15　研究に関する問い合わせ先、苦情の窓口

この研究に関するお問い合わせは、下記の研究責任者までご連絡ください。苦情がある場合は、自治医科大学附属さいたま医療センター総務課学務係（電話 048-647-2111・内線 2341）で受け付けます。

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研究参加同意書

自治医科大学附属さいたま医療センター長　殿

私は、「注射用アムホテリシンＢリポソーム製剤（L-AMB）の血中濃度と有効性・安全性の検討」研究について、研究者（　　）から、説明文書を用いて次の事項について説明を受けました。

（説明を受け理解した項目の□の中にご自分でチェックの印をつけてください。）

□ 研究の意義と目的
□ 研究への参加をお願いする理由
□ 研究への参加は任意であり、参加の同意をしなくても不利益を受けないこと
□ 研究への参加に同意した後でも、いつでも不利益を受けることなく同意を撤回できること
□ 研究の方法
□ 研究の期間
□ 研究者の所属、職名及び氏名
□ 予想される研究の結果、研究に参加することにより期待される利益及び起こりうる危険並びに必然的に伴う不快な点、研究終了後の対応
□ 研究計画の閲覧
□ 個人情報の保護
□ 知的財産権の帰属
□ 研究結果の公表
□ 研究の資金源
□ 研究に伴う補償
□ 研究に関する問い合わせ先、苦情の窓口

以上の説明を十分に理解したので、被験者として研究に参加することに同意致します。

＿＿＿＿＿年＿＿＿月＿＿＿＿日

住所＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

氏名＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

（氏名は自署、または記名・押印）

厚生労働科学研究費補助金（免疫アレルギー疾患等予防・治療研究事業）
分担研究報告書

イトラコナゾールの血中濃度モニタリング

森　有紀　　国家公務員共済組合連合会　虎の門病院　血液内科

研究要旨　同種造血幹細胞移植後の深在性真菌症に対するイトラコナゾール内用液の予防投与における血中濃度モニタリングの有用性を検討した。造血幹細胞移植患者に対して、イトリゾール®内用液 1%200mg(20mL)1 日 1 回の経口予防投与を開始し、内服困難時はイトリゾール®注 1% 200mg1 日 1 回の静注投与に変更可として投与を継続し、移植前、移植 1 週間後及び 1 ヶ月後に高速液体クロマトグラフィーを用いてイトラコナゾールの血中濃度を測定した。イトラコナゾールの血中濃度の中央値は、移植前が 275.23ng/mL、移植 1 週間後が 297.88ng/mL、移植 1 ヶ月後が 492.89ng/mL で、症例間、また同一症例内でも移植前後の時期によってばらつきが大きく、血中濃度が有効域に達していた症例は 50-60%程度に過ぎなかった。本研究より、同種造血幹細胞移植患者における深在性真菌感染症予防としてイトラコナゾールを用いる場合は、定期的に血中濃度をモニターする必要があることが示唆された。

A．研究目的

同種造血幹細胞移植後の真菌感染症は、診断が難しく、かつ一旦発症すると治療に難渋し致死率が非常に高いことから、その予防対策に重点が置かれている。従来フルコナゾールが移植後の真菌感染予防薬として広く用いられて来たが、現在ではより広い抗菌スペクトルを有する新規アゾール系薬剤の開発が進み、その１つであるイトラコナゾールは、従来のカプセル剤よりも吸収効率が大幅に改善された内用液や注射薬の出現により、その予防効果が強く期待される。しかし、これらを用いても、イトラコナゾールの有効血中濃度（250ng/mL－500mg/mL)に達しない症例も一部残存し、また一度の測定で有効域の血中濃度が確認されても、移植時のように患者の状態が急激に変化する場合は、これが常に維持されているか否かは不明である。また、イトラコナゾールは、チトクロム P450 酵素系を介して代謝される薬剤との相互作用を有し、特に移植領域においては免疫抑制剤であるカルシニューリン阻害薬（シクロスポリン及タクロリムス）との相互作用が問題となる。一般的にはイトラコナゾールによりカルシニューリン阻害薬の血中濃度が上昇するとされているが、カルシニューリン阻害薬がイトラコナゾールの薬物動態に与える影響は明らかでない。更に、イトラコナゾールは、フルコナゾールに比べて毒性が強く、この副作用が薬物血中濃度に依存して出現している可能性もある。以上より本研究では、イトラコナゾールの予防効果を最大限に引き出しつつ毒性をコントロールするための、薬物血中濃度モニタリングの有用性を評価した。

B．研究方法

真菌感染症が疑診または確定診断されていない20才以上の造血幹細胞移植患者を対象とし、移植日より 10 日以上前からイトリゾール®内用液 1% 200mg(20mL)1 日 1 回の経口投与を開始した。内服継続困難時はイトリゾール®注 1%200mg1 日 1 回の静注投与に変更可とし、原則として移植後 100 日目まで投与を継続した。イトラコナゾールのトラフ値の測定を、移植前（カルシニューリン阻害薬開始前)、移植 1 週間後及び 1 ヶ月後に行なった。末梢より血液 5mL を採血して血漿を分離した後、-20℃で凍結保存し、高速液体クロマトグラフィーを用いてイトラコナゾール血漿中濃度を測定した。

＜倫理面への配慮＞

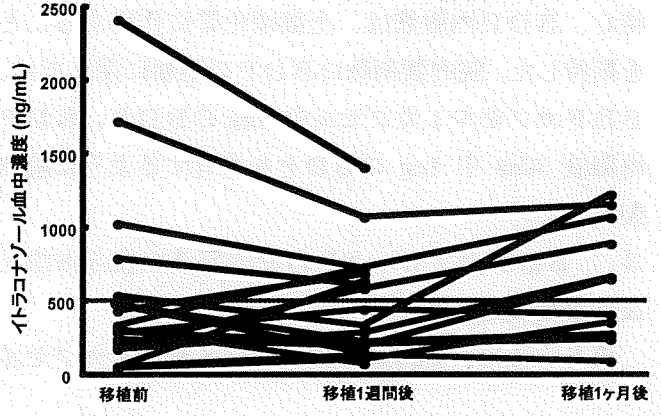
本試験は GCP を準用し、かつヘルシンキ宣言に基づいた倫理的原則を遵守して実施した。治験審査

委員会で承認の得られた同意説明文書に基づいて文書及び口頭による十分な説明を行い、被験者の自由意志による同意を得た。被験者のプライバシーの保護に十分配慮し、登録時に連続番号を用いて匿名化を行なった。検査及び血中濃度測定後に余った検体は速やかに破棄した。

C. 研究結果

血中濃度モニタリングを実施した症例は 22-69 才の患者 19 例（男性 13、女性 6）で、原疾患は、急性白血病 12 例、骨髄異形成症候群 1 例、慢性骨髄性白血病 2 例、成人 T 細胞性白血病 1 例、悪性リンパ腫 2 例、多発性骨髄腫 1 例であった。移植ソースは臍帯血が 11 例、非血縁骨髄が 6 例、血縁末梢血幹細胞が 2 例で、移植前処置は、フルダラビンを含む非骨髄破壊的前処置が 16 例、エンドキサンを含む骨髄破壊的前処置が 3 例であった。

