じめて第Ⅱ期の接種を受けた者が中学1年生になる．5年間の時限措置は終了したが，明らかな既往歴のある者以外は，2回のMRワクチン接種によって確実に疾患を予防することが望ましいという基本的な考えかたは今後も変りがない．2012～2013年にかけて報告された風疹の流行をみても，予防ワクチンを接種しておくことの大切さが再認識された．

今後の学校における麻疹対策においては，小学校入学時点で2回の定期予防接種の完了の徹底，学校内の感受性者対策および麻疹発生時の迅速な感染拡大防止策を実施することが中心となり，そのためには，就学時健康診断や児童生徒の健康診断，職員の健康診断における予防接種歴の確認および接種推奨を行うことなどがより重要となる．とくに就学時健康診断においては，罹患歴と予防接種歴を，原則として母子健康手帳や予防接種済証をもって確認し，未罹患で，かつ，麻疹の予防接種を必要回数である2回接種していない者に対する接種勧奨が非常に重要である．また，外国への修学旅行の際の麻疹についての情報提供なども，ひき続き実施していくべきである[3]．

学校や幼稚園における感染症対策

感染症対策においては，感染症が発生してからの対策ももちろん重要であるが，その予防がもっとも大切である．手洗いの励行や身の回りを清潔に保つなど，日常生活から習慣づける予防行動はもちろんのこと，ワクチンで予防できる疾患は多く，必要な予防接種について接種の推奨が不可欠である．感染症の種類によっては，教職員の予防接種も重要な課題であり，教職員も含めて必要な予防接種を受ける環境を整えていきたい．各種感染症に対する学校・幼稚園の管理体制の構築や，医療機関や行政との連携の強化など，教育現場における感染症の発生と流行の防止をはかる努力の継続を忘れてはならない．

文　献

1）学校保健安全法施行規則
http://law.e-gov.go.jp/htmldata/S33/S33F03501000018.html
2）学校保健安全法
http://law.e-gov.go.jp/htmldata/S33/S33HO056.html
3）文部科学省：学校において予防すべき感染症の解説．2012年12月
http://www.mext.go.jp/a_menu/kenko/hoken/1334054.htm
4）関谷紀貴，藤　由香，田原寛之・他：宮崎県における髄膜炎菌感染症集団発生事例．病原微生物検出情報（IASR）32:298-299，2011
http://idsc.nih.go.jp/iasr/32/380/kj3802.html
5）CDC:Meningococcal Disease. Epidemiology and prevention of vaccine-preventable diseases（The Pink Book）. 12th ed., Public Health Foundation, 193-204, 2011
6）American Academy of Pediatrics:Meningococcal Infectios. Red Book 2012. 29th ed., American Academy of Pediatrics, 500-509, 2012
7）Hayden FG, Fritz RS, Lobo MC et al.:Local and systemic cytokine responses during experimental human influenza A virus infection. Relation to symptom formation and host defense. J Clin Invest 101:643-649, 1998
8）Hayden FG, Treanor JJ, Fritz RS et al.:Use of the oral neuraminidase inhibitor oseltamivir in experimental human influenza;randomized controlled trials for prevention and treatment JAMA 282:1240-1246, 1999
9）Bhattarai A, Villanueva J, Palekar RS et al.:Viral Shedding Duration of Pandemic Influenza A H1N1 Virus during an Elementary School Outbreak—Pennsylvania, May—June 2009. Clin Infect Dis 52（Suppl. 1）:S102-108, 2011
10）Hall CB, Douglas RG Jr:Nosocomial influenza infection as a cause of intercurrent fevers in infants. Pediatrics 55:673-677, 1975
11）Frank AL, Taber LH, Wells CR et al.:Patterns of shedding of myxoviruses and paramyxoviruses in children. J Infect Dis 144:433-441, 1981
12）American Academy of Pediatrics:Influenza. Red Book 2012. 29th ed., American Academy of Pediatrics, 439-454, 2012
13）厚生労働省：保育所における感染症対策ガイド

ライン（2012年改訂版）．平成24年11月
http://www.mhlw.go.jp/bunya/kodomo/pdf/hoiku02.pdf
14）American Academy of Pediatrics: Pertussis. Red Book 2012. 29th ed., American Academy of Pediatrics, 553-566, 2012
15）Polgreen PM, Bohnett LC, Cavanaugh JE et al.: The duration of mumps virus shedding after the onset of symptoms Clin Infect Dis 46: 1447-1449, 2008
16）Okafuji T, Yoshida N, Fujino M et al: Rapid diagnostic method for detection of mumps virus genome by loop-mediated isothermal amplification. J Clin Microbiol 43: 1625-1631, 2005
17）American Academy of Pediatrics: Mumps. Red Book 2012. 29th ed., American Academy of Pediatrics, 514-518, 2012

著者連絡先
〒700-8505　岡山県岡山市北区中山下2-1-80
川崎医科大学小児科学
中野貴司

抗インフルエンザ薬

中野貴司*

はじめに

流行期には誰もがインフルエンザに罹る可能性があり，年齢や基礎疾患の有無を問わない。また，乳幼児や高齢者，あるいは基礎疾患を有する者ではインフルエンザが重症化しやすい。合併症のひとつであるインフルエンザ脳症は，年少児に発症しやすいことが知られている。肺炎や呼吸不全は高齢者で頻度が高いが，2009年に出現したA(H1N1)pdm09ウイルスでは，小児でも呼吸器合併症が目立った。そして，インフルエンザ発症時点では，これら合併症発現の有無を予測することは難しい場合が多い。

抗インフルエンザ薬は，発熱などのインフルエンザに伴う諸症状の持続や程度を軽減する効果がある。インフルエンザは通常の感冒より症状が強く，治療により患者の不快感を和らげたいという思いから抗インフルエンザ薬が処方される機会は多い。薬剤による重症化予防効果については，対照群を設定した研究は難しいが，2009年に発生したパンデミックの際には，抗インフルエンザ薬が最も使用されたといわれるわが国の患者の予後は，世界各国の中できわめて良好であった。一部の薬剤は，高齢者や慢性心疾患・肺疾患・腎疾患・代謝疾患などインフルエンザが重症化するリスクの高い宿主に対して，感染源である患者との濃厚接触が想定される際は，健康保険は適用されないが予防投与も認められている。

抗インフルエンザ薬の使用に際しては，耐性ウイルスの出現に注意する必要がある。また，わが国で大きく取り上げられた年長児の飛び降り，飛び出しなど安全性に関する留意事項もある。きわめて一般的な疾患に対する治療薬であるがゆえに，抗インフルエンザ薬については有効性と安全性の観点からさまざまな議論が行われている。本稿では，各薬剤の特徴について概説する。

NAKANO Takashi
* 川崎医科大学附属川崎病院小児科
〔〒700-8505 岡山市北区中山下2-1-80〕
TEL 086-225-2111 FAX 086-232-8343

Ⅰ．M2イオンチャンネル阻害薬

アマンタジン

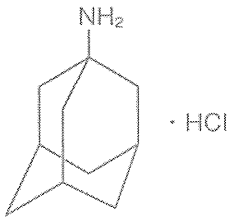
1）概要，作用機序

抗ウイルス薬として1959年に開発されたが，Parkinson病の症状を改善することがわかり，抗パーキンソン薬として広く用いられた。その後，1998年11月にわが国でも抗インフルエンザウイルス作用が効能として追加された（**図，表**）[1]。しかし，本薬剤の使用はきわめて速やかなウイルスの耐性獲得につながることが報告され，現在はほとんど用いられない。

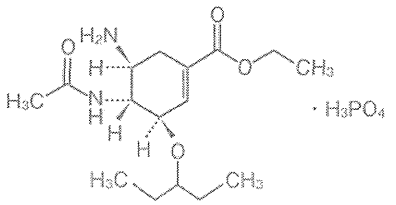
作用機序は，M2イオンチャネルの阻害薬である。インフルエンザウイルスは生体の細胞表面に吸着し，エンドサイトーシスで細胞内にとりこまれ，M2イオンチャネルが活性化される。アマンタジンは，このウイルス脱殻の段階を阻害することにより，ウイルス粒子の細胞核内への輸送を阻止する。A型インフルエンザウイルスだけがもつM2蛋白に作用するため，本薬剤はB型ウイルスに対しては効果がない。

2）小児への投与

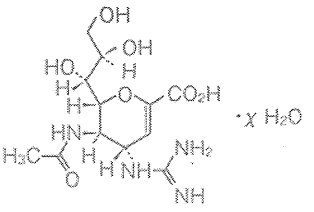
経口内服薬で，50 mg錠剤，100 mg錠剤，10%細粒剤がある。小児に対して5 mg/kg/日を2分割，一日上限量100 mgという投与量が通常用いられるが，小児適応は未承認である。健康成人に



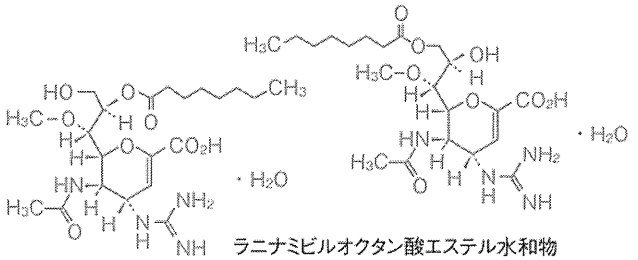
アマンタジン塩酸塩
(amantadine hydrochloride)
分子式　$C_{10}H_{17}N \cdot HCl$
分子量　187.71



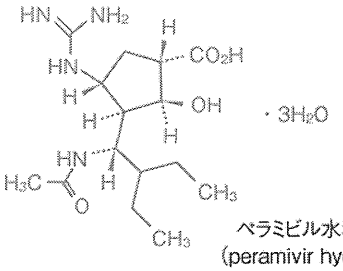
オセルタミビルリン酸塩
(oseltamivir phosphate)
分子式　$C_{16}H_{28}N_2O_4 \cdot H_3PO_4$
分子量　410.40



ザナミビル水和物
(zanamivir hydrate)
分子式　$C_{12}H_{20}N_4O_7 \cdot xH_2O$



ラニナミビルオクタン酸エステル水和物
(laninamivir octanoate hydrate)
分子式　$C_{21}H_{36}N_4O_8 \cdot H_2O$
分子量　490.55



ペラミビル水和物
(peramivir hydrate)
分子式　$C_{15}H_{28}N_4O_4 \cdot 3H_2O$
分子量　382.45

図　わが国で使用されている抗インフルエンザ薬の一般名と化学構造式
（各薬剤の添付文書，インタビューフォーム，中野[1] 2011 より引用）

本剤を投与して検討した結果では，血漿中濃度が最高値に達するのは投与後約 3 時間，血漿中濃度半減期は 10～12 時間であった[2]。発症後 48 時間以内に投与を開始しないと，十分な効果は得られないとされる。

