts to examine whether a traditional creatinine clearance (CLcr)-based nomogram for individualizing vancomycin maintenance doses may be valid in patients receiving long-term vancomycin therapy. In this context, the present study was conducted to address these questions based on a retrospective review of data retrieved from our therapeutic drug monitoring records.

MATERIALS AND METHODS

Data Collection

Data were collected retrospectively from the medical records of patients who received intermittent intravenous infusion of vancomycin for the treatment of documented infections at Kanto Medical Center NTT East Corporation between December 1, 2000, and April 30, 2006. Patients aged 20 years or older having at least one set of peak and trough plasma vancomycin concentrations at steady state were

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included in this study. Patients undergoing hemodialysis and those given teicoplanin in combination with vancomycin or vancomycin orally were excluded from analysis. Patient demographic data, blood biochemistry, vancomycin doses, and concomitantly administered drugs were retrieved from medical records. We defined clinically relevant renal dysfunction during vancomycin therapy as a 50% or greater increase in serum creatinine at the end of vancomycin administration compared with the pretreatment value.[11] The protocol of the present study was approved by the Ethics Committee of Kanto Medical Center NTT East Corporation before the study was started.

Drug Administration and Sample Analysis

Vancomycin was given to patients at doses ranging from 1.5 to 30.9 mg/kg per day by intravenous infusion over 60 minutes. Doses were individualized according to the patient's renal function using the nomogram of Moellering et al.[12] Durations of vancomycin therapy were decided based on clinical responses to the drug in individual patients and were infused intravenously over 60 minutes. Peak vancomycin plasma concentrations were measured at 1 hour postinfusion (C_{peak}) and plasma trough concentrations were measured 30 minutes before the next dose (C_{trough}). Plasma samples were collected at presumed steady state. We considered the pharmacokinetics of vancomycin would have reached steady state when at least 4 days (more than five half-lives of the drug in patients with normal renal function) had elapsed from the beginning of vancomycin therapy. Vancomycin plasma concentrations were assayed by fluorescence polarization immunoassay (TDX; Abbott Diagnostics, Tokyo, Japan).

Pharmacokinetic Analysis

Plasma concentration–time data of vancomycin were analyzed using a one-compartment model according to the method of Sawchuk et al.[13] Briefly, the elimination rate constant (K_{el}) and half-life ($t_{1/2}$) of the drug were calculated based on a set of C_{peak} and C_{trough} in each patient. C_{peak} represents plasma vancomycin concentration at the end of infusion. C_{trough} represents the concentration at the end of the previous dosing interval. The volume of distribution (Vd) of the drug was calculated according to the following equation[13]:

$$Vd = \frac{k_0}{k_{el}} \frac{(1 - e^{-k_{el} \cdot t_{in}})}{(C_{peak} - C_{trough} e^{-k_{el} \cdot t_{in}})}$$

in which k_0 is the constant infusion rate of vancomycin and t_{in} is the duration of drug infusion. The CL_{VCM} was calculated as $Vd \times K_{el}$.

CLcr was estimated according to the formula of Cockcroft and Gault.[14] The pharmacokinetic data obtained from patients at different phases of vancomycin therapy were classified according to the length of vancomycin therapy: Group 1 (4–7 days; n = 31), Group 2 (8–14 days; n = 22), Group 3 (15–21 days; n = 13), Group 4 (22–28 days; n = 8), and Group 5 (29 days or longer; n = 11). When CL_{VCM} was measured, the ratio of CL_{VCM}/CLcr was also calculated concurrently.

Statistical Analysis

Multiple comparisons of the mean values for vancomycin pharmacokinetic parameters obtained from Groups 1 to 5 were undertaken by analysis of variance followed by the Dunnett test. In addition, correlation between CL_{VCM} and the duration of vancomycin therapy was examined by Pearson's correlation analysis. For patients treated concomitantly with an aminoglycoside or amphotericin B, the mean values for CLcr, CL_{VCM}, and CL_{VCM}/CLcr ratio obtained from patients given the drugs for less than 14 days and the corresponding values obtained from those given the drugs for 14 days or longer were compared by Mann-Whitney *U* test. All statistical analysis was performed using SPSS (release 11.0; SPSS Inc., Tokyo, Japan). A *P* value of <0.05 was considered significant throughout the study.