イトラコナゾールの血中濃度の中央値は、移植前が 275.23ng/mL(8.00-2376.53ng/mL)、移植 1 週間後が 297.88ng/mL(37.90-1371.99ng/mL)、移植 1 ヶ月後が 492.89ng/mL(53.04-1185.17ng/mL)であった。イトラコナゾールの血中濃度は、図 1 のように症例間、及び同一症例内でも移植前後の時期によってばらつきが大きく、血中濃度が 250ng/mL 以上又は 500ng/mL 以上に達した症例は、それぞれ、移植前は 52.6 % 及び 26.3 %、移植 1 週間後は 52.6 %及び 42.1 %、移植 1 ヶ月後は 66.7 %及び 58.3 %であった。イトラコナゾールに起因すると思われる重篤な副作用を呈した症例はなかった。1 例で移植後 23 日目に侵襲性肺アスペルギルス症の発症を認めたが、この症例の血中濃度は、移植前が 438.77ng/mL であったのに対し、移植 1 週間後は 112.38ng/mL と低値であった。



D. 考察

内用液や注射薬を用いても、有効域に達していない症例が存在し、かつ移植前に測定した血中濃度が有効域であっても移植後には血中濃度が有効域に達していない症例があり、患者間あるいは同一患者内であっても時期によってイトラコナゾールの血中濃度に大きなばらつきがあることが分かった。

E. 結論

以上より、同種造血幹細胞移植患者における深在性真菌感染症予防としてイトラコナゾールを用いる場合は、定期的に血中濃度をモニターする必要がある。また有効血中濃度が得られない症例に関しては、増量又は他剤への変更を検討する必要があると考えられた。

F. 研究発表

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G. 知的財産権の出願・登録状況

1. 特許取得

該当なし

2. 実用新案登録

該当なし

3. その他

該当なし

厚生労働科学研究費補助金（免疫アレルギー疾患等予防・治療等研究事業）
分担研究報告書

腸管移植片対宿主病(GVHD)患者に対する経口ベクロメタゾン投与時の血中濃度に関する検討

山本　久史　　国家公務員共済組合連合会　虎の門病院　血液内科　医員

研究要旨　腸管ＧＶＨＤに対してベクロメタゾン（ＢＤＰ）を経口投与した患者を対象として、ＢＤＰおよびその代謝物の血中濃度を高速液体クロマトグラフィーを用いて LC-MS/MS で測定し経口ＢＤＰの消化管からの吸収について検討した。全対象症例 5 例よりＢＤＰの主要活性代謝物である 17ＢＭＰが検出された。5 例中 4 例における 17ＢＭＰの血中濃度は 618～1,749 pg/mℓ であり吸入剤を投与した時と同程度あるいはそれ以下であった。1 例は、17ＢＭＰの血中濃度が 2,439±161 pg/mℓ を示し吸入剤投与時以上に血中濃度が上昇した。本症例における 17ＢＭＰの血中濃度は、健常人にＢＤＰ 4mg を単回経口投与した際の最高血中濃度と比較して高値を示したことからＧＶＨＤ患者では健常人よりも血中濃度が上昇する症例が存在する事が示唆された。腸管ＧＶＨＤの stage が高い症例に 17ＢＭＰの血中濃度が高値である症例が認められたことから、腸管粘膜障害とＢＤＰ吸収の亢進との関連が示唆された。以上の結果から、腸管ＧＶＨＤに対する経口ＢＤＰ投与は、必ずしも全身的副作用が無視できるものではない点に留意すべきと考えられた。

A．研究目的

移植片対宿主病（Graft-versus-host disease：ＧＶＨＤ）は、同種造血幹細胞移植後の重篤な合併症の一つである。ステロイド薬の全身投与は、ＧＶＨＤに対する標準的な治療法であるが、感染症の増加など副作用が問題となる。

ジプロピオン酸ベクロメタゾン（beclomethasone dipropionate：ＢＤＰ）は、本邦において気管支喘息・アレルギー性鼻炎に対する吸入剤として一般的に使用されている薬剤である。ＢＤＰは、肺や腸管から吸収後、エステラーゼにより急速に分解され主要活性代謝物である 17-モノプロピオン酸ベクロメタゾン（beclomethasone-17-monopropionate：17ＢＭＰ）に代謝され、17ＢＭＰはステロイド活性を持たないベクロメタゾン（beclomethasone ：ＢＯＨ）へ代謝される。ＢＤＰは初回通過効果により大部分が代謝されることから、全身的な作用は少ないことが報告されている。こうした体内動態の特性から、経口ＢＤＰは、副作用を軽減した腸管 GVHD の治療薬として、我々を含めた数グループからその安全性と有効性が報告されている。一方で腸管ＧＶＨＤに対する経口ＢＤＰ治療中に、副腎機能抑制などの全身的な作用を示唆させる報告も散見されている。今回、我々は、腸管ＧＶＨＤ患者における経口ＢＤＰの吸収の程度を確認するため、ＢＤＰおよびその代謝物の血中濃度について検討した。

B．研究方法

1．　対象

平成 18 年 10 月から平成 19 年 1 月までの期間に当院で同種造血幹細胞移植を受け、腸管 GVHD（急性、慢性を含む）に対して経口ＢＤＰを投与された患者を対象とした。

2．　院内製剤経口ＢＤＰと規格

院内製剤経口ＢＤＰは、MP Biomedical 社のジプロピオン酸ベクロメタゾンの原末を使用し、院内製剤した。カプセル剤とシロップ剤の２つの剤型とし、ＢＤＰカプセルは、小腸で溶解後、小腸及び大腸に活性薬物が供給され下部消化管で作用することを期待し、ＢＤＰ内服液は、上部消化管に作用することを期待した。院内製剤経口ＢＤＰの規格に関しては、ＢＤＰカプセル１カプセル中 1mg のＢＤＰ、ＢＤＰ内服液 30mℓ 中 1mg のＢＤＰを含有するように院内製剤した。

3．　ＢＤＰカプセルとＢＤＰ内服液の投与方法と血中濃度測定時間

投与方法は、全対象症例においてＢＤＰカプセル

は1回1カプセルを1日4回（6時、11時、16時、21時）、ＢＤＰ内服液は1回30mℓを1日4回（カプセル服用の15分後）、経口投与した。血中濃度測定は、ＢＤＰ投与開始後3日目以降でＢＤＰカプセル服用約4時間後（BDP内服液服用後3時間45分）を目安とした。

4．　ＢＤＰ、17ＢＭＰ、ＢＯＨの血中濃度測定方法

ＢＤＰ、17ＢＭＰ、ＢＯＨの血中濃度測定は、Applied Biosystems/MDS SCIEX 社の API 3200 ™ LC-MS-MS system （LC-MS/MS）で行った。高速液体クロマトグラフィー（high-performance liquid chromatography：HPLC）のカラムはSymmetry Shield ™RP8 5μm2.1×150mm Column（Waters Corps.）を用い、LC-MS/MSで測定を行った。