3）安全性

副作用として，幻覚，妄想，せん妄，錯乱，ミオクローヌス，不随意運動などの精神神経症状が出現することがあり，てんかん発作を誘発または悪化させることもある。投与患者において自殺企図の報告があり，精神障害のある患者や中枢神経系に作用する薬剤を投与中の患者ではとくに注意する。アマンタジンは大部分が未変化体として尿中に排泄されるので，腎機能障害のある患者では薬剤蓄積による副作用発現の可能性がある。本剤は通常の血液透析では少量しか除去されないので，透析患者には投与しない。催奇形性が疑われる症例報告や動物実験の報告があり，妊婦には投与しない。ヒト母乳中に移行するため，授乳婦にも投与しない。

Ⅱ．ノイラミニダーゼ阻害薬

1．オセルタミビル（商品名タミフル®）

1）概要，作用機序

2001 年 2 月に承認された経口内服のノイラミニダーゼ阻害薬であり，有効成分はオセルタミビルリン酸塩で，体内で活性体に変化して抗インフルエンザウイルス効果を示す。成人用カプセル剤に加えて，2002 年 4 月に 3% ドライシロップ製剤が承認された（図，表）[1]。

インフルエンザウイルスが生体の細胞から細胞へ感染・伝播していくためには，ウイルス表面に存在するノイラミニダーゼが不可欠であるが，オセルタミビルはこの作用をブロックすることにより増殖したウイルスが細胞外へ出ていくことを阻害する。ノイラミニダーゼは A・B 型ウイルスに共通して存在するので，両方の型のウイルスに効果を有する。

2）小児への投与

1 歳未満乳児に対する投与の安全性および有効

表　わが国で使用されている抗インフルエンザ薬

一般名	アマンタジン塩酸塩	オセルタミビルリン酸塩	ザナミビル水和物	ラニナミビルオクタン酸エステル水和物	ペラミビル水和物
商品名	シンメトレル® など	タミフル®	リレンザ®	イナビル®	ラピアクタ®
剤　型	50 mg 錠，100 mg 錠，10%細粒	75 mg カプセル，3%ドライシロップ	1ブリスターにザナミビル5 mg を含有する吸入剤	1容器中にラニナミビルオクタン酸エステル20 mg を含有する吸入剤	点滴静注用製剤150 mg，300 mg
投与法	内服	内服	吸入	吸入	点滴静注
有効なウイルス型	A型のみ	A型&B型	A型&B型	A型&B型	A型&B型
作用機序	M2イオンチャネル阻害薬	ノイラミニダーゼ阻害薬	ノイラミニダーゼ阻害薬	長時間作用型ノイラミニダーゼ阻害薬	長時間作用型ノイラミニダーゼ阻害薬
治療効果を期待できる投与開始時期	発症後48時間以内	発症後48時間以内	発症後48時間以内	発症後48時間以内	発症後48時間以内
用法と用量（治療，成人）	100 mg/日・1〜2回に分割	150 mg/日・2分割（1回1カプセルを1日2回）	1回10 mg（5 mg ブリスターを2ブリスター）・1日2回吸入	1回40 mg（2容器分）を単回吸入	300 mg を15分以上かけて単回点滴静注。重症では600 mg まで増量可
用法と用量（治療，小児）	5 mg/kg/日・2分割，上限100 mg/日，ただし適応未承認	4 mg/kg/日・2分割，上限150 mg/日。1歳未満児に対する安全性未確立	5歳以上の小児に対して，1回10 mg（5 mg ブリスターを2ブリスター）・1日2回吸入	10歳以上の場合，1回40 mg（2容器分）を単回吸入。10歳未満の場合，1回20 mg（1容器分）を単回吸入	10 mg/kg を15分以上かけて単回点滴静注。上限量600 mg
標準的な治療期間	5日間	5日間	5日間	単回投与	単回投与。重症には反復も可
用法と用量（予防，成人）*	原則としては推奨されない	75 mg/日・1分割	1回10 mg（5 mg ブリスターを2ブリスター）・1日1回吸入	有効性，安全性は確立していない（承認申請中）	有効性，安全性は確立していない
用法と用量（予防，小児）*	原則としては推奨されない	2 mg/kg/日・1分割，上限75 mg/日。1歳未満児に対する安全性未確立	5歳以上の小児に対して，1回10 mg（5 mg ブリスターを2ブリスター）・1日1回吸入	有効性，安全性は確立していない（承認申請中）	有効性，安全性は確立していない
予防投与の期間*	—	7〜10日間	10日間	—	—
予防に有効な期間*	薬剤投与期間中	薬剤投与期間中	薬剤投与期間中	—	—
備　考	幻覚，妄想，せん妄，自殺企図，てんかん誘発，異常行動・言動，腎障害患者への注意。妊婦，授乳婦，透析患者への投与禁忌。耐性ウイルス誘導の頻度が高い	因果関係は不明であるが，本薬剤投与による小児での異常行動・言動発現への注意により，2007年3月以降10歳代の者への投与は原則的に差し控え。腎障害患者への投与量減量。アナフィラキシー	小児での異常行動・言動への注意。気管支攣縮，ショックや失神への注意。アナフィラキシー	小児での異常行動・言動への注意。気管支攣縮，ショックや失神への注意。アナフィラキシー	小児での異常行動・言動への注意。腎障害患者への投与量減量

* 抗インフルエンザ薬の予防投与に対して，健康保険は適用されない。
* 予防投与の対象は，原則として，インフルエンザ患者の同居家族または共同生活者で，高齢者や基礎疾患を有するなどインフルエンザ重症化のリスクがある者　（中野[1]，2011）

性は確立していない[3]。小児における薬物動態の検討では，活性体の血漿中濃度が最高値に達するのは投与後4～5時間，血漿中濃度半減期は8～14時間であった。低年齢児のほうが，血中最高濃度に達するまでの時間・半減期とも長い傾向にあった。

治療効果を期待するためには，インフルエンザ発症後48時間以内に投与を開始する。国内小児でのオープン臨床試験では，咳・鼻症状が改善し，体温37.4℃以下に回復するまでの時間は中央値72.5時間であった。体温のみに着目すると，体温が37.8℃未満に回復するまでに21.3時間，37.4℃以下に回復するまでに35.3時間であった。

米国とカナダでは，小児を対象にプラセボ対照臨床試験によりインフルエンザ罹病期間の比較検討が行われた。本剤5日間投与群（n＝217）におけるインフルエンザ罹病期間の中央値は101.3時間（95%信頼区間88.8～118.3時間）で，プラセボ対照群（n＝235）の137.0時間（同124.5～149.6時間）と比較して有意（p<0.0001）に短縮していた。

3）予防投与

感染源への曝露後，できるだけ早く投与を開始することが望ましい。国内外での成人を対象とした比較対照試験では，プラセボ群におけるインフルエンザ発病率が5～10%であったのに対して，投与群では1%前後であり統計学的に有意な予防効果が確認された。わが国の小児の成績では，2002/03シーズンに小児病棟でのインフルエンザ患者院内発症に際して，発端児3例との病棟内濃厚接触児のべ29名を対象とした調査研究がある[4]。オセルタミビル予防内服（体重あたり2mgを1日1回，7～10日間投与）を行った13例から発症例はなく副反応も認められなかったが，予防内服を行わなかった16例中11例（69%）はインフルエンザを発症した。

4）安全性

小児・未成年者への投与に際して，「飛び降り・飛び出しなどの異常行動」との関連が論議されている。2007年3月に厚生労働省が発した通達により，因果関係は不明であるものの本剤服用後に異常行動を発現し転落などの事故にいたった例が報告されていることより，10歳以上20歳未満の患者に対しては，合併症や既往歴からハイリスク患者と判断される場合を除いては，原則として本剤の使用を差し控えることになっている。

また，小児・未成年者への投与に際しては，インフルエンザ罹患そのものでも発現する恐れのある異常行動から事故が発生することを防止するための予防的な対応として，①異常行動の発現のおそれがあること，②自宅において療養を行う場合，少なくとも2日間，保護者らは小児・未成年者が一人にならないよう配慮することについて患者・家族に対し説明を行うこと，が定められている。

腎排泄型の薬剤であり，腎機能が低下している場合は血漿中濃度が高くなる。成人における海外での検討では，クレアチニンクリアランスが30mL/分以下の患者では，治療・予防ともに投与間隔を長くする，あるいは1回投与量を減じるという目安が定められている。頻度は高くないが，アナフィラキシーの報告もある。

2．ザナミビル（商品名リレンザ®）

1）概要，作用機序

2001年2月に承認された吸入用のノイラミニダーゼ阻害薬であり，吸入薬の特性として，インフルエンザウイルスの感染局所である上気道へ薬剤が直接高濃度に到達する。A・B型の両方の型のウイルスに効果を有する（図，表）[1]。

本剤は吸入局所でそのまま作用すると考えられるが，単回吸入後の薬物体内動態を成人・小児で解析した結果では，血漿中濃度が最高値に達するのは投与後1～1.5時間，血漿中濃度半減期は2～2.5時間で，小児では成人よりも上記2指標とも時間が短縮する傾向にあった[5]。本剤は，体内で代謝を受けずに未変化体のまま尿中に排泄される。腎機能低下の患者ではその排泄は遅延するが，常用量投与では忍容試験で得られた値をはるかに下回る程度までしか血漿中濃度は上昇しないため，腎機能低下の患者においても投与量の調整は必要ないとされる。

2）小児への投与

成人・小児とも，1回2ブリスター（10mg）を

1日2回吸入する[5]。低年齢児では適切に吸入できるかどうかの手技的なチェックが大切である。

インフルエンザ発症後48時間以内に投与を開始することで治療効果が期待できる。国内で5～14歳の小児を対象としたオープン臨床試験では，インフルエンザ主要症状の軽減までに要した日数の中央値は4.0日であった。海外での5～12歳までの小児を対象としたプラセボ対照臨床試験の結果[6]では，主要症状の軽減までに要した日数の中央値がザナミビル投与群(n＝164)では4.0日で，対照群（n＝182）の5.25日と比較して統計学的に有意（p<0.001）な効果が認められた。

3）予防投与

曝露後できるだけ早い時期に開始することはオセルタミビルと同様である。国内外での成人を対象とした比較対照試験で，有意な発病予防効果が確認されている。家族内でのインフルエンザ患者発生に際して，5歳以上の家族全員をザナミビル投与群あるいはプラセボ群に割り付け，予防投薬の効果を検討した研究[7]でも，ザナミビル投与群では有意にインフルエンザの家族内二次感染が抑制されていた。