RESULTS

We retrieved the data from 123 patients who received an intravenous administration of vancomycin during the survey period. Among them, 85 patients met the inclusion criteria comprising 66 men and 19 women ranging in age from 21 to 92 years [mean (± standard deviation), 65 ± 15 years]. Before vancomycin treatment, the mean body weight was 55.3 (± 13.7) kg, mean serum creatinine 0.80 (± 0.43) mg/dL, and mean estimated CLcr 84 (± 39) mL/min. Table 1 shows the demographic and relevant clinical data of the patients according to the length of vancomycin therapy. There were no significant differences in the variables examined.

Eight of 85 patients (9%) showed a 50% or greater increase in serum creatinine during antiinfective chemotherapy, including vancomycin: one patient in Group 1 (3%), three in Group 2 (14%), one in Group 3 (8%), one in Group 4 (13%), and two in Group 5 (18%). Overall, the incidence of renal dysfunction was low across the groups and did not appear to be time-dependent. At the end of vancomycin therapy, the mean serum creatinine concentration was 0.90 (± 0.65) mg/dL and ranged from 0.26 to 5.44 mg/dL. Nineteen patients received potentially nephrotoxic drugs concomitantly with vancomycin: aminoglycosides in 15 patients, amphotericin B in two patients, and both in two patients. The mean duration of concurrent aminoglycosides and amphotericin B were 6.3 (± 4.4) days and 12.3 (± 6.9) days, respectively. When the data obtained from these patients were analyzed separately, five of 19 patients (26%) showed a 50% or greater increase in serum creatinine concentration during vancomycin therapy. Consequently, five of eight patients who showed renal impairment during vancomycin treatment were coadministered these nephrotoxic agents.

Table 2 shows the clinical characteristics and pharmacokinetic parameters of vancomycin in the five groups of patients allocated according to the length of vancomycin therapy. There was no difference in the daily vancomycin dose among the patient groups. However, renal function as determined by serum creatinine concentration tended to be reduced as the length of vancomycin exposure was prolonged, although there was no significant difference. Patients who received vancomycin for on average 50 days (Group 5) had

TABLE 1. Demographic and Relevant Clinical Characteristics of Patients Treated With Vancomycin

Variable	Group 1	Group 2	Group 3	Group 4	Group 5
Number of patients	31	22	13	8	11
Age (years)	62 ± 15	65 ± 18	71 ± 11	62 ± 15	67 ± 11
Gender (male/female)	24/7	15/6	9/4	8/0	9/2
Initial body weight (kg)	56.4 ± 12.4	53.6 ± 15.3	56.7 ± 15.2	57.1 ± 16.1	57.7 ± 12.5
Baseline serum creatinine level (mg/dL)	0.79 ± 0.31	0.76 ± 0.28	0.75 ± 0.32	0.72 ± 0.20	1.08 ± 0.94
Baseline creatinine clearance (mL/min)	80.0 ± 29.7	73.9 ± 30.4	76.0 ± 28.6	84.6 ± 30.6	69.3 ± 32.4

All values are mean ± standard deviation.
Group 1, treated with vancomycin for 4 to 7 days; Group 2, treated for 8 to 14 days; Group 3, treated for 15 to 21 days; Group 4, treated for 22 to 28 days; Group 5, treated for 29 days or longer.

approximately 40% higher serum creatinine concentration (1.27 ± 0.78 mg/dL) compared with patients in Group 1.

Pharmacokinetic Parameters

Vancomycin pharmacokinetic parameters obtained from patients who received different durations of vancomycin therapy are summarized in Table 2. Significant increases in mean (± standard deviation) C_{peak} and C_{trough} were observed as the duration of vancomycin exposure increased. The mean C_{peak} and C_{trough} concentrations were significantly ($P < 0.05$) elevated in Group 5 compared with Group 1. In addition, the differences observed in vancomycin peak and trough plasma concentrations were not a result of the differences in vancomycin doses administered to the various patients, because the dose-corrected values showed essentially similar changes (Table 2).

There was also a significant change in mean (± standard deviation) CL_{VCM} between the groups; the value obtained from Group 5 was significantly reduced compared with Group 1. No significant differences in Vd were observed between groups. When CL_{VCM}/CLcr ratios were calculated, the mean value was significantly ($P < 0.05$) reduced in Group 5 compared with Group 1 (Table 2).

In addition, a significant negative correlation ($r = -0.337$, $P < 0.01$) was found between CL_{VCM}/CLcr ratio and duration of vancomycin exposure. In the analysis of patients given nephrotoxic agents concomitantly with vancomycin, no significant differences in CLcr, CL_{VCM}, and CL_{VCM}/CLcr ratio were observed between patients treated for less than 14 days and those treated for longer than 14 days.