＜倫理面への配慮＞

対象例すべてから文書によるインフォームドコンセントを取得した。対象患者の個人情報はデータ取得後直ちに連結不能な暗号化がなされ、当該分担研究者によって厳格に管理された。

Ｃ．研究結果

1．　患者背景と採血時間

対象患者数は5例で、年齢中央値は62歳（28-68）であった。原疾患は急性骨髄性白血病3例、急性リンパ性白血病1例、慢性骨髄性白血病1例で、移植細胞源は、臍帯血2例、骨髄1例、同種末梢血2例であった。採血時間は、症例2～5ではＢＤＰカプセル服用4.5時間後であったが、症例1ではＢＤＰカプセル服用1.5時間後であった。

2．　ＢＤＰ、17ＢＭＰ、ＢＯＨの血中濃度

ＢＤＰカプセル服用1.5時間後に測定した症例1は、17ＢＭＰの血中濃度が2439±161 pg/mℓまで上昇した。ＢＤＰカプセル服用4.5時間後に測定した4例全例で17ＢＭＰは、618～1749 pg/mℓの範囲の血中濃度で検出された。ＢＤＰは全例、ＢＯＨは2例で検出感度以下であった。

3．　17ＢＭＰの血中濃度と腸管ＧＶＨＤのstageの関連

腸管ＧＶＨＤの重症度と経口ＢＤＰの吸収を検討するため、17ＢＭＰの血中濃度と腸管ＧＶＨＤの関連性を検討した。症例1は、服用1.5時間後に採血したため解析対象から除外した。解析症例4例のうち、腸管ＧＶＨＤに伴う症状は全例下痢でありstage1は1名、stage2は1名であり17ＢＭＰの血中濃度はそれぞれ696pg/mℓ、618pg/mℓであった。stage3は2名であり17ＢＭＰの血中濃度は1166pg/mℓ、1749pg/mℓであった。腸管ＧＶＨＤのstageが高い症例では17ＢＭＰの血中濃度が高値である傾向が認められた。

Ｄ．まとめ・考察

全対象患者5例でＢＤＰの主要活性代謝物である17ＢＭＰが血中で検出された。1995年のMcDonaldらの研究では、経口ＢＤＰを投与した腸管ＧＶＨＤ患者20例中11例（55%）に副腎抑制を認めたことより、経口ＢＤＰは吸収され、全身的な副作用が発症しうることが示唆されている。今回、我々の研究結果は、McDonaldらが副腎抑制により間接的にＢＤＰの吸収を示唆した報告を支持する結果となった。

健常人でのＢＤＰ1000μｇの単回吸入投与の報告によると17ＢＭＰの血中濃度は、2103pg/mℓであった。また、ＢＤＰの吸入剤であるキュバール®において、ＢＤＰ400μｇを軽度から中程度の気管支喘息患者に単回吸入投与した際の17ＢＭＰの血中濃度は1,419pg/mℓであった。今回の研究では、1回2mg（カプセル1mg、内服液1mg）を1日4回投与したが、5例中4例において17ＢＭＰの血中濃度は618pg/mℓから1,749pg/mℓであった。これは、ＢＤＰ1回400～1,000μｇを単回吸入投与した時（1,419～2,103 pg/mℓ）と同程度あるいはそれ以下の血中濃度であることが確認された。吸入剤投与時のＢＤＰ1日投与量が1500μｇまでの場合、副腎皮質系抑制の有意な危険性はないとの報告がある事より吸入剤と同程度の血中濃度であれば副腎皮質系抑制の危険性は低いと考える。1例は、17ＢＭＰの血中濃度は2,439±161 pg/mℓで吸入剤投与時以上の血中濃度の上昇がみられた。

健常人を対象とした研究で経口ＢＤＰ服用後の17ＢＭＰの最高血中濃度到達時間が4時間であったことより本研究は、血中濃度測定時期をＢＤＰ

カプセル服用約 4 時間後としたが、実際の測定時間は、症例 1 はＢＤＰカプセル服用 1.5 時間後、症例 2 から 5 は 4.5 時間後であった。ＢＤＰカプセル服用 1.5 時間後に測定した症例 1 の 17ＢＭＰの血中濃度は 2,439pg/mℓ と 5 症例のうち最高値を示した。症例 1 は、ＢＤＰ開始時の腸管ＧＶＨＤの stage が 3 であり高度の腸管粘膜障害を呈していた。腸管輸送(運動)能が保持されていて、粘膜障害も高度でない、stage 1 及び 2 の患者では健常者と同程度の Tmax である可能性があるものの、stage 3 以上の腸管ＧＶＨＤ患者では、最高血中濃度到達時間は、健常人と異なる可能性を有すると考える。

腸管ＧＶＨＤの stage1 と stage2 の症例における 17ＢＭＰの血中濃度は、それぞれ 696pg/mℓ、618pg/m であり健常人にＢＤＰ 2mg を単回経口投与し服用 4 時間後の最高血中濃度（703 pg/mℓ）と比較して同程度の血中濃度であった。腸管ＧＶＨＤの stage3 の症例 2 名の 17ＢＭＰの血中濃度は 1166pg/mℓ、1749pg/mℓ であり、健常人の血中濃度と比較しそれぞれ 1.7 倍、2.5 倍と高値を示したことからＧＶＨＤの stage が高い患者では健常人よりも血中濃度が上昇する可能性が示唆された。また、腸管ＧＶＨＤの stage が高い症例で 17ＢＭＰの血中濃度が高値である傾向が認められた原因として、腸管ＧＶＨＤの重症度つまり腸管粘膜障害の程度が高まるにつれて経口ＢＤＰの吸収が亢進し血中濃度が上昇した可能性がある。特に重度の腸管ＧＶＨＤが発症している症例は、経口ＢＤＰによる全身的な副作用の発現の可能性を考慮すべきである。今後、多数例での検討が必要である。

Ｅ．結論

腸管ＧＶＨＤに対して経口ＢＤＰを投与する際には、全身的作用を留意すべきと考えられた。

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Ｇ．知的財産権の出願・登録状況

1. 特許取得

該当なし

2. 実用新案登録

該当なし

3. その他

該当なし

Ⅲ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

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Ⅳ. 研究成果の刊行物・別刷

Bone Marrow Transplantation (2009), 1–7

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ORIGINAL ARTICLE

Pharmacokinetics of CsA during the switch from continuous intravenous infusion to oral administration after allogeneic hematopoietic stem cell transplantation

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We investigated the serial changes in the blood CsA concentration during the switch from continuous intravenous infusion to twice-daily oral administration in allogeneic hematopoietic stem cell transplant recipients ($n=12$). The microemulsion form of CsA, Neoral, was started at twice the last dose in intravenous infusion in two equally divided doses. The area under the concentration–time curve during oral administration (AUC_{PO}) was significantly higher than the AUC during intravenous infusion (AUC_{IV}) (median 7508 vs 6705 ng/ml × h, $P=0.050$). The median bioavailability of Neoral, defined as ($AUC_{PO}/DOSE_{PO}$) divided by ($AUC_{IV}/DOSE_{IV}$), was 0.685 (range, 0.45–1.04). Concomitant administration of oral voriconazole ($n=4$) significantly increased the bioavailability of Neoral (median 0.87 vs 0.54, $P=0.017$), probably due to the inhibition of gut CYP3A4 by voriconazole. Although the conversion from intravenous to oral administration of CsA at a ratio of 1:2 seemed to be appropriate in most patients, a lower conversion ratio may be better in patients taking oral voriconazole. To obtain a similar AUC, the target trough concentrations during twice-daily oral administration should be halved compared with the target concentration during continuous infusion.