4）安全性

吸入後，まれに気管支攣縮や呼吸機能低下の報告がある。インフルエンザ罹患時は気道過敏性が亢進する可能性があり，気管支喘息の患者で気管支拡張薬の吸入薬を併用する場合には，ザナミビルを投与する前に使用する。失神やショック症状の報告もあるが，これら症状の発現には，インフルエンザに伴う発熱や脱水などの全身状態の悪化に加え，本剤を強く吸入したこと，または長く息を止めたことが誘因となった可能性がある。患者に吸入法を十分に理解させ，座位などくつろいだ状態で吸入するよう指導することが大切である。また，万が一このような症状が現れた場合に適切な対処ができるよう，失神などに伴う二次的な外傷が起こらないような場所で吸入させるなどの配慮も大切である。頻度は高くないが，吸入後にアナフィラキシーの報告もある。

小児・未成年者における異常行動は，インフルエンザ罹患自体で発現する可能性があり，事故防止の対応として「少なくとも2日間は患者が一人にならないよう配慮すること」に関する注意は必要である。

3．ラニナミビル（商品名イナビル®）

1）概要，作用機序

2010年10月に承認された吸入用のノイラミニダーゼ阻害薬で，長時間作用型という特性を有し単回の吸入で効果が期待できる。A・B型の両方の型のウイルスに効果を有する（図，表）[1]。予防投与については，2013年8月現在承認申請中である。

薬剤に含まれる有効成分はラニナミビルオクタン酸エステル水和物で，吸入投与後，気道上皮細胞内で加水分解により活性代謝物ラニナミビルに変換されると考えられている[8]。活性代謝物ラニナミビルが気道上皮細胞内に長く留まり，長時間効果を発揮するすると考えられるが，吸入後の薬物体内動態を解析した結果では，成人単回吸入による薬物体内動態の解析では，血漿中濃度が最高値に達するのは投与後約4時間，血漿中濃度半減期は70時間前後であった。小児においても，単回吸入1・4・24時間後に血漿中濃度を測定した結果，4時間後の濃度が最も高値であり，吸入144時間後においても血漿中にラニナミビルは検出された。

2）小児への投与

成人および10歳以上の小児では1回40 mg（2容器分）を単回吸入，10歳未満の小児では1回20 mg（1容器分）を単回吸入する[8]。

インフルエンザ発症後48時間以内に投与することで治療効果を期待できることは，他の薬剤と同様である。日本，台湾，韓国，香港で成人を対象に実施されたオセルタミビルリン酸塩を対照薬とした臨床試験の結果[9]では，インフルエンザ罹病時間の中央値が，ラニナミビル40 mg群（n＝334）で73.0時間，対照薬オセルタミビル群（n＝336）で73.6時間という結果であり，ラニナミビル単回吸入の有効性はオセルタミビルと同等であった。国内の3～9歳小児を対象とした臨床試験[10]では，インフルエンザ罹病時間の中央値がラニナミビル20 mg群（n＝61）では56.4時間，対照であるオセルタミビル群（n＝62）では87.3時

間であり，ラニナミビル群で罹病時間が短かった。10～19歳においては，原則禁忌の扱いであるオセルタミビルを対照群に設定ができなかったためオープン試験であるが，ラニナミビル40 mg投与群のインフルエンザ罹病時間の中央値は76.0時間であった。

3）安全性

長時間作動薬であるが，それにより問題となる安全性への懸念事項は，現状で報告されていない。吸入薬剤であり，失神や気管支攣縮への注意事項はザナミビルと同様で，アナフィラキシーの頻度も他薬剤と同程度と考えられる。小児と未成年者における異常行動は，インフルエンザ罹患自体で発現する可能性があり，事故防止の対応として「少なくとも2日間は患者が一人にならないよう配慮すること」も同様である。

4．ペラミビル（商品名ラピアクタ®）

1）概要，作用機序

2010年1月に承認された点滴静注用のノイラミニダーゼ阻害薬であり，ラニナミビル同様に長時間作用型薬剤である。A・B型の両方の型のウイルスに効果を有する（図，表）[1]。予防投与の有効性・安全性は確立していない。

健康成人に単回点滴静注して血漿中濃度を解析した結果，平均滞留時間は約3時間でペラミビルは速やかに消失した。反復投与での体内動態は単回投与時とほとんど変わらず，蓄積性は認められなかった。咽頭分泌液および鼻腔分泌液中には，血漿中に比しAUCとして3～9%が移行することが確認され，限外濾過法により測定したヒト血清蛋白結合率は，1～100 μg/mLの濃度範囲において0.3～1.8%であった。血漿および尿中に代謝物は検出されず，未変化体のみが検出された。ラットにおいて放射性同位元素でペラミビルを標識して行った実験では，すべての組織中放射能濃度は投与5分後に最高濃度を示し，作用部位である肺および気管においても良好な分布が認められ，主排泄臓器の腎臓ではより高い分布が認められた。すべての組織中放射能濃度は投与48時間後までに定量限界未満となり，組織への蓄積性および残留性は低いことが示唆された。また，脳内への移行性はきわめて低かった。

2）小児への投与

通常は1日1回10 mg/kgを15分以上かけて単回点滴静注するが，症状に応じて連日の反復投与も可能である。小児における1回投与量の上限は，成人最大投与量である600 mgまでとする。

発症後48時間以内に治療を開始することについては，他の薬剤と同様である。国内での成人を対象としたペラミビル単回点滴静注によるプラセボ比較対照試験では，プラセボ対照群（n＝100）でのインフルエンザ罹病期間中央値81.8時間（95%信頼区間68.0～101.5時間）に比して，300 mg群（n＝99）は59.1時間（同50.9～72.4時間），600 mg群（n＝97）は59.9時間（同54.4～68.1時間）と統計学的に有意（$p<0.05$）な治療効果が確認された。オセルタミビル投与群を対照群として設定した成人の検討では，オセルタミビル群（n＝365）でのインフルエンザ罹病期間中央値が81.8時間（95%信頼区間73.2～91.1時間）であったのに対して，300 mg群（n＝364）は78.0時間（同68.4～88.6時間），600 mg群（n＝362）は81.0時間（同72.7～91.5時間）という結果であった。反復投与については，糖尿病，慢性呼吸器疾患，免疫抑制薬投与中などのハイリスク因子を有する患者を対象とした成績が報告されており，インフルエンザ罹病期間の短縮傾向が認められた。小児を対象とした国内臨床試験では，4か月～15歳の115例に対してペラミビル10 mg/kgが1日1回1～2日間投与された。インフルエンザ罹病期間の中央値は27.9時間（95%信頼区間21.7～31.7時間）であった。

3）安全性

腎排泄型の薬剤で，腎機能障害の患者では高い血漿中濃度が持続するおそれがあるので，クレアチニンクリアランスが50 mL/分より低い患者では腎機能の低下に応じて投与量を減量する。本剤は，血液透析により速やかに血漿中から除去される。小児と未成年者における異常行動に関する諸注意事項は他のノイラミニダーゼ阻害薬と同様である。

Ⅲ. 薬剤耐性ウイルス

治療効果に期待したい一方で，耐性ウイルスの監視は常に継続する必要がある。M2チャンネル阻害薬のアマンタジンは，耐性ウイルスがきわめて高頻度であるため，現在ほとんど用いられないことは既述した。

オセルタミビルに耐性となるアミノ酸変異H274Yをもつ H1N1 ソ連型ウイルスが2007年から世界中で出現増加し，わが国でも2008/2009流行期に分離された H1N1 ソ連型ウイルスのほぼ全例がオセルタミビル耐性であった。この耐性ウイルスはザナミビル，ラニナミビルには感受性であるが，ペラミビルには感受性が低下していた。

その後，A(H1N1)pdm09 ウイルスによるパンデミックが発生し，H1N1 ソ連型ウイルスは世界から姿を消した。同じ亜型である A(H1N1)pdm09 ウイルスの同様の耐性獲得が懸念されたが，現状では幸い臨床的に大きな問題となるレベルにはいたっていない。

不適切な薬剤の使用が耐性ウイルスの出現増加に関与していないかも注意してゆく必要がある。予防投与はうまく利用すれば，基礎疾患を有するなどのハイリスク者には有用な手段であるが，薬剤の濫用につながらないよう適切な使用を考慮したい。

Ⅳ. 感染伝播への影響

抗インフルエンザ薬治療により症状が早期に軽快すると，ウイルス排泄者が早期に集団生活に復帰してかえって感染を拡大しないかという議論がしばしば行われる。一方，治療によるウイルス排泄への影響を検討した研究では，抗インフルエンザ薬投与によりウイルス排泄量は少なくなり，排泄期間は短縮したという報告がなされている[8,12,13]。

たとえば，学校保健安全法施行規則による出席停止期間は，集団生活におけるエチケットを守るための決まりである。他人へインフルエンザを伝播させないように努めるという気持ちを，患者・医療者ともが常に忘れないことが大切である。抗インフルエンザ薬による治療は，適切に運用すれば他人へ感染を伝播する期間を短縮し，社会や集団における感染制御策として役立つと考えられる。

Key Points

1. 内服，吸入，点滴静注のノイラミニダーゼ阻害薬が広く用いられ，長時間作用型の薬剤もある。
2. 重症化や合併症を予防する効果の評価はなかなか難しいが，多数が罹患する疾患だからこそ，病原体特異的な治療に期待したい。
3. 安全性について異常行動との関連がしばしば議論されるが，インフルエンザ罹患そのものでも異常行動は出現するので，とくに発症後2日間は一人にしない配慮が大切である。
4. 薬剤耐性ウイルスへの監視は今後も継続して必要であり，感染制御の観点からも適正使用を心がけたい。

