3. 指向性検査法

【遺伝子型検査】

患者血漿中から抽出したHIV genome RNAおよびPBMCから抽出したproviral DNAを鋳型として、*env*領域をPCR法で増幅した。Gateway BP反応を用いて、pFG01にクローニングし、sequence解析を行った。*vpu*を含む*env*以外のORFにstop codonは認めなかった。V3領域の塩基配列からGeno2Pheno法を用いて、指向性を決定した。また、*env*全長のsequenceをもとにMEGAを用いて系統樹解析を行った。

【表現型検査】

また、pFG01からさらにGateway LR反応を用いてpFG02 Rlucに*env*領域をcloningした。これを、293T細胞にtransfectionし、recombinant HIV-1を含む培養上清を得た。これを、CD4、CD4+CXCR4、CD4+CCR5を発現しているNP-2細胞に感染させ、48時間後にcell lysateを調整し*Renilla* luciferase活性を測定し、感染の有無を評価した（図2）。感染の有

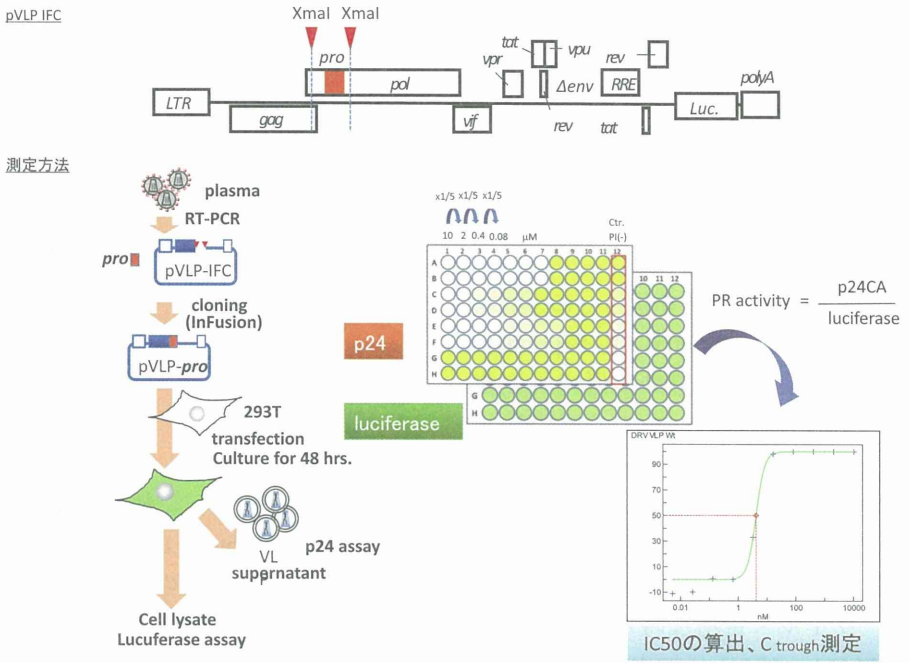


図1：VLP ELISA法の概略

血漿中のHIV-1 genome RNAからprotease領域を増幅し、pVLP INFにInFusion systemを用いてクローニングする。293T細胞にtransfectionし、上清中のp24、cell lysate中のluciferase活性からprotease活性を評価する。96穴プレートを用いて感受性検査を実施し、IC50を算出する。