DISCUSSION

In the present study, we report that patients receiving vancomycin for longer than 4 weeks showed a significant reduction in CL_{VCM} but no significant change in CLcr. As a result, the mean CL_{VCM}/CLcr ratio in patients receiving the drug for an extended period of over 4 weeks was significantly reduced compared with patients receiving the drug for less than 7 days (Table 2). These data indicate that CLcr may not be a reliable index for individualizing vancomycin dose in patients receiving the drug for a prolonged period of 4 weeks or longer. With the emergence of infective endocarditis by multidrug-resistant methicillin-resistant *S. aureus* and coagulase-negative *Staphylococcus* species, vancomycin is often given to patients over an extended period (longer than 4 weeks) in contemporary clinical settings. In this context, our

TABLE 2. Pharmacokinetic Data Obtained From Patients Who Were Given Vancomycin for Different Durations

Variable	Group 1 (n = 31)	Group 2 (n = 22)	Group 3 (n = 13)	Group 4 (n = 8)	Group 5 (n = 11)
Length of exposure (day)	5.3 ± 1.3	10.0 ± 2.1	17.2 ± 1.8†	24.9 ± 2.0†	49.5 ± 24.0†
Dose (mg/kg/day)	21.8 ± 6.9	24.0 ± 11.2	20.0 ± 8.4	18.9 ± 10.8	18.2 ± 14.5
$t_{1/2}$ (hours)	12.7 ± 5.1	19.0 ± 12.8	17.4 ± 8.8	16.7 ± 7.2	60.1 ± 86.7†
Vd (L/kg)	1.14 ± 0.41	1.28 ± 0.42	1.24 ± 0.34	1.15 ± 0.42	1.47 ± 0.74
Peak concentration (μg/mL)	23.6 ± 9.1	27.7 ± 8.2	24.1 ± 6.9	25.1 ± 4.5	32.1 ± 8.8*
(corrected for 1 g/day; μg/mL)	(21.5 ± 10.4)	(27.3 ± 14.3)	(26.7 ± 12.9)	(33.4 ± 18.4)	(68.7 ± 114.4†)
Trough concentration (μg/mL)	8.9 ± 6.2	11.1 ± 5.4	8.2 ± 3.2	7.4 ± 2.9	19.2 ± 9.9†
(corrected for 1 g/day; μg/mL)	(7.6 ± 4.3)	(12.4 ± 9.9)	(8.7 ± 4.4)	(10.0 ± 8.1)	(47.5 ± 95.2†)
Serum creatinine level (mg/dL)	0.76 ± 0.34	0.89 ± 0.46	0.81 ± 0.40	0.87 ± 0.42	1.42 ± 1.46*
CLcr (mL/min)	83.1 ± 29.3	71.8 ± 35.3	73.3 ± 30.3	76.4 ± 33.8	65.8 ± 31.2
CL_{VCM} (mL/min)	67.6 ± 41.0	53.8 ± 32.4	54.2 ± 26.6	52.2 ± 34.8	34.0 ± 23.6*
CL_{VCM}/CLcr ratio	0.82 ± 0.32	0.79 ± 0.30	0.77 ± 0.24	0.69 ± 0.32	0.49 ± 0.21†

Data are means ± standard deviation.
$t_{1/2}$, half-life; Vd, volume of distribution; CL_{VCM}, systemic clearance of vancomycin; CLcr, creatinine clearance; Group 1, treated with vancomycin for 4 to 7 days; Group 2, treated for 8 to 14 days; Group 3, treated for 15 to 21 days; Group 4, treated for 22 to 28 days; Group 5, treated for 29 days or longer.
*$P < 0.05$.
†$P < 0.01$ compared with the corresponding values in Group 1.

findings emphasize the importance of plasma concentration monitoring of vancomycin to assure optimal dosing of vancomycin in such situations.

The necessity to individualize vancomycin dose has been advocated based on clinical data indicating that therapeutic failure is more common with trough plasma vancomycin concentrations below 10 μg/mL for the treatment of bacterial endocarditis and sepsis,[15,16] whereas excessively elevated plasma concentrations are associated with increased risks of renal impairment and other adverse reactions than therapeutic concentrations (20–40 μg/mL)[1,5,17] for most susceptible organisms. Fernández de Gatta et al[4] reported that patients exhibiting renal impairment after vancomycin therapy had significantly elevated serum vancomycin concentrations (especially trough concentrations) compared with those exhibiting no signs of renal impairment. In the present study, we also observed that the mean C_{peak} was significantly ($P < 0.05$) higher in Group 5 than in Group 1 despite the fact that we individualized vancomycin doses according to CLcr measured for each patient. Nevertheless, the mean C_{peak} obtained from Group 5 was lower than the presumed toxic concentration of vancomycin (eg, greater than 60 μg/mL).