文献

1) 中野貴司：インフルエンザウイルス．小児科 **52**：1101-1109，2011
2) シンメトレル錠50 mg/シンメトレル錠100 mg/シンメトレル細粒10%添付文書，ノバルティス ファーマ（株），2013年4月改訂（第15版）
3) タミフルドライシロップ3%添付文書，中外製薬（株），2012年5月改訂（第23版）
4) 新庄正宜，佐藤清二，菅谷憲夫，他：小児病棟におけるインフルエンザ接触者へのオセルタミビル予防内服効果．感染症誌 **78**：262-269，2004
5) リレンザ添付文書，グラクソ・スミスクライン（株），2013年1月改訂（第14版）
6) Hedrick JA, Barzilai A, Behre U, et al：Zanamivir for treatment of symptomatic influenza A and B infection in children five to twelve years of age：a randomized controlled trial. Pediatr Infect Dis J **19**：410-417, 2000
7) Monto AS, Pichiero ME, Blanckenberg SJ, et al：Zanamivir prophylaxis：an effective strategy for the prevention of influenza types A and B within households. J Infect Dis **186**：1582-1588, 2002
8) イナビル吸入粉末剤20 mg添付文書，第一三共（株），2012年2月改訂（第5版）
9) Watanabe A, Chang SC, Kim MJ, et al：Long-acting neuraminidase inhibitor laninamivir octanoate versus oseltamivir for treatment of influenza：A

double-blind, randomized, noninferiority clinical trial. Clin Infect Dis **51**：1167-1175, 2010
10) Sugaya N, Ohashi Y：Long-acting neuraminidase inhibitor laninamivir octanoate (CS-8958) versus oseltamivir as treatment for children with influenza virus infection. Antimicrob Agents Chemother **54**：2575-2582, 2010
11) ラピアクタ点滴静注液バッグ300 mg/ラピアクタ点滴静注液バイアル150 mg添付文書，塩野義製薬(株)，2013年7月改訂（第6版）
12) 柏木征三郎，工藤翔二，渡辺　彰，他：インフルエンザウイルス感染症に対するリン酸オセルタミビルの有効性および安全性の検討―プラセボを対照とした第Ⅲ相二重盲検並行群間比較試験成績．感染症誌 **74**：1044-1061，2000
13) Hayden FG, Treanor JJ, Betts RF, et al：Safety and efficacy of the neuraminidase inhibitor GG167 in experimental human influenza. JAMA **275**：295-299, 1996

Jpn. J. Infect. Dis., 66, 260-261, 2013

Laboratory and Epidemiology Communications

Clinical Manifestations of Coxsackievirus A6 Infection Associated with a Major Outbreak of Hand, Foot, and Mouth Disease in Japan

Masaaki Kobayashi[1], Tomohiko Makino[2], Nozomu Hanaoka[2], Hiroyuki Shimizu[3], Miki Enomoto[2,4], Nobuhiko Okabe[5], Kazuhiko Kanou[2], Masami Konagaya[2], Kazunori Oishi[2], and Tsuguto Fujimoto[2]*

[1]*Kobayashi Pediatric Clinic, Fujieda 426-0067;*
[2]*Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo 162-8640;*
[3]*Department of Virology II, National Institute of Infectious Diseases, Tokyo 208-0011;*
[4]*Hyogo Prefectural Institute of Public Health and Consumer Sciences, Hyogo 652-0032; and*
[5]*Kawasaki City Institute for Public Health, Kanagawa 210-0821, Japan*

Communicated by Takaji Wakita

(Accepted April 24, 2013)

A serious outbreak of hand, foot, and mouth disease (HFMD) occurred in Japan in the summer of 2011 (1,2). A major cause of this epidemic was coxsackievirus A6 (CA6), and the clinical manifestations of the disease differed from those of typical HFMD. The Infectious Diseases Control Law mandates medical doctors in Japan to officially report cases exhibiting the following symptoms immediately after diagnosis: fever with sore throat and appetite loss, followed by the appearance of reddish vesicles on the hands and feet approximately 2 days after the onset of fever. The vesicles usually disappear within 7 to 10 days without specific treatment. Reported cases should satisfy both of the following clinical criteria: (i) vesicles measuring 2–5 mm with blisters on the palm of the hand and sole or dorsum of the foot and oral mucosa and (ii) vesicles that heal without crust formation.

During the 2011 outbreak, it was challenging for pediatricians to judge whether the majority of HFMD cases should be diagnosed as a subtype of conventional HFMD or a new type caused by CA6 infection. We recommend that pediatricians should be alert while making diagnosis because the rashes caused by CA6 are similar to those caused by chickenpox. Establishing differential diagnoses is necessary in order to prevent misdiagnosis and inappropriate treatment. The critical point in differentiating CA6 from other infections is the specific clinical course and spread of eruptions with scabbing. For this purpose, in 2011, we summarized the detailed clinical features of HFMD caused by CA6 infection (CA6-associated HFMD).

On the basis of laboratory confirmation (1), we reviewed 28 cases of CA6-associated HFMD that occurred in Japan between June 2011 and July 2011. CA6 strains were detected in samples of feces and/or pharyngeal swabs and tested using reverse transcription (RT)-PCR and sequencing of the VP1 region (AB649286–AB649291).

Clinical samples were collected during the course of medical care in hospitals and laboratory tests were performed for the purpose of diagnosis and treatment. Informed consent for this study was obtained from all the patients' guardians, and the clinical samples were diagnosed for national surveillance.

Patient age ranged from 9 months to 9 years (mean, 29.1 months), and 75% of patients were aged under 3 years. There was no statistically significant gender difference. All HFMD cases were associated with a fever (38.0–40.2°C; mean, 39.0°C) that lasted for 1.5 days on an average. The appearance of rashes on the oral mucosa was noted from the second day of onset of fever, similar to that observed in cases of herpangina with less oral pain and reluctance to eat. The appearance of reddish vesicles on the extremities and buttocks was noted on the third day of onset; these vesicles were mostly found on the upper arms, thighs, lips, neck, and buttocks, while they were found less frequently on the hands and soles of the feet. The vesicles were flat and umbilicated. Some lesions grew to be more than 10 mm in diameter, and scabbing was observed within several days (Fig. 1).

As described above, the criteria for notification in Japan require that the vesicles associated with HFMD heal without crust formation. However, in this study, crust formation was observed within several days of disease onset in all 28 cases. The notification criteria for HFMD include vesicles that heal without crust formation. Our results showed that CA6-associated HFMD did not fulfill the notification criteria for HFMD. However, it was impossible to find crust formation in the early stage of the illness. In some cases, a careful differential diagnosis from chickenpox, impetigo contagiosa, herpes simplex disease, and varicella-zoster disease was required. The critical features of CA6-associated HFMD were the successive appearance of herpangina-like oral mucosal lesions, widely spread rashes, and crust formation during the healing process.

We followed 16 recovered cases for 2–8 weeks and found that 6 (37.5%) experienced onychomadesis, which was a significant finding. All cases healed without severe sequelae, except for the 6 cases that developed onychomadesis. The incidence of CA6-associated HFMD with crust formation is thought to be high (almost 100%), with the rate of onychomadesis estimated to be over 30%. Further studies are therefore

*Corresponding author: Mailing address: Infectious Disease Surveillance Center, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan. Tel: +81-3-5285-1111, Fax: +81-3-5285-1129, E-mail: fujimo-t@nih.go.jp

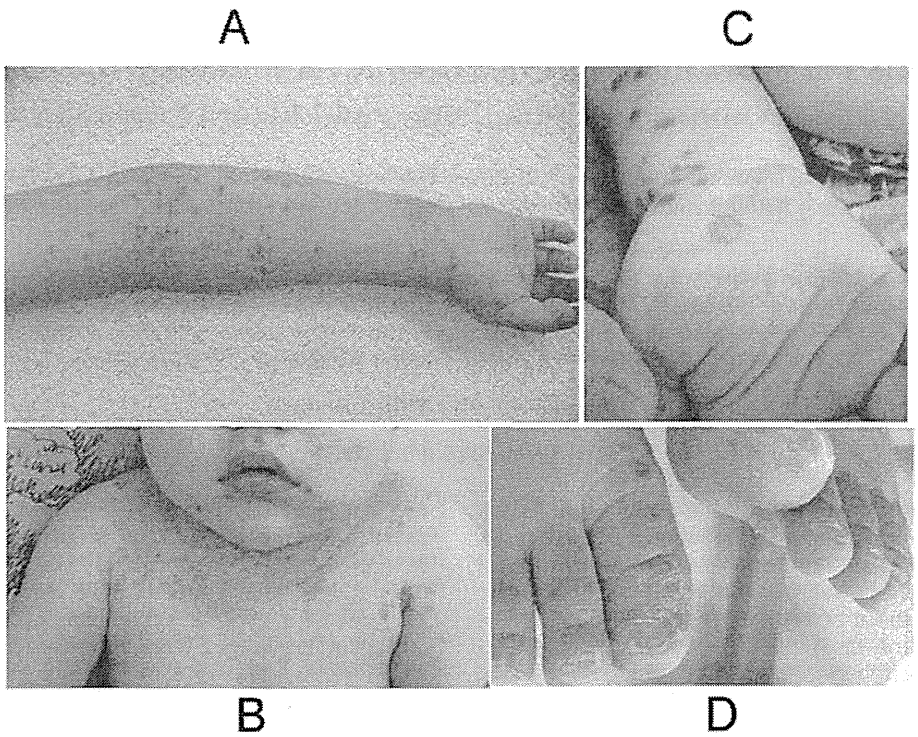


Fig. 1. Vesicles on the upper arm (A) with rashes spread widely on the trunk (B). Flat and umbilicated vesicles as opposed to the typical manifestations of HFMD (C). Onychomadesis (D).

required to evaluate these incidence rates.

Rashes associated with CA6-associated HFMD are spread more widely on the extremities and trunk, unlike those associated with typical HFMD where they are mostly localized on the palms of the hands and soles of the feet. One notable clinical symptom of CA6-associated HFMD is onychomadesis, an outbreak of which was reported in Finland in 2008 (3). Similar cases were reported from Ehime Prefecture in Japan in 2009 (4), Taiwan in 2011 (5), the United States in 2012 (6), and from other countries. Although the number of patients with CA6-associated HFMD decreased in Japan in 2012, the clinical manifestations of CA6 infection should be continuously and carefully monitored in the national surveillance program. Comparisons of the clinical manifestations of CA6-associated HFMD with those of other types of enterovirus-associated HFMD are in progress, including studies on CA16 and enterovirus 71, in a large number of patients.

This article appeared partly in the Infectious Agents Surveillance Report (IASR), vol. 32, p. 230–231, 2011 in Japanese.

Conflict of interest None to declare.

REFERENCES

1. Fujimoto, T., Iizuka, S., Enomoto, M., et al. (2012): Hand, foot, and mouth disease caused by coxsackievirus A6, Japan, 2011. Emerg. Infect. Dis., 18, 337–339.
2. National Institute of Infectious Diseases and Tuberculosis and Infection Control Division, Ministry of Health, Labour and Welfare (2012): Hand, foot and mouth disease in Japan, 2002–2011. Infect. Agents Surveillance Rep., 33, 55'–56'.
3. Osterback, R., Vuorinen, T., Linna, M., et al. (2009): Coxsackievirus A6 and hand, foot, and mouth disease, Finland. Emerg. Infect. Dis., 15, 1485–1488.
4. Watanabe, Y. and Tohyama, M. (2012): Onychomadesis associated with hand, foot, and mouth disease. J. Pediatr. Dermatol., 30, 179–183 (in Japanese).
5. Wei, S.H., Huang, Y.P., Liu, M.C., et al. (2011): An outbreak of coxsackievirus A6 hand, foot, and mouth disease associated with onychomadesis in Taiwan, 2010. BMC Infect. Dis., 11, 346.
6. Flett, K., Youngster, I., Huang, J., et al. (2012): Hand, foot, and mouth disease caused by coxsackievirus A6. Emerg. Infect. Dis., 18, 1702–1704.