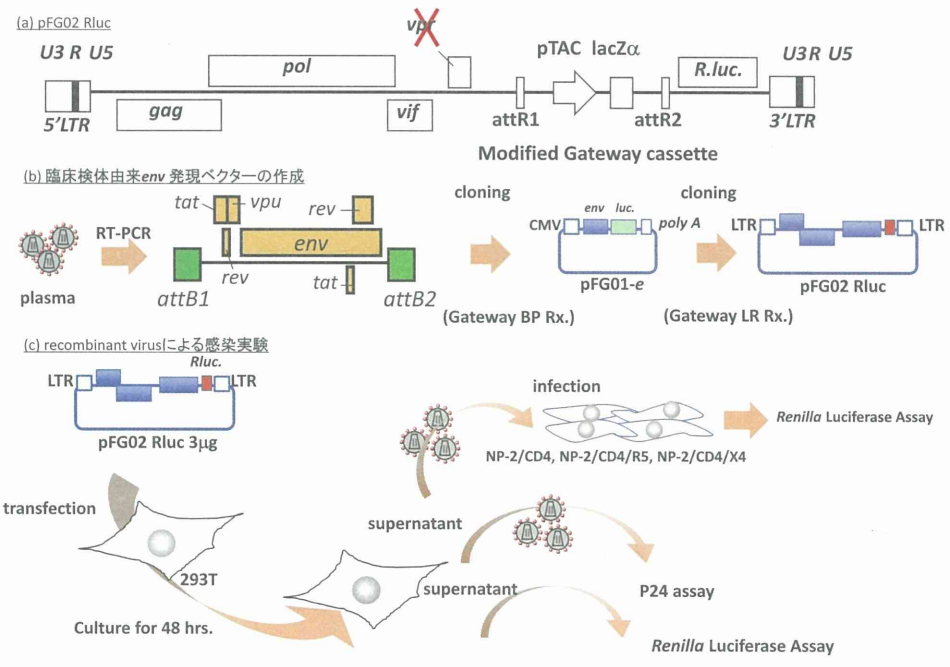


図2：recombinant HIV-1を用いた指向性感受性検査法の概略

臨床検体由来*env*を含むpFG01から*env*をcloning可能なpFG02（a）。Gateway cloning systemによるpFG02へのcloning方法（b）と、recombinant HIV-1を使った指向性表現型検査法（c）。pFG01、pCTLRlucのtransfectionの効率はそれぞれluciferase、*Renilla* luciferaseを測定することで評価することが可能である。

無は、CD4のみを発現しているNP-2のRluc活性値に対するCD4+CXXR4またはCD4+CCR5のRluc活性値の比を求めた。*env*(-)のpFG01のRluc活性値を用いてウイルス量を補正し評価した。

検査はduplicateで3回実施し、平均値をデータとして使用した。また、CXCR4指向性HIV-1としてHXB-2、CCR5指向性HIV-1としてJRFLの*env*領域を組み込んだvirusを用いた。

(倫理面への配慮)

検体はインフォームドコンセントを得て採取した。また、検体は連結匿名化し、個人が特定されないように配慮した。

C．研究結果

1. subtype C薬剤耐性HIV感染症例に対するsalvage療法

薬剤耐性遺伝子型検査の結果を図3-aに示す。LPV高度耐性が既知のsubtype B HIV-1由来proteaseのアミノ酸配列（320）と、国立感染症研究所佐藤浩徳先生から分与いただいたSubtype Cの感染性分子クローンとしてpIndieC1のアミノ酸配列を示す。母親から分離されたクローンである NMC 256には耐性関連変異を認めなかったが、児は転院時に採取された検体から得られたクローンNMC 271には既にV82A変異を認め、ウイルス量増加時に採取した検体NMC307ではM46I、I54Vが加わった。VLP ELISA法を用いて表現型検査を行うと、NMC 307ではLPVに対してLPV高度耐性の320と同等の強い耐性を示し、遺伝子型によるGSSと乖離を示した（図3-b）。

これらの解析をもとに母児ともにDRVをkey drugとする抗HIV療法を行ったところ良好な経過を得た（図4-a, b）。

2. 指向性検査法の検討

【遺伝子型検査結果】

NMC191、NMC192それぞれの検体から得たHIV genome RNAとproviral DNAからそれぞれ6クローンを得た。図5-a, bと図6-a, bにV3領域のアミノ酸配列と指向性、false positive rate（FPR）および系統樹解析の結果を示す。Geno2Phenoによる解析ではNMC 191はX4指向性、NMC 192はR5指向性と判定された。

【recombinant HIV-1を用いた表現型検査】

pFG02を用いてreombinant HIV-1を作成し、感染実験を行った。NMC 191由来の*env*を組み込んだウイルスはdual tropicを示した（図5-c）。また、NMC192での解析ではR5指向性を示した（図6-c）。

D．考察

VLP ELISA法は、これまで薬剤耐性関連変異が高度に蓄積したsubtype B HIV-1感染症例で有用性を検討してきたが、今回の検討で、non-subtype C HIV-1のprotease活性の評価にも利用可能であることが示された。Protease inhibitorは、subtypeによっては、治療未経験例でも感受性が低下している場合がある。現在、コンピュータシミュレーションによる感