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Keywords: CsA; pharmacokinetics; bioavailability; drug interaction

Introduction

CsA is the most widely used immunosuppressive agent for the prophylaxis of GVHD after allogeneic hematopoietic stem cell transplantation (HSCT). It is usually administered by intravenous infusion for at least several weeks after allogeneic HSCT because of the damage done to the oral and gastrointestinal mucosa by the conditioning regimen. However, the dose, target blood level, and schedule of administration vary among protocols and have not been optimized.[1] It has been shown that the blood concentration of CsA affects the incidences of acute GVHD and adverse events,[2] and an increase in the target blood concentration from 300 to 500 ng/ml in the continuous infusion of CsA significantly decreased the incidence of acute GVHD.[3] On the basis of these results, we are currently administering CsA by continuous infusion with target concentrations of 500 ng/ml for standard-risk patients and 300 ng/ml in high-risk patients. When patients can tolerate oral intake, CsA is switched from intravenous to oral administration at a dose ratio of 1:2. Neoral, a microemulsion formulation of CsA, has improved bioavailability and is the most commonly used oral product.[4] However, the appropriateness of this conversion rate has been inconsistent among earlier studies.[5,6] Parquet *et al.* reported that doubling the last intravenous dose provided the best therapeutic range concentration, whereas the concentration/dose ratio was similar in intravenous administration and oral administration and thus, 1:1 conversion seemed appropriate in the McGuire's study. In addition, no data are available regarding the detailed pharmacokinetics in allogeneic HSCT recipients. Therefore, in this study, we investigated the serial changes in the CsA blood concentration during the switch from intravenous to oral administration and assessed the bioavailability of Neoral.

Patients and methods

Patients

Patients who underwent allogeneic HSCT with GVHD prophylaxis consisting of the continuous infusion of CsA and short-term MTX were included. This single-center prospective study was approved by the Institutional Review Board of Jichi Medical University, and each patient provided their written informed consent to be enrolled in the study.

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Transplantation procedure
The conditioning regimen was mainly a combination of cyclophosphamide (60 mg/kg for 2 days) and TBI (2 Gy twice daily for 3 days) ($n=8$). Patients with severe aplastic anemia ($n=3$) were prepared with fludarabine, cyclophosphamide, and anti-thymoglobulin with or without a low dose of TBI at 2 Gy.[7] A reduced-intensity regimen with fludarabine and melphalan was used for a 58-year-old patient with acute lymphoblastic leukemia ($n=1$). GVHD prophylaxis consisted of the continuous infusion of CsA with a starting dose of 3 mg/kg/day and short-term MTX (10–15 mg/m^2 on day 1 and 7–10 mg/m^2 on days 3 and 6, and optionally on day 11 in HSCT from a donor other than an HLA-matched sibling). The dose of CsA was adjusted to maintain the blood CsA concentration between 450 and 550 ng/ml in standard-risk patients ($n=9$) or 250 and 350 ng/ml in high-risk patients ($n=3$) according to the disease status.[3] Acute GVHD was graded as described earlier.[8] Prophylaxis against bacterial, fungal, and Pneumocystis jiroveci infection consisted of levofloxacin, fluconazole (FLCZ), and sulfamethoxazole/trimethoprim (ST) or inhalation of pentamidine. In three patients, micafungin (MCFG) was used instead of FLCZ because of persistent fever despite broad-spectrum antibiotic therapy, development of Candidemia, and high risk for invasive aspergillosis, respectively. As prophylaxis against herpes simplex virus infection, acyclovir (ACV) was given from days −7 to 35, followed by a long-term low-dose administration of ACV for varicella zoster reactivation.[9] Pre-emptive therapy with ganciclovir for cytomegalovirus infection was performed by monitoring cytomegalovirus antigenemia.[10]

Study schedule
When patients were able to tolerate oral intake, CsA was switched from continuous infusion to oral administration. Intravenous infusion was stopped just before the first oral administration. The initial dose of Neoral was twice the last daily dose of continuous infusion, and was given in two equally divided doses based on the reported bioavailability of Neoral of about 0.4 (40%) in allogeneic HSCT recipients.[5] On the last day of the continuous infusion of CsA (day −1), the serum CsA concentration was measured at 9:00, 15:00, and 21:00. After the patient was switched to Neoral, the CsA concentration was measured just before (C_0), and 1 (C_1), 2 (C_2), 3 (C_3), 4 (C_4), 6 (C_6), and 12 (C_{12}) hours after the oral administration of Neoral on the first day (day 0) and between day 3 and day 5. The CsA concentration was measured using the CYCLO-Trac SP-whole blood kit (DiaSorin, Inc., Stillwater, MN, USA).[11] In brief, 200 µl of whole blood sample was mixed with 800 µl of methanol and centrifuged at 1600 *g* for 5 min. The methanolic supernatant (50 µl in duplicate) was mixed with 100 µl of ^{125}I-ligand and 1 ml of anti-CYCLO-Trac Immune Sep (pre-mixed mouse monoclonal antibody, donkey anti-mouse serum, and normal mouse serum). After centrifuging, the ligand was discarded by decanting and the amount of radioactivity of the pellet was determined. Data were analyzed by logit-log reduction. The standard curve was obtained using the CsA standard sera provided in the kit. The intra-assay coefficient of variance was <15%. The inter-assay coefficient of variance was <14%. The limit of detection was 4.0 ng/ml. The results of this assay showed good correlation with those obtained by high-performance liquid chromatography ($r=0.98$).

During the study, the dose of CsA could be modified at the discretion of each physician. Vital signs and laboratory variables including renal and liver function tests were evaluated on days 0, 3, 7, and 14. Concomitant medications that could potentially interact with CsA were recorded.

Statistical considerations
The area under the concentration–time curve (AUC) (0–12 h) of CsA was calculated by the trapezoidal method. We estimated the bioavailability of Neoral by dividing ($AUC_{PO}/DOSE_{PO}$) by ($AUC_{IV}/DOSE_{IV}$). Toxicities after switching from intravenous to oral administration were evaluated compared with the baseline data on day 0. Renal toxicity was defined as an elevation of the creatinine (Cr) level above × 1.5 the baseline value. Liver dysfunction was defined as an elevation of alanine aminotransferase (ALT) above × 2 the baseline value, or elevation of the total bilirubin (T-bil) level by 2 mg per 100 ml compared with the baseline value. Comparisons were made using the Wilcoxon signed-rank test for continuous variables. The Pearson correlation coefficient was used to analyze the correlation between AUC and the CsA concentration at each measurement point after logarithmic transformation. The effect of concomitant medications on CsA pharmacokinetics was first analyzed by a univariate analysis with the Mann–Whitney *U*-test, and then those with at least borderline significance ($P<0.10$) were subjected to a multivariate analysis using multiple regression modeling. A *P*-value of <0.05 was considered to be significant.