Journal of Clinical Virology 59 (2014) 132–134

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Case Report

Echovirus 3 as another enterovirus causing life-threatening neonatal fulminant hepatitis☆

Ippei Miyata[a], Nozomu Hanaoka[b], Nobuhiko Okabe[c], Tsuguto Fujimoto[b], Seisuke Sakamoto[a], Mureo Kasahara[a], Akihiko Saitoh[a,d,*]

[a] National Center for Child Health and Development, Japan
[b] National Institute of Infectious Diseases, Japan
[c] Kawasaki City Institute for Public Health, Japan
[d] Niigata University Graduate School of Medical and Dental Sciences, Japan

ARTICLE INFO

Article history:
Received 29 September 2013
Received in revised form
27 November 2013
Accepted 30 November 2013

Keywords:
Echovirus infections
Infant
Hepatitis
Liver transplantation
Umbilical cord

1. Why this case is important?

The hereby reported case presents with two features of prominence: first, this is the first case of neonatal fulminant hepatitis caused by Echovirus 3 (E-3). Second, this case is a rare survival case of a neonate from a devastating manifestation.

2. Case description

The patient is a female baby born in mid-July, at gestational age of 38 w 0 d, weighing 2372 g, whose Apgar score was 9. The labor was induced due to oligohydramnios. The patient developed fever of 38.5 °C on the third day of her life. Septic work up revealing pleocytosis of cerebrospinal fluid (CSF), ampicillin and cefotaxime were initiated. On the fourth day of her life, she developed asphyxia and was intubated. Acyclovir was added for suspected herpes simplex virus (HSV) infection. On the fifth day, she developed coagulopathy and thrombocytopenia and was transferred to a pediatric intensive care unit (PICU) of a nearby pediatric medical center. Initial blood culture, CSF culture results as well as plasma PCR test for HSV-1 and HSV-2 was negative at the point of first transfer. Furthermore, all drugs were withdrawn because they suspected drug-induced hepatitis. Conditions did not improve despite continued supportive care. Due to the severity of the disease, she was transferred to our institution on the 10th day of her life for possible indication of liver transplantation.

Prenatal history demonstrated that her mother suffered a day of fever and diarrhea three days preceding the labor. Enterovirus infection was strongly suspected due to this history of preceding maternal illness and the summer season. Continuing intensive care, serum was subjected to PCR, which turned out to be positive for enteroviruses, negative for HSV-1, HSV-2, Epstein-Barr virus, cytomegalovirus, and human parechovirus. Due to worsening jaundice and persistent coagulopathy refractory to intensive treatment, liver biopsy was performed on the 20th day of life. Histopathological examination of the biopsied specimen revealed regeneration of hepatocytes, indicating prolonged supportive care expecting significant regeneration. This specimen was also subjected to PCR, which also proved positive for enteroviruses.

However, coagulopathy did not improve despite further prolonged supportive care, thus living donor liver transplantation was performed on the 32nd day of life. Coagulopathy, jaundice,

☆ The work was conducted at the following two institutions: National Center for Child Health and Development, and National Institute of Infectious Diseases.

* Corresponding author at: Department of Pediatrics, Niigata University Graduate School of Medical and Dental Sciences, 1-757 Asahi-machi, Chuo-ku, Niigata 951-8510, Japan. Tel.: +81 25 227 2222; fax: +81 25 227 0778.
E-mail address: asaitoh@med.niigata-u.ac.jp (A. Saitoh).

1386-6532/$ – see front matter
http://dx.doi.org/10.1016/j.jcv.2013.11.015

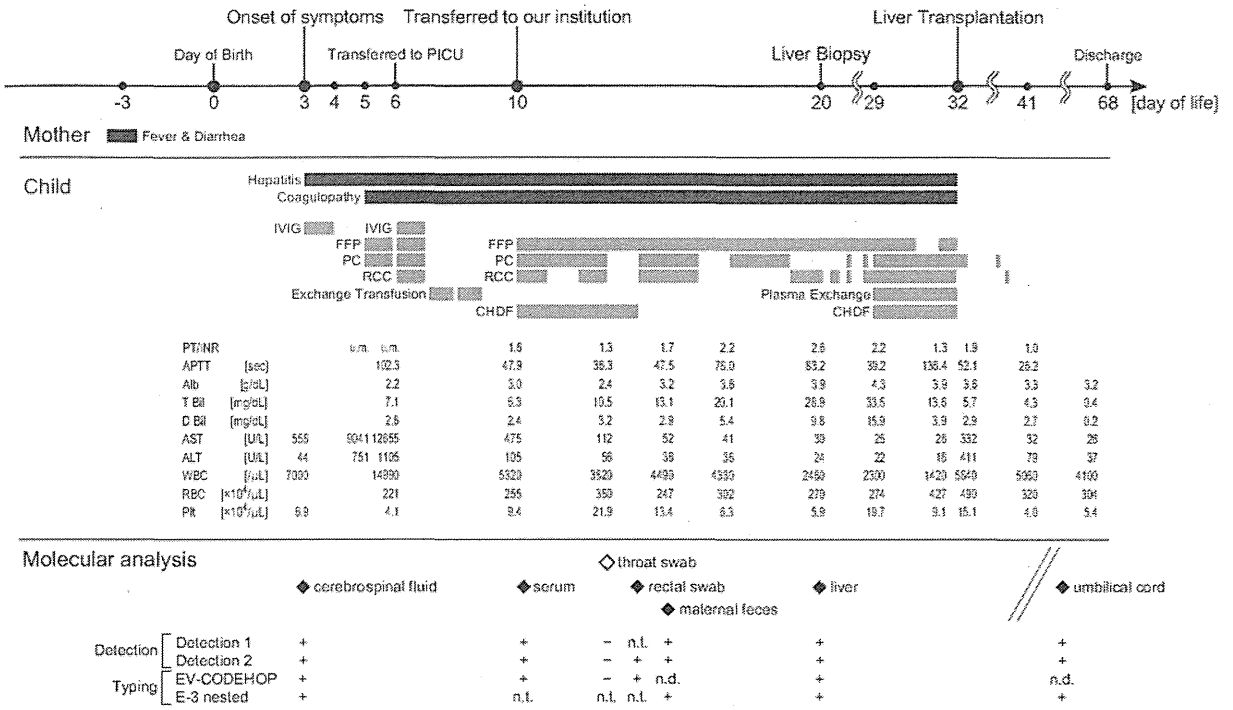


Fig. 1. Clinical course of the case and points of specimen collection for PCR analysis of enterovirus. PICU, pediatric intensive care unit; IVIG, intravenous immunoglobulin; FFP, fresh frozen plasma; PC, platelet concentrates; RCC, red blood cells concentrates; CHDF, continuous hemodiafiltration; PT/INR, international normalized ratio for prothrombin time; APTT, activated partial thromboplastin time; Alb, albumin; T.Bil, total bilirubin; D.Bil, direct bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; WBC, white blood cells; RBC, red blood cells; Plt, platelets; u.m., unmeasurable; n.t., not tested; n.d., not determined. Diamonds represent the point of specimen collection; these specimens were subjected to molecular analysis. Specimens positive for enterovirus (Echovirus 3) are indicated by filled diamonds; negative, open diamonds. Detection 1, method based on Ref. [1]; detection 2, method based on Ref. [3]; EV-CODEHOP, method based on Ref. [4]; E-3 nested, nested PCR as described in this manuscript.

hepatic failure improved promptly following liver transplantation. Postoperative course was uncomplicated and she was able to discharge home on the 68th day of life (postoperative day 36). Clinical course of this patient is summarized in Fig. 1, with points of specimen collection as well.

3. Molecular analyses

The following specimens were subjected to real-time PCR detection for enteroviruses according to the literature [1]; CSF, serum, throat swab, biopsied liver from the patient; feces from the mother; preserved umbilical cord. Nucleic acid from CSF, serum, throat swab, rectal swab was extracted by silica membrane-based spincolumns. RNA from biopsied liver, feces was extracted using ISOGEN (Nippon-Gene, Tokyo, Japan), according to the manufacturer-supplied protocol for tissue specimens. RNA from preserved umbilical cord was extracted as described elsewhere [2]. All but the throat swab proved positive for enterovirus.

In addition to the specimens subjected to real-time PCR detection, rectal swab specimen from the patient was assayed and confirmed for enterovirus by another method [3]. All specimens but the throat swab proved positive for enterovirus by this method as well.

All specimens were further subjected to molecular typing as described in the literature [4]; a portion of the VP1 region is amplified for sequence analysis, using degenerated primers that target relatively conserved sequences which flank the amplified portion which varies among various enteroviruses. Among the enterovirus positive specimens, RNA from maternal feces and preserved umbilical cord could not be analyzed by this general method, and required nested PCR employing specific primers for this case. These primers were designed based on the sequence data obtained from filial rectal swab and serum specimens: the sequences of outer primers are 5′-ACATACCTCCCAGGTCGTG-3′ and 5′-ATGTACATGATCTGGTGCGTC-3′; inner primers are 5′-AGGTCGTGCCAGGCGATAC-3′ and 5′-TGATCTGGTGCGTCAAAACGG-3′. All enterovirus positive specimens proved positive for E-3 by BLAST search of the determined partial sequences (accession numbers AB823760–AB823765). Each of these sequences further proved to be 100% identical with E-3 strain OC10-731 (accession number AB647324).

4. Other similar and contrasting cases in the literature

Enteroviruses are known to occasionally cause severe fulminant diseases that can be fatal among neonates in the following two major presentations which tend to overlap: encephalomyocarditis syndrome and hemorrhage-hepatitis syndrome [5,6]. The enteroviruses that are most associated with such devastating neonatal hepatic manifestations are Coxackievirus B and Echovirus 11 [5–9]; cases caused by Echovirus 21 [10], Echovirus 30 [11], and Echovirus 33 [12] are also reported in the literature, but not E-3. The authors are not aware of any devastating neonatal hepatic manifestations caused by E-3 in the literature that resemble the overwhelming clinical course reported herein. Although a few case reports of hepatic involvement accompanying E-3 are found in the literature, these cases responded favorably to supportive care [13,14]. The only fatal outcomes associated with E-3 in the literature are a case of a 12-year-old girl presenting with progressing weakness and myalgia who suddenly collapsed [15] and a case of a

7-year-old boy diagnosed as Reye's syndrome [16]. The autopsy findings of these fatal cases were "signs of toxic hepatitis" and "marked fatty degeneration of the liver" respectively, suggesting consequences of sepsis or septic shock.