(a) 遺伝子型検査

HXB2	PQVTLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIGGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF
320	--I------I----VA----------D--------I--------V---------P--------VLS---I----T--V----M--L------
pIndieC1	--I--------S-R---Q----------VNL--K----------P-----K----------I------M---L------
NMC256	--I--------S-K-A----------LK-PG----------EEVPL----------M---L------
NMC271	--I--------S-K-A----------LK-PG----------EEVP----------A------M---L------
NMC307	--I------F-S-K-A----------LK-PG-----I--------V-----EEVP----------A------M---L------

(b) 感受性検査

Clones	subtype	LPV		DRV		Major Resistance Mutations	Minor Resistance Mutations
		VLP*	GSS	VLP*	GSS		
HXB2	B	24.2	0	3.7	0	-	-
320	B	1611.2	65	19.0	2	M46L, I54V, V82T, L90M	L10I, L63P, A71V, G73S, V77I, I85V, I93L
NMC256	C	19.6	0	3.8	0	None	None
NMC271	C	86.0	25	2.6	0	V82A	None
NMC307	C	1658.6	51	8.1	2	M46I, I54V, V82A	L10F

図3：薬剤耐性検査の結果

遺伝子型検査の結果を（a）、感受性検査の結果を（b）に示す。HXB2のIC50に対する各クローンのIC50の比をVLP ELISA法の結果として示す。* GSS : Genotypic susceptibility score

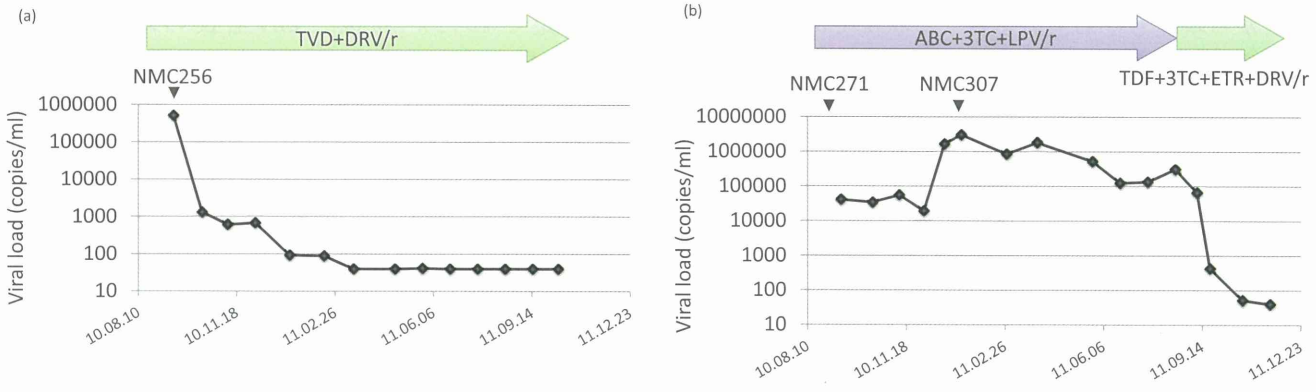
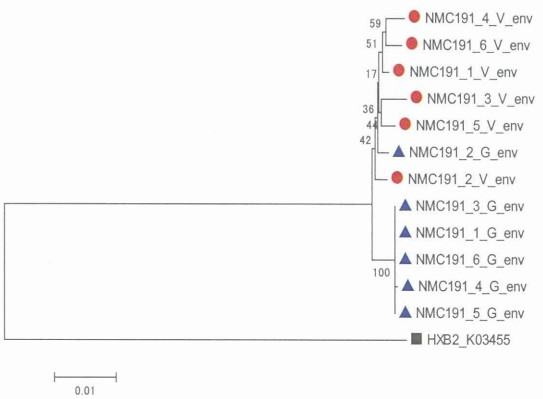


図4：subtype C感染例の治療経過
母親の治療経過を（a）、児の治療経過を（b）に示す。児については、DRV/rを含む協力な抗HIV療法を行い、ウイルス量の抑制に成功した。