Results

Patients
Between January 2008 and April 2009, 12 patients were enrolled in the study. There were 7 males and 5 females with a median age of 34.5 years (range, 16–58). Underlying diseases included acute myeloblastic leukemia ($n=4$), acute lymphoblastic leukemia ($n=3$), severe aplastic anemia ($n=3$), chronic myelogenous leukemia ($n=1$), and myelodysplastic syndrome ($n=1$). Five patients received bone marrow graft from an unrelated donor, whereas 1 and 6 patients, respectively, received bone marrow and peripheral blood stem cell graft from a related donor. There was an HLA mismatch in three donor-recipient pairs.

Pharmacokinetic analysis
The median duration from transplantation to the switch from intravenous to oral administration was 40 days (range, 27–60). The dose of CsA and the pharmacokinetic parameters during intravenous and oral administration are shown in Table 1. Neoral was started at approximately twice the last dose of intravenous infusion, except that 1 patient (No. 8) received Neoral at the same dose as in intravenous infusion, as the mean CsA concentration on the last day of intravenous infusion was >700 ng/ml.

Table 1 Dose of CsA and pharmacokinetic parameters during the intravenous and oral administration of CsA

Patient no.	*Day −1*			*Day 0*					*Steady state (Days 3–5)*				
	$DOSE_{IV}$ (mg/day)	C_{mean} (ng/ml)	AUC_{IV} (ng/ml × h)	$DOSE_{PO}$ (mg/day)	C_{max} (ng/ml)	T_{max} (h)	C_{min} (ng/ml)	AUC_{IV-PO} (ng/ml × h)	$DOSE_{PO}$ (mg/day)	C_{max} (ng/ml)	T_{max} (h)	C_{min} (ng/ml)	AUC_{PO} (ng/ml × h)
1	96	590	7110	200	1300	2	370	9525	160	1400	3	550	10625
2	140	643	7680	280	1600	3	480	10860	250	1000	2	320	7080
3	130	553	6630	260	2700	3	360	12555	160	1200	2	290	7790
4	173	663	7950	360	1900	2	340	11785	360	2500	1	420	12420
5	192	677	7920	400	1500	3	240	8685	400	1500	2	280	8355
6	125	577	6780	260	1200	2	360	8300	260	1200	3	360	8450
7	80	527	6330	160	650	0	390	5725	160	800	2	280	6105
8	192	717	8730	200	930	2	360	8100	200	990	4	300	7225
9	240	477	5820	500	1600	3	280	9035	500	2400	2	290	11265
10	125	357	4350	260	840	2	210	5285	260	880	2	210	5310
11	58	257	3090	120	720	2	130	3375	120	360	4	110	2860
12	77	303	3690	160	1100	2	190	6025	160	1000	1	260	6590

Abbreviations: AUC_{IV} = area under the concentration–time curve (AUC) during continuous infusion; AUC_{PO} = AUC during oral administration; $DOSE_{IV}$ = dose of CsA during continuous infusion; $DOSE_{PO}$ = dose of CsA during oral administration.

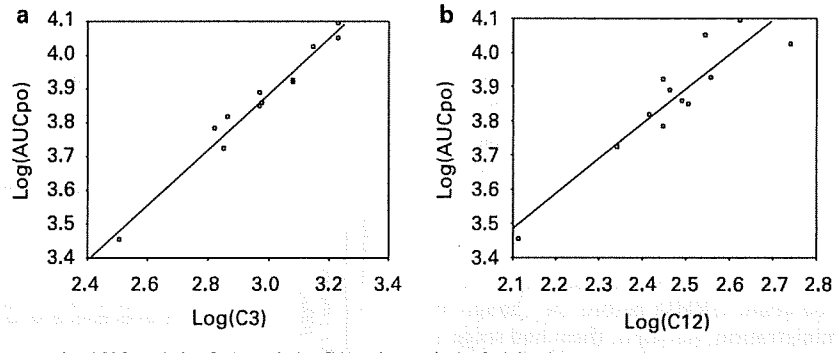

Figure 1 Correlation between the AUC and the CsA peak (a: C_3) and trough (b: C_{12}) levels.

In three patients (Nos. 1, 2, and 3), the dose of CsA was reduced on day 1 due to the high CsA concentration on day 0 (the day when Neoral was started).

The median AUC value was 6705 ng/ml × h (AUC_{IV}; range, 3090–8730) before the conversion from intravenous to oral administration (day −1), 8493 ng/ml × h (AUC_{IV-PO}; range, 3375–12 555) on day 0, and 7508 ng/ml × h (AUC_{PO}; range, 2860–12420) on days 3–5, respectively. AUC_{PO} was considered to be the AUC of Neoral in the steady state, as AUC_{IV-PO} was affected by the intravenous administration of CsA and at least 3 days are required for the CsA concentration to stabilize after a change in the administration route. As a result, not only AUC_{IV-PO} but also AUC_{PO} was significantly higher than AUC_{IV} ($P = 0.050$), even though the dose of Neoral was reduced in three patients and the conversion ratio was 1:1 in another patient. The median bioavailability of Neoral was 0.685 (range, 0.45–1.04).

Relationship between AUC and the CsA concentration at each measurement point

Although the CsA concentration at each measurement point significantly correlated with AUC_{PO} after logarithmic transformation, the strongest correlation was observed between C_3 and AUC_{PO} (Figure 1a and Table 2, correlation coefficient 0.984, $P < 0.001$). The AUC_{PO} could be predicted from the trough concentration (C_0 or C_{12}), which is widely measured in daily practice, by the following formula based on the linear regression model: $\text{Log}(AUC_{PO}) = 1.020 \times \text{Log}(C_{12}) + 1.344$ (Figure 1b). Accordingly, each trough concentration between 50 and 250 ng/ml corresponds to the CsA concentration during the continuous intravenous infusion of CsA with the same AUC, calculated by dividing the predicted AUC by 12, between 99 and 514 ng/ml (Table 3). Thus, when the continuous intravenous administration of CsA with a target concentration of 500 ng/ml was switched to twice-daily oral administration, the target trough level should be about 250 ng/ml to obtain the same AUC. Also, the target blood concentration of 300 ng/ml during continuous infusion corresponds to the target trough concentration at 150 ng/ml during twice-daily oral administration. This estimation was different from that in kidney transplantation by Nakamura *et al.* (Table 3).[12]

Influence of possible confounding factors on the bioavailability of Neoral

With regard to laboratory data, there were no statistically significant correlations between the bioavailability of Neoral and the serum Cr level, ALT level, and T-bil level

Table 2 Correlation coefficients between the AUC and the cyclosporine concentration at each measurement point

	Correlation coefficient	P-*value*	*Conversion formula*
C0	0.869	<0.001	$Log(AUCPO) = 0.846 \times Log(C0) + 1.747$
C1	0.874	<0.001	$Log(AUCPO) = 0.465 \times Log(C1) + 2.539$
C2	0.953	<0.001	$Log(AUC_{PO}) = 0.718 \times Log(C_2) + 1.693$
C3	0.984	<0.001	$Log(AUC_{PO}) = 0.821 \times Log(C_3) + 1.424$
C4	0.918	<0.001	$Log(AUC_{PO}) = 0.876 \times Log(C_4) + 1.319$
C6	0.961	<0.001	$Log(AUC_{PO}) = 1.314 \times Log(C_6) + 0.258$
C12	0.921	<0.001	$Log(AUC_{PO}) = 1.020 \times Log(C_{12}) + 1.344$

Abbreviation: AUC_{PO} = area under the concentration–time curve during oral administration.