5. Discussion

E-3 is related mostly with neurologic diseases, such as paralysis [17–19], aseptic meningitis [5,16,19–21] and meningoencephalitis [22]. In our case, CSF pleocytosis was present on the day of symptom onset. Furthermore, E-3 was proved from the remainder of this CSF, suggesting concomitant aseptic meningitis. However, the liver was involved in our case, presenting as fulminant hepatitis. The case fatality rates of nenonatal enterovirus with hepatic involvements and/or accompanying coagulopathy range between 24% and 100% in the previous literatures [8,23–25]. The course of our case was refractory to supportive intensive care, and liver transplantation was crucial for survival. Three years have passed since the transplantation, and the patient has been growing without any sequelae.

Although the cause of fulminant hepatitis in children are more likely to remain undetermined [26–28], we were able to identify E-3 as a cause of this case. Methods based on nucleic acid amplification played an indispensable role in diagnosis and clinical decision making. Furthermore, analysis of preserved umbilical cord provided crucial information to determine the infection route of this case to be presumably pre-natal. The negative result from the patient's throat swab further supports this conclusion, indicating post-partum oral infection to be less likely. These facts serve as evidence of pre-natal infection. This is the first report of typing enteroviral RNA from preserved umbilical cord, as well as confirming the sequence to be identical with the sequence determined from maternal and filial specimens. Our experience adds to proving the potency of preserved umbilical cord as a potential resource for retrospective molecular investigation of perinatal infections.

Funding

This research was supported by a grant from the National Center for Child Health and Development (21A-2).

Competing interests

None declared.

Ethical approval

Approved by the Internal Review Board at the National Center for Child Health and Development in Tokyo.

Acknowledgements

The authors acknowledge Dr. Noriyasu Iwase for technical assistance.

References

[1] Mohamed N, Elfaitouri A, Fohlman J, Friman G, Blomberg J. A sensitive and quantitative single-tube real-time reverse transcriptase-PCR for detection of enteroviral RNA. J Clin Virol 2004;30:150–6.
[2] Miyata I, Saitoh A. Detection of enteroviral RNA from preserved umbilical cord. J Clin Virol 2013;56:274–5.
[3] Fujimoto T, Izumi H, Okabe N, Enomoto M, Konagaya M, Chikahira M, et al. Usefulness of real-time reverse transcription-polymerase chain reaction for the diagnosis of echovirus aseptic meningitis using cerebrospinal fluid. Jpn J Infect Dis 2009;62:455–7.
[4] Nix WA, Oberste MS, Pallansch MA. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. J Clin Microbiol 2006;44:2698–704.
[5] White DO, Fenner FJ. Picornaviridae medical virology. 4th ed. San Diego: Academic Press; 1994. p. 381–406.
[6] Abzug MJ. Nonpolio enteroviruses. In: Kliegman RM, Stanton BF, St Geme 3rd JW, Schor NF, Behrman RE, editors. Nelson textbook of pediatrics. 19th ed. Philadelphia: Saunders; 2011. p. 1088–94.
[7] Kaplan MH, Klein SW, McPhee J, Harper RG. Group B coxsackievirus infections in infants younger than three months of age: a serious childhood illness. Rev Infect Dis 1983;5:1019–32.
[8] Modlin JF. Perinatal echovirus infection: insights from a literature review of 61 cases of serious infection and 16 outbreaks in nurseries. Rev Infect Dis 1986;8:918–26.
[9] Lake AM, Lauer BA, Clark JC, Wesenberg RL, McIntosh K. Enterovirus infections in neonates. J Pediatr 1976;89:787–91.
[10] Pedrosa C, Lage MJ, Virella D. Congenital echovirus 21 infection causing fulminant hepatitis in a neonate. BMJ Case Rep 2013;2013. http://dx.doi.org/10.1136/bcr-2012-008394.
[11] Pino-Ramirez RM, Pertierra-Cortada A, Iriondo-Sanz M, Krauel-Vidal X, Munoz-Almagro C. Neonatal echovirus 30 infection associated with severe hepatitis in twin neonates. Pediatr Infect Dis J 2008;27:88.
[12] Huang QS, Carr JM, Nix WA, Oberste MS, Kilpatrick DR, Pallansch MA, et al. An echovirus type 33 winter outbreak in New Zealand. Clin Infect Dis 2003;37:650–7.
[13] Leggiadro RJ, Chwatsky DN, Zucker SW. Echovirus 3 infection associated with anicteric hepatitis. Am J Dis Child 1982;136:744–5.
[14] Imai T, Itoh S, Okada H, Onishi S. Aplastic anemia following hepatitis associated with echovirus 3. Pediatr Int 2002;44:522–4.
[15] Selwyn S, Montgomery H, Gray NA. Fatal illness associated with E.C.H.O. virus type 3 infection. Scott Med J 1963;8:162–3.
[16] Haynes RE, Cramblett HG, Hilty MD, Azimi PH, Crews J. ECHO virus type 3 infections in children: clinical and laboratory studies. J Pediatr 1972;80:589–95.
[17] Stevenson J, Hambling MH. Paralysis in echovirus-3 infection. Lancet 1968;1:525–6.
[18] Williams H, MacArthur P, Bell EJ, Lamb R. Paralysis in echovirus-3 infection. Lancet 1968;1:425.
[19] Bell E. Echovirus-3 meningoencephalitis. Lancet 1971;297:703.
[20] Peters AH, O'Grady JE, Milanovich RA. Aseptic meningitis associated with Echovirus type 3 in very young children. Am J Dis Child 1972;123:452–6.
[21] Sekiguchi J, Kubo H, Kaida A, Takakura K, Goto K, Hase A, et al. High incidence of human echovirus type 3 among children in Osaka, Japan during the summer of 2010. Jpn J Infect Dis 2011;64:533–5.
[22] Person DA, Smith TF, Herrmann EC. Outbreak of echovirus-3 meningoencephalitis. Lancet 1971;1:238.
[23] Krajden S, Middleton PJ. Enterovirus infections in the neonate. Clin Pediatr (Phila) 1983;22:87–92.
[24] Abzug MJ. Prognosis for neonates with enterovirus hepatitis and coagulopathy. Pediatr Infect Dis J 2001;20:758–63.
[25] Lin TY, Kao HT, Hsieh SH, Huang YC, Chiu CH, Chou YH, et al. Neonatal enterovirus infections: emphasis on risk factors of severe and fatal infections. Pediatr Infect Dis J 2003;22:889–94.
[26] Squires Jr RH, Shneider BL, Bucuvalas J, Alonso E, Sokol RJ, Narkewicz MR, et al. Acute liver failure in children: the first 348 patients in the pediatric acure liver failure study group. J Pediatr 2006;148:652–8.
[27] Farmer DG, Venick RS, McDiarmid SV, Duffy JP, Kattan O, Hong JC, et al. Fulminant hepatic failure in children: superior and durable outcomes with liver transplantation over 25 years at a single center. Ann Surg 2009;250:484–93.
[28] Mahadeb P, Gras J, Sokal E, Otte JB, Lerut J, Detaille T, et al. Liver transplantation in children with fulminant hepatic failure: the UCL experience. Pediatr Transplant 2009;13:414–20.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 363790, 6 pages
http://dx.doi.org/10.1155/2014/363790

Research Article

Simple and Cost-Effective Restriction Endonuclease Analysis of Human Adenoviruses

Arun Kumar Adhikary,[1,2] Nozomu Hanaoka,[2] and Tsuguto Fujimoto[2]

[1] *Unit of Microbiology, Faculty of Medicine, AIMST University, Semeling, 08100 Bedong, Kedah Darul Aman, Malaysia*
[2] *Infectious Disease Surveillance Center, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan*

Correspondence should be addressed to Tsuguto Fujimoto; fujimo-t@nih.go.jp

Received 15 January 2014; Accepted 5 February 2014; Published 9 March 2014

Academic Editor: Andreas Jenke

Restriction endonuclease analyses (REAs) constitute the only inexpensive molecular approach capable of typing and characterizing human adenovirus (HAdV) strains based on the entire genome. However, the application of this method is limited by the need for time-consuming and labor-intensive procedures. We herein developed a simple and cost-effective REA for assessing HAdV. The method consists of (1) simple and cost-effective DNA extraction, (2) fast restriction endonuclease (RE) digestion, and (3) speedy mini agarose gel electrophoresis. In this study, DNA was isolated according to the kit-based method and 21.0 to 28.0 μg of viral DNA was extracted from prototypes (HAdV-1, HAdV-3, HAdV-4, and HAdV-37) in each flask. The amount of DNA ranged from 11.4 to 57.0 μg among the HAdV-3 ($n = 73$) isolates. The obtained viral DNA was found to be applicable to more than 10 types of REAs. Fast-cut restriction endonucleases (REs) were able to digest the DNA within 15 minutes, and restriction fragments were easily separated via horizontal mini agarose gel electrophoresis. The whole procedure for 10 samples can be completed within approximately six hours (the conventional method requires at least two days). These results show that our REA is potentially applicable in many laboratories in which HAdVs are isolated.

1. Introduction

Human adenoviruses (HAdVs) are divided into seven species, A (HAdV-A) through G (HAdV-G), based on various biological and morphological criteria, nucleic acid characteristics, and homologies [1]. Approximately one-third of HAdVs are associated with human diseases, being estimated to cause 8% of clinically relevant viral diseases globally. Common adenoviral diseases include respiratory infections in children and military recruits, infantile gastroenteritis, and ocular infections among healthy individuals. Less frequently, these pathogens can cause urinary tract infections, myocarditis, meningoencephalitis, and acute hemorrhagic cystitis [2]. Meanwhile, in neonates and immunocompromised individuals, HAdVs have been reported to cause fulminant fatal pneumonia, hepatitis, and/or encephalitis [3, 4]. Genetically variable strains are present within a type designated as the genome type or DNA variant [5]. The genome type is determined based on a panel of a restriction endonuclease analysis (REA) of the viral genome. The site of cleavage of DNA by a restriction endonuclease is sequence dependent, and the presence of mutations at potential cleavage sites, insertions, and deletions anywhere in the genome results in different patterns of fragments when separated on agarose gel, a phenomenon termed restriction fragment length polymorphism [6]. The profile of DNA fragments visualized via gel electrophoresis can be compared to other published profiles of adenovirus isolates in order to designate the genome type [7]. REA is currently the only inexpensive molecular approach capable of typing and characterizing HAdV strains based on the entire genome.

Two systems for naming HAdV genome type/DNA variants are currently in use. In one nomenclature system, the prototype strain is abbreviated as "p," while the other strains were designated as a, b, c, and so forth in order of discovery [5, 8]. In other classification systems, numerical codes for multiple restriction enzymes are used to denominate the genome type. In this method restriction endonucleases are

displayed in alphabetical order. The prototype restriction profile is designated as 1, and each profile distinct for a given endonuclease is designated as 2, 3, 4, and so forth in chronological order of each new profile [9].