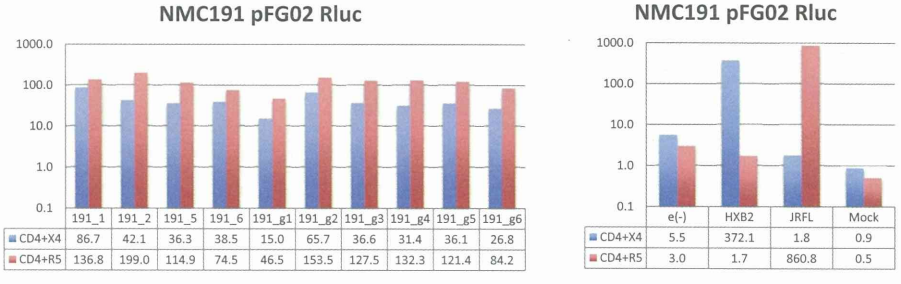
(a)

Sample	Sample name	V3 region	FPR	判定結果
Plasma (viral RNA)	NMC191_1_V_env	CTRPNNNTRKGIHLGPGRAWYATEKIIGDIRKAHC	1.7	X4
	NMC191_2_V_env	CTRPNNNTRKGINLGPGRAWYTTEKIIGDIRKAHC	1.7	X4
	NMC191_3_V_env	CTRPNNNTRKGINLGPGRAWYATEKIIGDIRKAHC	1.8	X4
	NMC191_4_V_env	CTRPNNNTRKGIHLGPGRAWYATEKIIGDIRKAHC	1.7	X4
	NMC191_5_V_env	CTRPNNNTRKGINLGPGRAWYATEKIIGDIRKAHC	1.8	X4
	NMC191_6_V_env	CTRPNNNTRKGIHLGPGRAWYATEKIIGDIRKAHC	1.7	X4
PBMC (proviral DNA)	NMC191_1_G_env	CTRPNNNTRKGIHLGPGRAWYATEKIIGDIRKAHC	1.7	X4
	NMC191_2_G_env	CTRPNNNTRKGINLGPGRAWYATEKIIGDIRKAHC	1.8	X4
	NMC191_3_G_env	CTRPNNNTRKGIHLGPGRAWYATEKIIGDIRKAHC	1.7	X4
	NMC191_4_G_env	CTRPNNNTRKGIHLGPGRAWYATEKIIGDIRKAHC	1.7	X4
	NMC191_5_G_env	CTRPNNNTRKGIHLGPGRAWYATEKIIGDIRKAHC	1.7	X4
	NMC191_6_G_env	CTRPNNNTRKGIHLGPGRAWYATEKIIGDIRKAHC	1.7	X4

(b)



(c)



	191_1	191_2	191_5	191_6	191_g1	191_g2	191_g3	191_g4	191_g5	191_g6
CD4+X4	86.7	42.1	36.3	38.5	15.0	65.7	36.6	31.4	36.1	26.8
CD4+R5	136.8	199.0	114.9	74.5	46.5	153.5	127.5	132.3	121.4	84.2

	e(-)	HXB2	JRFL	Mock
CD4+X4	5.5	372.1	1.8	0.9
CD4+R5	3.0	1.7	860.8	0.5

図5：NMC191の指向性検査結果
Geno2Phenoによる解析結果（a）、系統樹解析結果（b）および表現型検査結果（c）の結果を示す。縦軸の1.0は、NP-1 CD4と同じ*Renilla* luciferase活性を示す場合、すなわち、感染性がないことを示す。NMC 191から得られたクローンは全てdual tropicを示した。

受性評価系の構築も同時に行っているが、non-subtype B HIV-1 proteaseの構造データが限られていることから現在もなお検討を要する状況である。ウイルス分離、組み替えHIV-1の作成には施設や手技上の問題もある。簡易はVLP ELISA法を応用した感受性検査は適応範囲の広い検査法であると考えられた。

NMC 191から得た12クローンを用いてpseudotype HIV-1を作成、表現型検査を試みたが、NMC 191由来のenvを発現させると非常に強い細胞毒性により十分なウイルス量が得られない場合があったり、クローン間、実験間のばらつきが非常に大きかった。

それに比べて、pFG02を用いてrecombinant HIV-1を作成する方法が、容易に安定した結果を得る事ができた。Gateway systemはBP反応、LR反応をone tubeで連続して行うことが可能であり、現在、他の検体を用いて指向性表現型検査を実施している。