Table 3 Target cyclosporine concentration during continuous infusion to obtain a similar AUC during twice-daily oral administration with each target trough concentration

Trough level of CsA during twice-daily oral administration (ng/ml)	*Corresponding CsA concentration during continuous infusion*	
	Nakamura et al.[12]	*Current study*
50	128	99
100	255	202
150	383	305
200	510	409
250	638	514

Abbreviation: AUC = area under the concentration–time curve.

($P = 0.867$, $P = 0.159$, and $P = 0.770$, respectively). Four patients had developed acute GVHD before the change in the route of CsA administration, but all of them had stage 1 skin GVHD that was successfully controlled by topical steroid. None of the patients had gastrointestinal involvement and thus the influence of gut GVHD on the bioavailability of Neoral could not be evaluated.

With regard to drug interactions, the effects of the following drugs on the bioavailability of Neoral were evaluated; antifungal agents including FLCZ, itraconazole (ITCZ), voriconazole (VRCZ), and MCFG, antibacterial agents including ST, vancomycin, fluoroquinolones (FQ), and cefepime, antiviral agents including ACV and ganciclovir (DHPG), and other drugs including amlodipine, sulpiride, gabapentin, and prednisolone (PSL) (Table 4). FLCZ ($n = 3$), ITCZ ($n = 3$), and VRCZ ($n = 4$) were exclusively administered orally. These agents had been started at least 7 days before the change in the route of CsA administration. By the Mann–Whitney *U*-test, VRCZ, FQ, and ST were shown to have significant effects with at least borderline significance ($P = 0.048$, $P = 0.061$, and $P = 0.100$, respectively). Among these, only VRCZ was identified as an independent significant factor by a multivariate analysis ($P = 0.017$). The median bioavailability of Neoral in patients taking VRCZ was 0.87 (range, 0.76–1.04), whereas it was only 0.54 (range, 0.45–0.94) in those without VRCZ.

Clinical course after the change in the route of CsA administration

One patient (No. 2) developed liver dysfunction with an elevation of ALT from 28 IU/l at baseline to 300 IU/l 2

Table 4 Clinical and laboratory data at the conversion that could influence the cyclosporine pharmacokinetics

Patient no.	AUC_{IV} / $DOSE_{IV}$	AUC_{PO} / $DOSE_{PO}$	*Bioavailability*	*aGVHD*			*Cr (mg per 100 ml)*	*Liver function*		*Concomitant medications*	
				Grade	*Stage*			*ALT (IU/l)*	*T-bil (mg per 100ml)*	*Antifungal agents*	*Others*
					Skin	*Liver*	*Gut*				
1	74	66	0.89	I	(1	0	0)	1.14	40	0.24	VRCZ 400 mg po	VCM, ST, ACV, PPI
2	55	28	0.51	0	(0	0	0)	0.65	28	0.9	ITCZ 200 mg po	ACV, PPI, FQ
3	47	49	1.04	0	(0	0	0)	0.81	182	0.77	VRCZ 400 mg po	ST, ACV, PPI, amlodipine gabapentin
4	46	35	0.76	I	(1	0	0)	0.98	28	1.06	VRCZ 400 mg po	ST, ACV, PPI, PSL
5	41	21	0.51	0	(0	0	0)	0.89	43	0.33	FLCZ 200 mg po	ACV, PPI
6	54	33	0.61	0	(0	0	0)	0.65	92	0.79	ITCZ 200 mg po	DHPG, PPI, amlodipine
7	79	38	0.48	I	(1	0	0)	0.72	85	0.59	ITCZ 200 mg po	DHPG, PPI, amlodipine
8	45	36	0.8	0	(0	0	0)	0.60	78	0.78	FLCZ 200 mg po	ACV, PPI
9	24	23	0.94	0	(0	0	0)	0.66	96	0.65	MCFG 150 mg iv	CFPM, ACV, PPI, amlodipine
10	35	20	0.57	0	(0	0	0)	1.43	46	0.37	FLCZ 200 mg po	CFPM, ACV, PPI
11	53	24	0.45	I	(1	0	0)	0.84	16	0.53	MCFG 150 mg iv	ACV, PPI, FQ, sulpiride
12	48	41	0.85	0	(0	0	0)	1.19	20	0.55	VRCZ 400 mg po	ACV, PPI

Abbreviations: ACV = acyclovir; ALT = alanine aminotransferase; AUC_{IV} = area under the concentration–time curve (AUC) during continuous infusion; AUC_{PO} = AUC during oral administration; CFPM = cefepime; DHPG = ganciclovir; $DOSE_{IV}$ = dose of CsA during continuous infusion; $DOSE_{PO}$ = dose of CsA during oral administration; FLCZ = fluconazole; FQ = fluoroquinolones; ITCZ = itraconazole; MCFG = micafungin; PPI = proton pump inhibitors; PSL = predonisolone; ST = sulphametoxazole-trimetoprim; VCM = vancomycin; VRCZ = voriconazole.

Table 5 Serial changes in laboratory data and blood pressure after the change in the route of CsA administration

	Mean (minimum–maximum)			
	Serum creatinine (mg per 100 ml)	*ALT (IU/l)*	*Total bilirubin (mg per 100 ml)*	*Blood pressure level (mm Hg)*
Day 0	0.87 (0.60–1.43)	64.4 (16–182)	0.63 (0.24–1.06)	Systolic 130 (114–173) Diastolic 82 (63–103)
Day 3	0.86 (0.32–1.63)	50.1 (10–106)	0.62 (0.27–1.47)	Systolic 124 (109–150) Diastolic 79 (51–103)
Day 7	0.92 (0.69–1.31)	44.6 (10–103)	0.61 (0.30–1.17)	Systolic 122 (109–132) Diastolic 80 (51–103)
Day 14	0.83 (0.67–1.29)	65.8 (10–300)	0.64 (0.27–0.96)	Systolic 121 (113–135) Diastolic 76 (68–89)

Abbreviation: ALT = alanine aminotransferase.

weeks after the conversion. The AUC of CsA was rather lower after conversion, and thus CsA was not considered to be the causative agent of liver dysfunction. Otherwise, no notable changes in laboratory and clinical data were observed (Table 5).