Molecular epidemiological studies have been conducted using genome typing, and more than 20 genome types of HAdV-7 have been reported to date. Among them, HAdV-7 h and HAdV-7d are reported to be virulent. HAdV-7 h became a predominant genotype in South America in 1986 and has circulated in North America since 1998 [10, 11]. HAdV-7d is associated with an 18% fatality rate in Korea among infants and children who presented with clinical evidence of lower respiratory tract infections; while HAdV-3 includes 51 genome types, many of which are associated with fatal infections. For example, HAdV-3a17 exhibits a 3.6% fatality rate among pediatric patients [12]. Ten genome types of HAdV-8 have been reported. HAdV-8e circulates worldwide and is related to many outbreaks of epidemic keratoconjunctivitis, whereas HAdV-8j is a localized strain [13, 14]. Therefore, it is clear that the HAdV genome types differ in virulence, and characterizing these strains is thus both clinically and epidemiologically important.

However, genome typing methods are time consuming and labor intensive, with their success being primarily dependent upon the extraction of a fairly large amount of viral DNA. DNA can be extracted from culture fluid, virus-infected cell lines, or both. The standard protocol for DNA extraction using ultracentrifugation involves the purification of viral particles obtained from infected cells, which requires time-consuming steps, such as cell disruption, cesium chloride centrifugation, and dialysis [15]. Another widely used method, developed by Hirt, or modified Hirt's methods, consists of extracting the HAdVs from infected cell DNA without prior purification of the virions [16, 17]. In these methods, overnight NaCl precipitation of cellular genomic DNA is required. Moreover, the above methods for preparing HAdV DNA require many steps and cannot be performed within one day. Methods employing slightly quicker extraction of DNA using a 75 cm^2 culture flask via short ultracentrifugation or concentration of the virus using a membrane filter followed by subsequent treatment with proteinase K to hydrolyze the viral proteins with final ethanol precipitation of viral DNA have been reported [18, 19]. However, the amount of DNA obtained using these methods is not sufficient for multiple REA digestion.

Considering the clinical and epidemiological importance of genome typing, we developed a one-day REA method that significantly reduces the time required to extract DNA from adenovirus-infected A549 cells using a commercial DNA extraction kit, rapid DNA digestion, and quick electrophoresis.

2. Materials and Methods

2.1. HAdV Strains. Prototype strains of HAdV-1, HAdV-3, HAdV-4, and HAdV-37 were obtained from the American Type Culture Collection (ATCC, Rockville, MD). A total of 73 HAdV-3 strains isolated over 22 years in Japan were used.

2.2. Viral Culture and Extraction of Viral DNA. The stock viruses were grown in A549 cells. A sample of each stock virus was further grown in the same cell line in a 25 cm^2 tissue culture flask (Becton Dickinson). The cells were infected according to standard procedures at an approximate multiplicity of infection of 10. The cells were observed every day for cytopathic effects (CPEs). When a CPE of 80% or more was detected, the cells were dislodged via tapping and/or with a rubber policeman, and the cells with medium were collected in a 15 mL conical centrifuge tube. The tube was then centrifuged at 1,500 ×g for five minutes to form cell pellets. The supernatant was discarded, and the cells were mixed via vortexing with the remaining medium (approx. 200 μL). The viral DNA was extracted using the High Pure Viral Nucleic Acid Kit (Roche). First, 200 μL of binding buffer supplemented with 50 μL of proteinase K (200 μg/mL) was mixed well, after which the content was immediately transferred to a 1.5 mL microcentrifuge tube and incubated at 72°C for 10 minutes. Then, 100 μL of binding buffer was added to the tube and mixed well. Poly A included in the kit was not used. The content was subsequently transferred to a spin column combined with the collection tube, and the column was centrifuged at 8,000 ×g for one minute then combined with a new collection tube. The old collection tube and contents were discarded. Four hundred and fifty microliters of wash buffer was added to the column and centrifuged at 8,000 ×g for one minute. The column was then combined in a new collection tube, and the collection tube and contents were discarded. Then, 450 μL of washing buffer was again added to the column and centrifuged a second time at 8,000 ×g for one minute followed by 13,000 ×g for 10 seconds in order to drain out the washing buffer. The column was combined with a 1.5 mL microcentrifuge tube, and 100 μL of elution buffer was added to the column and centrifuged at 8,000 ×g for one minute, after which the column was disposed. The contents remaining in the microcentrifuge tube comprised viral genomic DNA.

2.3. Quantitation of DNA. The concentrations of genomic DNAs were measured using a BioSpec-nano spectrophotometer (Shimadzu Corporation, Kyoto, Japan). In short, 2 μL (path length: 0.7 mm) of viral DNA was mounted in sample stage, and the instrument automatically measured the DNA concentration. The purity of DNA was considered significant for an optical density ratio (OD ratio) OD 260/OD 280 of approximately 1.8 and less than 2.0. The copy numbers of randomly selected viral DNA (n = 5) were measured using real-time PCR [20] after dilution (×100). To confirm the quality of the extracted DNA, 1.0 μL of the extracted DNA was electrophoresed on 1% horizontal submerged agarose mini gel. The electrophoresis was performed in TAE buffer at 100 V for 50 minutes (Mupid, Advance, Tokyo, Japan).

2.4. DNA Restriction Endonuclease Analysis and Agarose Gel Electrophoresis. Both usual RE and RE fast digestion of HAdV-1p, HAdV-3p, HAdV-4p, and HAdV-37p were performed with usual REs and high fidelity (HF) REs, such as *BamHI*, *BglII*, and *HindIII* (New England Biolabs),

which have the power to complete digestion within 5–15 minutes. A total of 1 μg of DNA was incubated with 10 units of fast digest RE in 20 μL of reaction mixture at an appropriate temperature and time (recommended for each RE). The fragmented DNA and molecular weight markers are loaded in separate wells on 1.2% horizontal submerged agarose mini gel (size, 106 mm (W) × 60 mm (L)) made with a thicker comb (6 mm × 12 well), and electrophoresis was performed in TAE buffer at 50 V for one hour and 50 minutes (Mupid). The electrophoresis machine was cooled on ice or in a cooler (Cosmo-bio, Tokyo, Japan) to keep the buffer cool during electrophoresis. After electrophoresis, the DNA in the gel was stained for 30 minutes in GelRed (Biotium, Hayward, CA) solution made in TAE buffer. Lambda DNA-HindIII digest marker (New England Biolabs) photographs were taken under UV light. For electrophoresis, a prototype strain was always added as a control.

3. Ethical Considerations

This study did not use clinical samples but rather isolates obtained using anonymous information that cannot be associated with the individual patient. Therefore, the study protocol did not require ethics committee approval. For HAdV isolation, Ethical Committee has approved the experiments (ID25-35).

4. Results

4.1. Viral Culture and Extraction of Viral DNA. Mixing the cell pellets via vortexing is important before adding the binding buffer (6 M guanidine-HCl, 10 mM Tris-HCl, 10 mM urea, 20% Triton X-100 (*w/v*), pH4.4 (+25°C)). If the binding buffer is added without creating a cell suspension, a clump of cell lysate is formed, which can clog the mesh of the spin column. The entire procedure, from collection of the infected cells from a 25 cm^2 flask to extraction of DNA, takes one hour and 30 minutes for 10 samples. The CPE of prototypes HAdV-1, HAdV-3, HAdV-4, and HAdV-37 was visible within 24 hours and included 80% of the cells within 48 hours (Figure 1). This method has been well studied for identifying the most clinically important HAdVs that grow well in cell culture and give rise to clearly visible CPEs; however, it has not been thoroughly evaluated for identifying fastidious HAdVs, such as HAdV-41.

4.2. Quantitation of Genomic DNA. The mean concentration of DNA among the 73 HAdV-3 isolates was 338 ± 111 ng/μL (mean ± standard deviation, range 114–576 ng/μL) with an OD ratio (OD 260/280) of 1.92 ± 0.04 (mean ± standard deviation, range 1.74–1.98), as expressed by the manufacturer. A clear background in the gel also suggests the extraction of a good amount of quality DNA. The extracted DNA included 2.1×10^{10}–9.1×10^{10} copies/μL (mean, 5.3×10^{10} copies/μL) of viral DNA.

4.3. DNA Restriction Endonuclease Analysis and Agarose Gel Electrophoresis. The *BamHI*, *BglII*, *HindIII*, and *SmaI* digestion patterns of HAdV-1p, HAdV-3p, HAdV-4p, and HAdV-37p were in agreement with previously published restriction patterns [21, 22]. The restriction pattern clearly distinguished these strains (Figures 2(a) and 2(b)). We used Mupid mini gel and big gel (125 mm (W) × 123 mm (L)) to separate the cleavage products. We found that, like big gel, mini gel can be used to separate the DNA fragments well and is adequate for use in the classification of genome types.

Table 1: Overall time, including preparation time, required for the restriction endonuclease analysis (REA) of HAdVs using 10 samples.

REA steps	Time required
(1) DNA extraction from infected cells	1 hour 30 minutes
(2) Quantification of DNA of genomic DNA and electrophoresis	1 hour 15 minutes
(3) Restriction endonuclease digestion	5–15 min by fast-cut enzyme (1 hour 30 minutes by conventional enzyme)
(4) Agarose gel electrophoresis	1 hour 50 minutes
(5) Gel stain	30 minutes
Total time	Approx. 6 hours

4.4. Overall Time for the Entire Procedure. Theoverall working time is dependent on the time required for DNA extraction, concentration measurement, digestion, and electrophoresis. The working time of the entire procedure is approximately six hours (Table 1).

5. Discussion

REA of viral DNA is established for studies of the molecular epidemiology of HAdVs. It is important to (1) understand the incidence and prevalence of different genome types, (2) identify the most prevalent genome types circulating worldwide or in a given country, (3) compare the most prevalent currently circulating HAdV genome types in order to identify possible common targets for intervention, and (4) analyze the frequency of genome types in order to identify their geographical distribution and patterns of circulation. However, performing fast REA of HAdVs poses a challenge with respect to standardizing the techniques used in various steps of the procedures, such as viral culture, lengthy DNA extraction, prolonged electrophoresis to separate DNA fragments, and the need for proper documentation of results. Occasionally, REAs must be repeated [23].