今回、検討した検体のうちNMC 191はdual tropicと判定された。今後、*env*全長の塩基配列とGeno2Phenoを初めとする遺伝子型検査と表現型検査の結果を比較検討することにより、指向性を規定するアミノ酸変異に関する知見が得られる可能性がある。また、臨床経過、MVCを使用した場合の治療効果を解析することにより、R5およびX4指向性HIV-1の臨床的意義が明らかになる可能性がある。

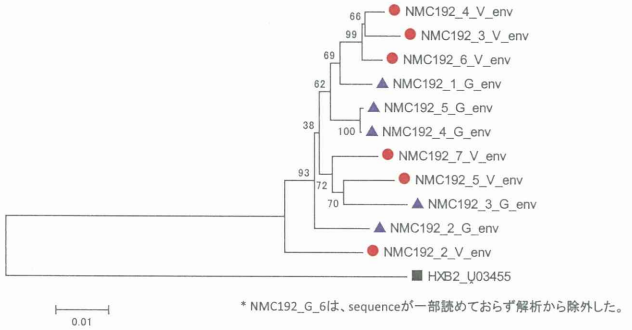
E．結論

これまでに確立したHIV-1 protease活性評価法であるVLP ELISA法は、non-subtype C HIV-1 proteaseに関しても活性の評価が可能であり、薬剤耐性感受性検査への応用が可能であった。

(a)

Sample	Sample name	V3 region	FPR	判定結果
Plasma (viral RNA)	NMC192_2_V_env	C⊕RP˜˜⊕RK˜IPLGPGR˜φY˜⊕GIIG˜IRQ˜HC	83.3	R5
	NMC192_3_V_env	C⊕RP˜˜⊕RK˜IHLGPGQ˜LY˜⊕GIIG˜IRQ˜HC	98.3	R5
	NMC192_4_V_env	C⊕RP˜˜⊕RK˜IRIGPGQ˜LY˜⊕GIIG˜IRQ˜HC	83	R5
	NMC192_5_V_env	C⊕RP˜˜⊕RK˜IRIGPGQ˜LY˜⊕GIIG˜IRQ˜HC	83	R5
	NMC192_6_V_env	C⊕RP˜˜⊕RK˜IRIGPGQ˜φY˜⊕GIIG˜IRQ˜HC	54.4	R5
	NMC192_7_V_env	C⊕RP˜˜⊕RK˜IRIGPGQ˜LY˜⊕GIIG˜IRQ˜HC	83	R5
PBMC (proviral DNA)	NMC192_1_G_env	C⊕RP˜˜⊕RK˜IRIGPGQ˜LY˜⊕GIIG˜IRQ˜HC	83	R5
	NMC192_2_G_env	C⊕RP˜˜⊕RK˜IRIGPGQ˜LY˜⊕GIIG˜IRQ˜HC	83	R5
	NMC192_3_G_env	C⊕RP˜˜⊕RK˜IRIGPG˜˜LY˜⊕GIIG˜IRQ˜HC	89.1	R5
	NMC192_4_G_env	C⊕RP˜˜⊕RK˜IRIGPGQ˜LY˜⊕GIIG˜IRQ˜HC	83	R5
	NMC192_5_G_env	C⊕RP˜˜⊕RK˜IRIGPGQ˜LY˜⊕GIIG˜IRQ˜HC	83	R5
	NMC192_6_G_env	C⊕RP˜˜⊕RK˜IRIGPGQ˜LY˜⊕GIIG˜IRQ˜HC	83	R5

(b)



* NMC192_G_6は、sequenceが一部読めておらず解析から除外した。

(c)

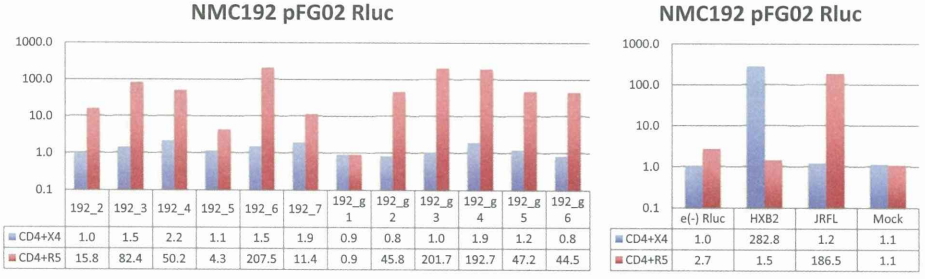


図6：NMC192の指向性検査結果　Geno2Phenoによる解析結果
(a)、系統樹解析結果（b）および表現型検査結果（c）の結果を示す。縦軸の1.0は、NP-1 CD4と同じ*Renilla* luciferase活性を示す場合、すなわち、感染性がないことを示す。NMC 192から得られたクローンの大部分はR5指向性を示した。