Four patients had developed grade I acute GVHD of the skin before the change in the route of CsA administration. During the 2 weeks after the switch, 3 of the 4 patients had persistent grade I skin GVHD, whereas GVHD was improved in 1 patient. Among the eight patients who did not have acute GVHD at the switch, one patient developed grade I acute GVHD of the skin, which was well controlled by topical steroid, and the other seven patients did not develop acute GVHD during the observation period. No clinically significant changes in vital or biological parameters occurred in the study patients. One patient (No. 9) developed nausea soon after conversion. An excessive increase in the CsA concentration was considered to be the cause of nausea and this symptom was improved after the dose of Neoral was reduced.

Discussion

Neoral is a microemulsion formulation of CsA that has improved bioavailability and reduced variability in pharmacokinetic parameters within and between patients compared with a conventional CsA formulation (Sandimmun).[4] Its bioavailability has been reported to be 0.38 (38%) in healthy volunteers.[13] However, allogeneic HSCT patients have complications that could influence the CsA pharmacokinetics, such as damaged gastrointestinal mucosa and multiple drug interactions. The results of this study showed that the median value of the bioavailability of Neoral was 0.685 (range, 0.45–1.04). Detailed analyses revealed that the oral administration of VRCZ strongly affected the bioavailability of Neoral (0.87 vs 0.54). Therefore, although the switch from intravenous to oral administration of CsA at a ratio of 1:2 seemed to be appropriate in most patients, a lower conversion ratio such as 1:1.1 or 1:1.2 may be better in patients taking oral VRCZ.

The drug interactions between CsA and azole antifungal agents including FLCZ, ITCZ, and VRCZ have been well recognized.[14] Azole antifungal agents are metabolized through the cytochrome P450-3A (CYP3A4) enzyme system, interfere with the metabolism of CsA, and thereby increase the exposure to CsA. Therefore, careful monitoring of the blood CsA concentration is recommended when these agents are added during CsA administration. On the other hand, there are considerable differences among azole antifungals with regard to their ability to inhibit CYP3A4.[14] Interestingly, the concomitant use of oral VRCZ significantly increased the bioavailability of Neoral. We confirmed that VRCZ was started at least 7 days before the switch from intravenous to oral administration of CsA and was continued at the same dose after the switch. Therefore, the drug interaction between CsA and VRCZ seemed to be stronger during oral administration than during the intravenous infusion of CsA. We hypothesized that this stronger interaction can be explained by the presence of the P450 enzyme system in the gastrointestinal mucosa. The CYP3A4 isoenzymes are the most abundant isoforms of CYP and it has been postulated that CsA is also metabolized in the intestine by gut CYP3A4 isoenzymes.[15] The administration of VRCZ might have inhibited the gut metabolism of CsA and increased the bioavailability of CsA. However, a prospective controlled study is required to confirm this hypothesis.

ITCZ, another strong inhibitor of CYP3A4, did not increase the bioavailability of Neoral. As the ratio of $AUC_{IV}/DOSE_{IV}$ was higher not only in patients taking VRCZ but also in patients taking ITCZ compared with other patients (median 47.5, 55, and 41), ITCZ might have inhibited liver CYP3A4 similar to VRCZ, but inhibited gut CYP3A4 less strongly than VRCZ. This might have been affected by the different bioavailable dose of these agents, as the bioavailability of ITCZ is lower than that of VRCZ, in addition to the fact that the dose of ITCZ was lower than that of VRCZ (200 vs 400 mg/day).

With regard to the route of VRCZ, it was exclusively administered orally in this study. Therefore, we could not conclude whether the intravenous administration of VRCZ would similarly affect the bioavailability of CsA. In earlier reports, the extent of drug interaction between CsA and azole antifungals varied according to the route of administration and the dose or kind of antifungal agent. Numerous reports have shown a significant interaction (>84%) between oral FLCZ with a dose of 200 mg/day or greater and oral CsA.[16,17] On the other hand, Osowski *et al.*[18] evaluated the drug interaction between intravenous FLCZ at 400 mg/day and intravenous CsA in HSCT recipients and there was a statistically significant but smaller increase (21%) in the serum CsA concentration.

Mihara *et al.*[19] reported that the mean steady-state whole-blood level of CsA significantly increased after the route of FLCZ administration was switched from intravenous to oral. These data suggest that the drug interaction between CsA and FLCZ was stronger when FLCZ was administered orally. With regard to other azole antifungal agents, not only oral but also intravenous administration of ITCZ significantly affected the blood concentration of CsA.[20–22] Concerning the interaction between VRCZ and CsA, Mori *et al.*[23] reported that the administration of VRCZ to patients receiving CsA resulted in a significant increase in the concentration/dose ratio of CsA, but the route of VRCZ administration did not affect the changes in the concentration/dose ratio. If we consider these findings together, it may be reasonable to suggest that the interaction between azole antifungal agents and CsA is stronger when the antifungals are given orally, but the difference becomes unclear with ITCZ and VRCZ, as the interactions of these agents are stronger than that of FLCZ and can be detected even when they are given intravenously. Therefore, when we interpret pharmacokinetic data of CsA, we must be cautious not only about concomitantly used agents but also the route of administration of both CsA and the other drugs. For example, Parquet *et al.* reported that a ratio of 1:2 in the switch from intravenous to oral administration was appropriate,[5] whereas a 1:1 ratio seemed to be appropriate in the study by McGuire *et al.*[6] In the former study, oral FLCZ was used concomitantly and thus their conclusion was consistent with our data. In the latter study, information on the use of antifungal agents was not described, and thus the data were difficult to interpret.

When we switch the route of CsA administration from continuous infusion to twice-daily oral administration, the target blood concentration should also be changed. Nakamura *et al.*[12] reported that the CsA blood concentration during continuous infusion was estimated to be 2.55 times the trough level during twice-daily oral administration of Neoral to obtain an equal AUC of CsA in kidney transplant patients. In this study, we concluded that the CsA concentration during continuous infusion should be doubled compared with the trough concentration during twice-daily oral administration in allogeneic HSCT recipients. Although the calculation method was different, the conclusion was consistent (mean 2.01) when we applied their methods. Although the reason for the difference between these studies remains unclear, it may have been due to the differences in the use of concomitant drugs or the status of the gastrointestinal tract.

In conclusion, when switching CsA from continuous infusion to oral administration, concomitant medications that could affect the bioavailability of CsA, especially azole antifungal agents, should be taken into account. Although a 1:2 ratio on switching may be appropriate in most patients, a lower conversion ratio is recommended in patients taking oral VRCZ.

Conflict of interest

The authors declare no conflict of interest.

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LETTER TO THE EDITOR

Target blood concentrations of CYA and tacrolimus in randomized controlled trials for the prevention of acute GVHD after hematopoietic SCT

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In a recent issue of *Bone Marrow Transplant*, Ram *et al.*[1] reported a systematic review and meta-analysis of prophylaxis regimens for GVHD. They combined three randomized controlled trials that compared the combination of CYA and MTX (CYA–MTX) with the combination of tacrolimus and MTX (TAC–MTX), and concluded that although TAC–MTX was superior to CYA–MTX in terms of acute GVHD reduction, the incidence of all-cause mortality was similar.[1–4] A large retrospective study in Japan also revealed that the incidence of grades II–IV acute GVHD was significantly lower in patients who received TAC than in those who received CYA in matched unrelated donor transplantation, whereas no such difference was observed in matched sibling donor transplantation.[5] Although it is difficult to directly compare the incidence of GVHD among these studies because of the difference in the study population, these findings suggest that although TAC is more effective than CYA for preventing acute GVHD, this benefit does not confer a survival benefit, probably because of increased toxicities.