Various continuous epithelial cell lines, including HEp-2, HeLa, KB, and A549, are useful for isolating HAdVs. However, comparative studies of various cell lines have shown that the A549 cell line is very permissive, allowing for the propagation of HAdVs [24, 25]. The cells usually swell, with nuclear enlargement, followed by

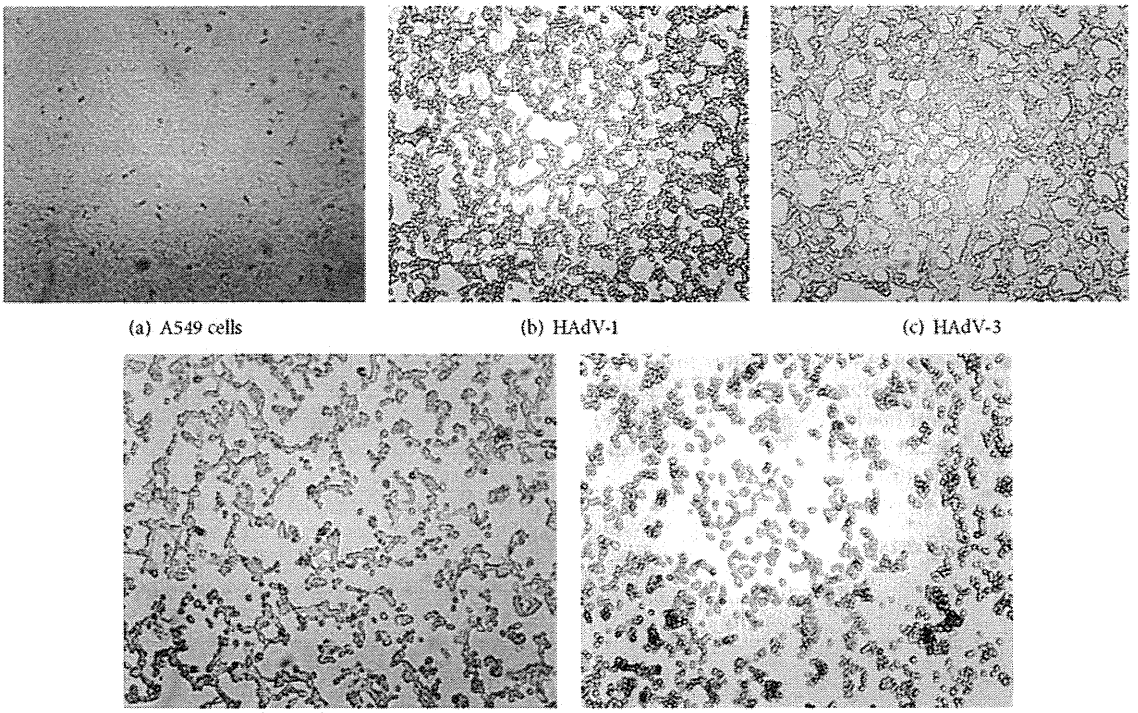


FIGURE 1: Typical HAdV CPE. (a) Noninfected A549 cells, (b) A549 cells infected with HAdV-1, (c) A549 cells infected with HAdV-3, (d) A549 cells infected with HAdV-4, and (e) A549 cells infected with HAdV-37.

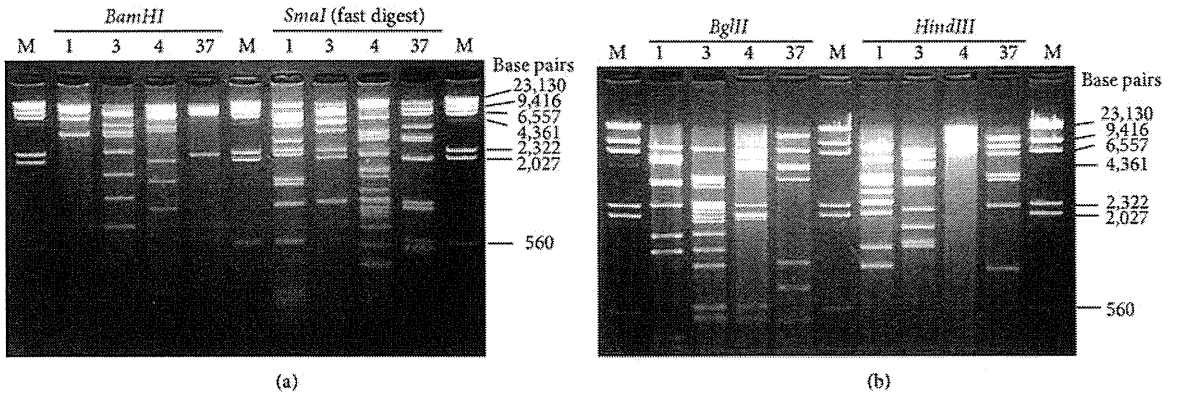


FIGURE 2: Picture of the REA. (a) REA pattern for *BamHI* and *SmaI* (fast digest). (b) REA pattern for *BglII* and *HindIII*. M shows Lambda DNA-HindIII digest marker. Numbers 1, 3, 4, and 37 show HAdV-1, -3, -4, and -37, respectively.

detachment from the surface [2]. The cytologic changes vary among the types of HAdVs, as shown in Figure 1.

The success of REA is principally dependent on the ability to extract a large amount of good quality viral DNA. In our kit-based extraction method, we have succeeded in extracting 11.4 μg to 57.0 μg of DNA (34 ± 11 μg, mean ± SD) from 25 cm^2 flasks, which is sufficient for analyses of multiple REAs which use 1 μg per reaction. It is easier to use 25 cm^2 flasks than 75 cm^2 flasks to prepare confluent cell cultures and also it saves space in the CO_2 incubator, with subsequent cost savings. In comparison to time-consuming and labor-intensive ultracentrifugation methods or Hirt's method, our procedure is appropriate for use in laboratories in which routine virology work is performed. This method also results in a significantly higher amount of DNA than other previously described DNA extraction methods [18, 19], which have limited use, as they are not cost effective and do not provide a sufficient amount of DNA. We attempted to extract DNA from 25 cm^2 flasks using modified Hirt's method and obtained an approximately 10 times lower amount of viral

genome (data not shown). This finding may be due to the many steps required in modified Hirt's method, resulting in the loss of viral DNA in each step.

Regarding the extraction of viral DNA, a visible CPE of 80% is a good result, yielding clear DNA in gel electrophoresis. However, it has not been thoroughly evaluated for fastidious HAdV, such as HAdV-41. In such case, PCR-RFLP [26] might be helpful. RE is cost effective and should not be underrepresented.

Traditionally, two hours of incubation are required for DNA cleavage using conventional endonucleases. Currently, fast-cut RE is available from various biomedical suppliers, with a similar or lower cost compared to the conventional one. These enzymes save time, as they cleave DNA within 5–15 minutes.

The ability to separate the digested DNA fragments via electrophoresis and documentation of gel images is very important for classifying the genome type. Sometimes it is difficult to distinguish important bands on these images and can be challenging to interpret the resulting restriction pattern based on comparisons with published restriction profiles [21, 27]. Therefore, we recommend the use of a prototype or known genome type of the respective isolates as a standard for quick interpretation of the results. We employed a mini horizontal agarose gel apparatus at lower voltage (50 V in this case), as it can separate the fragments clearly within two hours, which is both quick and cost effective.

Despite the clinical-epidemiological impact of REA, the number of REA-based genome typing studies is lower than expected, which may be due to the specialized and tedious nature of the procedure [7]. Therefore, PCR-based identification methods appear to be overtaking traditional serotyping and REA methods. It is important to note that PCR- and sequencing-based typing methods (molecular typing) focus on limited areas of the adenovirus genome and have the potential to overlook and mistype new HAdV variants that differ genetically in other gene regions. Recently, a genotyping method employing the complete sequence of HAdV was introduced [28]. However, this typing method is too expensive for routine study, at least in developing countries. Hence, until whole-genome sequencing (genotyping) becomes less expensive, REA will continue to have an important role in the epidemiological study of adenoviruses [29].

We have also successfully applied our REA method on different clinical isolates (data not shown) as well as on a new recombinant type. A novel HAdV-48 recombinant was isolated from eye swab of conjunctivitis patient [30] and our method was applicable to find the novel strain. We also confirmed that our REA is applicable to identify recombinant types such as HAdV-53 (AB605246) which is identical to type 22 in hexon loop region and type 8 in fiber region.

The extracted DNA exhibits adequate purity for REA and is found to be useful for direct nucleotide sequencing (data not shown). The time required to complete the extraction procedure is approximately one hour and 30 minutes for 10 samples. RE digestion and electrophoresis require an additional five hours. Fast digest RE (e.g., Takara Shuzo, Shiga, Japan) can even shorten the reaction time by more than an hour compared with the conventional method.

Our rapid REA method can significantly reduce all steps of the REA procedure, from extraction of DNA to electrophoresis of the cleavage products, thus allowing laboratories with virus culture facilities to easily perform REA of HAdVs at a lower cost. This method is potentially applicable in various research institutes as well as public health laboratories where HAdVs are routinely isolated.

6. Conclusion

To our knowledge, this is the first practical approach for developing a quick step-by-step REA procedure that includes extraction of good quality and adequate quantity of DNA from a small volume of infected A549 cells, fast digestion of DNA, and rapid electrophoresis of the cleavage products. The application of our newly developed REA method will allow public health and research laboratories to determine the genome type quickly and inexpensively, which will further aid epidemiological, clinical, and virological studies of HAdVs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

All authors contributed equally to this work.

Acknowledgments

The authors thank Ms. Nozomi Yamamoto and Ms. Masako Nakamura (Fukui Prefectural Institute of Public Health and Environmental Science) for providing clinical isolates used in this study. They also thank Ms. Masami Konagya for the technical help. This study is supported by Japan Society for the Promotion of Science (JSPS) as "the invitation fellowship for research in Japan (long term)" and JSPS KAKENHI Grant no. 24592662.

References

[1] A. J. Berk, "Adenoviridae," in *Fields Virology*, D. M. Knipe, P. M. Howley, J. I. Cohen et al., Eds., pp. 1704–1731, Lippincott Williams & Wilkins, Philadelphia, Pa, USA, 6th edition, 2013.

[2] W. S. M. Wold and M. G. Ison, "Adenoviruses," in *Fields Virology*, D. M. Knipe, P. M. Howley, J. I. Cohen et al., Eds., pp. 1732–1767, Lippincott Williams & Wilkins, Philadelphia, Pa, USA, 6th edition, 2013.

[3] T. Lion, K. Kosulin, C. Landlinger et al., "Monitoring of adenovirus load in stool by real-time PCR permits early detection of impending invasive infection in patients after allogeneic stem cell transplantation," *Leukemia*, vol. 24, no. 4, pp. 706–714, 2010.

[4] M. Tebruegge and N. Curtis, "Adenovirus infection in the immunocompromised host," *Advances in Experimental Medicine and Biology*, vol. 659, pp. 153–174, 2010.