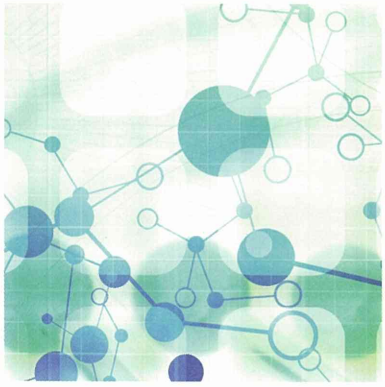
臨床検体由来envの指向性は、pFG02を用いてrecombinant HIV-1を作成することにより評価することが可能であった。現在用いられているGeno2Phenoによる遺伝子型検査の結果の妥当性評価等に有用である。

F. 研究発表

1) Hirano A, Ikemura K, Takahashi M, Shibata M, Amioka K, Nomura T, Yokomaku Y, Sugiura W: Lack of Correlation Between UGT1A1*6, *28 Genotypes, and Plasma Raltegravir Concentrations in Japanese HIV Type 1-Infected Patients. AIDS Res Hum Retroviruses. 2011 Nov 9. [Epub ahead of print]
2) Fujisaki S, Yokomaku Y, Shiino T, Koibuchi T, Hattori J, Ibe S, Iwatani Y, Iwamoto A, Shirasaka T, Hamaguchi M, Sugiura W: Outbreak of infections by hepatitis B virus genotype A and transmission of genetic drug resistance in patients coinfected with HIV-1 in Japan. J Clin Microbiol. 49(3):1017-24,2011

G. 知的財産権の出願・登録状況（予定を含む）

該当なし



抗HIV薬の心血管系への影響の解析

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研究要旨

日本人HIV感染者における動脈硬化症の実態を解明すべく頸動脈超音波検査および高感度CRP（hs-CRP）、血中ICMA-1、VCAM-1を用いて評価する研究を実施中である。また24時間自由行動下血圧計を用い、HIV感染血友病患者における高血圧の実態解明に関する研究を実施中である。未だ症例数は少ないものの5例中4例（80%）に脳血管イベントの危険因子となる早朝高血圧がみられた。

HIV感染者における動脈硬化症

A. 研究目的

抗HIV薬の進歩はHIV感染者の予後を大幅に改善したが、その一方で各種の副作用が問題となっている。特にプロテアーゼ阻害薬による脂質異常症は大きな課題で、PI投与により心筋梗塞のリスクが上昇するとの報告が存在する。またアバカビルは心筋梗塞のリスクを増大させ、血管内皮細胞を傷害することが報告されているが結論は出ていない。我々は日本人HIV感染者は、日本人全体に比し心・脳血管リスク因子である脂質異常症の有病率、喫煙率が有意に高く、32%の日本人HIV感染者が何らかの心・脳血管リスクを有することを明らかにした。今後はHIV感染者における動脈硬化症の改善、すなわち心・脳血管リスクを軽減させ、イベントを回避することがHIV感染者が長期療養を行う上で非常に重要な課題となると考えられる。本研究は日本人HIV感染者における動脈硬化症の実態を明らかにすることを目的として実施した。

B. 研究方法

頸動脈超音波検査および高感度CRP（hs-CRP）、血中ICMA-1、VCAM-1、HIV関連項目、心脳血管危険因子といったデータを横断的に収集した。動脈硬化の評価として頸動脈超音波検査により両総頸動脈の内膜中膜複合体厚（carotid intima media thickness（IMT））のうち最大IMT（max-IMT）とプラークの有無、高感度CRP（hs-CRP）、血中ICMA-1、VCAM-1を検討した。本研究では頸動脈超音波検査の精度向上と検査者によるバイアスを避けることを目的としてmax-IMTは頸動脈超音波検査画像解析ソフトIntimaScopeにより計測した。