However, before making such a conclusion, we have to consider whether CYA and TAC were administered at appropriate doses in these studies. Table 1 summarizes the design and results of the three randomized controlled trials. CYA was continuously infused with a target blood concentration between 150 and 450 ng/ml. This target concentration might have been too low, as the target concentration was equivalent to the target trough concentration that is widely accepted in European centers when CYA was administered twice daily.[6] The target steady-state concentration in the continuous infusion of CYA should be higher than the trough concentration in twice-daily administration to provide an equal area under the concentration-time curve.[7] In fact, in a retrospective study, the incidence of acute GVHD was significantly higher in patients who received a continuous infusion of CYA with a target concentration between 250 and 400 ng/ml than in those who received a twice-daily infusion of CYA with a target trough concentration between 150 and 300 ng/ml.[8] In contrast, the target concentration of TAC was between 10 and 30 ng/ml in the two randomized trials in the United States and between 20 and 25 ng/ml in the Japanese trial. These target concentrations were apparently higher than that in current transplantation practice. Couriel *et al.*[9] recommended a blood concentration of TAC between 8 and 12 ng per 100 ml based on their retrospective and prospective studies.

It has been shown that the blood concentrations of CYA and TAC affect the incidences of acute GVHD and adverse events.[10] In addition, an increase in the target blood concentration from 300 to 500 ng/ml in continuously infused-CYA significantly decreased the incidence of acute GVHD.[11] This difference was more prominent in transplantation from an unrelated donor, similar to the fact that the difference in the incidence of acute GVHD between patients who received CYA and those who received TAC was observed only in unrelated donor transplantation[5,11] (Table 2). Therefore, continuous infusion of CYA with a target concentration at 500 ng/ml may be as effective as TAC with a target concentration used in our daily practice. In contrast, although the relationship between the blood concentration of TAC and the incidence of acute GVHD was not clear, an increase in the blood concentration was associated with greater renal dysfunction.[10] Therefore, renal toxicity associated with TAC could be reduced by decreasing the target blood concentration to a range

Table 1 Summary of the randomized controlled trials of CYA and tacrolimus (TAC)

Study	*Group*	*Initial dose*	*Target concentration*	*Grades II–IV acute GHVD (%)*	*2-year survival (%)*
Hiraoka *et al.*[2]	CYA	Not fixed[a]	Not fixed[a]	48	65
	TAC	0.05 mg/kg per day continuous i.v.[b]	20–25 ng/ml	18 ($P<0.0001$)	63 ($P=0.93$)
Ratanatharathorn *et al.*[4]	CYA	3 mg/kg continuous i.v.	150–450 ng/ml	44	57
	TAC	0.03 mg/kg continuous i.v.	10–30 ng/ml	32 ($P=0.01$)	47 ($P=0.02$)
Nash *et al.*[3]	CYA	3 mg/kg continuous i.v.	150–450 ng/ml	74	50
	TAC	0.03 mg/kg continuous i.v.	10–30 ng/ml	56 ($P=0.0002$)	54 ($P=0.46$)

[a]Determined by each institution.
[b]0.15 mg/day orally was allowed.

Table 2 Retrospective comparison of CYA and tacrolimus (TAC)

Study	*Donor*	*Group*	*Target concentration*	*Grades II–IV acute GVHD (%)*
Yanada *et al.*[5]	HLA-matched sibling	CYA	Not fixed[a]	38
		TAC	Not fixed[a]	33
	HLA-matched unrelated donor	CYA	Not fixed[a]	58
		TAC	Not fixed[a]	36
Oshima *et al.*[11]	HLA-matched sibling	CYA	300 ng/ml	44
		CYA	500 ng/ml	33
	HLA-matched unrelated donor	CYA	300 ng/ml	59
		CYA	500 ng/ml	24

[a]Determined by each institution.

between 10 and 20 ng/ml, without increasing the incidence of acute GVHD. If we consider all of these points, neither CYA nor TAC was administered at an appropriate dose in the earlier three randomized controlled trials of CYA–MTX and TAC–MTX. To clarify this problem, a randomized controlled trial of CYA–MTX and TAC–MTX with target blood concentrations at 500 and 15 ng/ml, respectively, is being performed in the Kanto Study Group for Cell Therapy.

Conflict of interest

The authors declare no conflict of interest.

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Reverse seroconversion of hepatitis B virus after allogeneic hematopoietic stem cell transplantation in the absence of chronic graft-versus-host disease

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The appearance of hepatitis B surface antigen (HBsAg) in patients previously positive for antibody to this antigen (HBsAb) is called reverse seroconversion, a rare complication after hematopoietic stem cell transplantation (HSCT), which occurs almost exclusively after HSCT from an HBsAb-negative donor and the development of chronic graft-versus-host disease (CGVHD). However, we experienced a patient who developed reverse seroconversion 23 months after unrelated HSCT even in the absence of immunosuppressants use or CGVHD. Serum immunoglobulin level was persistently normal. Therefore, all HBsAb-positive recipients should be considered to be at risk for HBV reactivation, even in patients without any risk factors.

Keywords: Hematopoietic stem cell transplantation, hepatitis B virus, reverse seroconversion, graft-versus-host disease

Introduction

The reactivation of hepatitis B virus (HBV) may cause fulminant hepatitis after hematopoietic stem cell transplantation (HSCT) in patients positive for hepatitis B virus surface antigen (HBsAg). The prophylactic use of antiviral agents such as lamivudine or entecavir has reduced the risk of severe hepatitis, although the emergence of resistant mutations was observed during an extended treatment with lamivudine.[1,2] Recently, several papers have reported the reactivation of HBV even in HSCT recipients previously positive for antibody to HBsAg (HBsAb).[3–7] This is called reverse seroconversion (RS) and the risk factors for RS included HSCT from an HBsAb-negative donor, the development of chronic graft-versus-host disease (GVHD), and the use of immunosuppressants. However, we experienced a patient who developed RS 23 months after unrelated HSCT for acute lymphoblastic leukemia even in the absence of the use of immunosuppressants or the development of chronic GVHD. This experience suggested that close monitoring of HBV markers is required even for patients without any risk factors.

Case report

A 42-year-old woman was diagnosed as acute lymphoid leukemia with t(4;11)(q21;q23) translocation in June 2005. She was positive for HBsAb and antibody to HBV core antigen (HBcAb) and negative for HBsAg at the onset of leukemia, suggesting the resolution of prior HBV infection. She achieved complete remission with a single course of induction chemotherapy. After three cycles of post-remission chemotherapy, she underwent bone marrow transplantation in November 2005, from an HLA-matched unrelated donor who was negative for

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