Because vancomycin is eliminated mainly through renal excretion, several formulae or nomograms for estimating vancomycin dose using the patients' CLcr have been proposed and validated in patients receiving the drug for 2 to 3 weeks.[12] The present study revealed that the mean CL_{VCM}/CLcr ratio obtained from patients receiving vancomycin for longer than 4 weeks was significantly lower compared with patients receiving the drug for shorter periods, indicating that it is safer to individualize vancomycin doses by plasma concentration monitoring rather than relying solely on a CLcr-based prediction method.

The reason why an extended period of vancomycin exposure was associated with a greater reduction in CL_{VCM} than CLcr remains to be studied, because both substances are eliminated by the kidney primarily through glomerular filtration.[1,5,17,18] To our knowledge, no attempts have been made to study the pharmacokinetics of vancomycin in patients receiving the drug for a substantially prolonged period of over 4 weeks. Nevertheless, our data may be supported by previous studies[2,6] in which an elevation of plasma vancomycin concentration was documented in patients who received the drug for longer than 10 days but less than 4 weeks. Although Sym et al[6] did not perform a precise pharmacokinetic analysis, Pou et al[2] demonstrated that the mean CL_{VCM} was significantly reduced by 15% but CLcr was not in patients receiving vancomycin for more than 10 days. In the present study, we demonstrated that the CL_{VCM} was reduced by 50% in patients receiving the drug for 4 weeks or longer but Vd remained unchanged. In the 1990s, it was suggested that the measurements of serum vancomycin concentrations by fluorescence polarization immunoassay may be inaccurate as a result of interference in patients with renal failure. Our data were obtained using a modified fluorescence polarization immunoassay that is free from interference; therefore, our findings cannot be explained by analytical inaccuracy. Obviously, further studies are necessary to confirm our hypothetical explanation.

From the present observations, we were not able to provide a definitive explanation for the apparent abrupt reduction in pharmacokinetic parameters of vancomycin when administered for longer than 4 weeks (ie, Group 5) compared with a gradual decrease when administered for shorter durations (Groups 1–4). It is possible that patients requiring treatment for longer than 4 weeks differed from other patients from the beginning despite the observed similarities in demographic and renal function parameters. Although the mean estimated creatinine clearance at baseline obtained from Group 5 in which vancomycin was given for longer than 4 weeks appeared lower compared with other groups, the difference did not reach a statistically significant level.

There are a number of limitations in the present study. First, we did not follow the time courses of CL_{VCM} in the same patients over 4 weeks. Second, we assessed the renal functions of our patients by CLcr estimated from serum creatinine concentrations and not by CLcr based on 24-hour urine collection. The estimation of CLcr based on serum creatinine concentrations may be imprecise even under the best conditions. Hermida et al[19] reported that serum creatinine concentrations may not reflect the systemic clearance of amikacin and vancomycin accurately. CLcr estimated by serum creatinine concentrations using the Cockcrof & Gault method[14] may be systematically biased in patients with cirrhosis or severe malnutrition as a result of reduced synthesis of creatinine.[20] Nevertheless, our patients did not have either severe liver damage or malnutrition. Lastly, it is also possible that our findings may represent a mathematical artifact because we calculated CL_{VCM} using the formula $CL_{VCM} = Kel \times Vd$ (see "Methods"). Although we demonstrated no change in vancomycin Vd over time, it is possible that vancomycin tissue accumulation increases with longer durations of therapy with resultant slow redistribution from tissue stores back into blood blunting the Kel and, thus, increasing the drug $t_{1/2}$. Ideally, drug clearance should be determined using drug area under the curve (eg, dose/area under the curve), but under clinical conditions with only two plasma drug concentrations, accurate determination of the area under the curve is not possible underscoring the use of the clinically useful but mathematically dubious formula we used to estimate CL_{VCM}. Despite these limitations, our data strongly support the need for a critical study of vancomycin pharmacokinetics during prolonged therapeutic regimens.

CONCLUSION

Our data suggest that CL_{VCM} is reduced although CLcr remained unchanged when the drug was given for an extended period of over 4 weeks. Because the magnitude of the reduction in CL_{VCM} was greater than that of CLcr, vancomycin doses guided solely by CLcr may result in a substantial overestimation in such patients. As a safety precaution, individualized therapeutic drug monitoring is recommended for patients who require long-term vancomycin administration.

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