実施にあたっては国立国際医療研究センター倫理委員会の承認を得て実施した。

C. 研究結果及び
D. 考察

目標症例数400例に対し現在のエントリー症例数201例、このうち超音波検査有所見者数80例で現在もエントリー継続中である。この有所見者のうち頸動脈にプラークを有する症例3例が検査の後に、適切な治療が開始されたにも関わらず心脳血管イベントを発症した。（50歳台前半男性：急性脳梗塞、50歳台後半男性：急性脳梗塞、60歳台前半男性：不安定狭心症）

現在症例のエントリーを勧めているところであるが、心脳血管イベント発症予防のスクリーニング検査目的としても検査を実施すべきと考える。

E．結論

現在症例のエントリーを勧めているところであるが、心脳血管イベント発症予防のスクリーニング検査目的としても検査を実施すべきと考える。

HIV感染血友病患者における脳血管イベント予防を目的とした高血圧の実態解明

A．研究目的

日本人HIV感染者における脳血管イベントを取り巻く状況は「**HIV感染者における動脈硬化症**」に記したとおりであるが、さらに薬害血友病患者はその出血傾向により、頭蓋内出血を発症しやすい。加えて薬害血友病患者も高齢化しつつあり、高血圧を合併する患者も増加傾向にある。本研究はHIV感染血友病患者における脳血管イベント予防を目的とした高血圧の実態解明を目的として行う。

B．研究方法

24時間自由行動下血圧計を用いて血圧の評価を行った。また頸動脈超音波検査および高感度CRP（hs-CRP）、血中ICMA-1、VCAM-1、HIV関連項目、心脳血管危険因子といったデータを横断的に収集した。

実施にあたっては国立国際医療研究センター倫理委員会の承認を得て実施した。

C．研究結果及び
D．考察

現在のエントリー症例数5例、全員男性、年齢は43-64才で中央値52歳であった。24時間自由行動下血圧計による血圧平均値は昼間134/88mmhg、夜間128/80mmhgで早朝高血圧を有する症例は4例（80%）であった。早朝高血圧は脳血管イベント発症の危険因子とされており、それが高率であることから、高血圧の適切な管理が必要であることが示唆された。

E．結論

現在症例のエントリーを勧めているところであるが、脳血管イベント発症予防のスクリーニング検査目的としても検査を実施すべきと考える。

F．研究発表

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なし

2．学会発表

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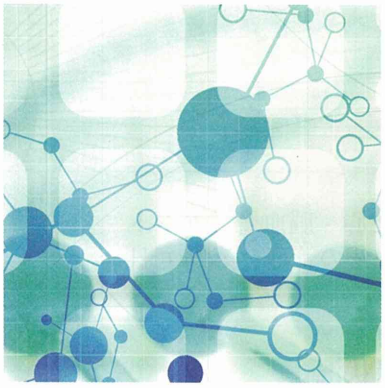
G．知的財産権の出願・登録状況

なし



新規薬剤の臨床効果の解析

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研究要旨

2006年以降に登場した新規抗HIV薬のうち、ラルテグラビルとダルナビルの併用療法の長期有効性に関する検討を行った。24週以上観察可能であった23例のうち20例で、最長144週の観察期間中良好なHIV抑制が維持されていたが、3例が治療中止に至り、うち1例ではラルテグラビル耐性変異の出現が確認された。観察期間中23例中13例で少なくとも1回のHIV血症が確認されたが、200 copies/mL以上の値が確認されたのはラルテグラビル耐性変異が出現した1例のみであった。

A．研究目的

抗HIV療法の進歩に伴いHIV感染者の生命予後が劇的に改善した結果、それぞれの感染者の服薬期間は長期化し、抗HIV薬の長期毒性や合併症による治療不耐、あるいは薬剤耐性の蓄積による治療失敗が大きな問題となっている。

2006年以降に登場した新規抗HIV薬（ダルナビルDRV、ラルテグラビルRAL、エトラビリンETR、マラビロクMVC）は薬剤耐性HIVに対して既存の薬剤より高い効果を有するほか、有害事象の面でも優れた特性を有する。このため新規抗HIV薬は既存薬の組み合わせによる治療が困難な症例における有力な選択肢となるが、これらの新規抗HIV薬に関する臨床試験のほとんどが海外のみで行われており、日本人における有効性・安全性のデータは少ない。本研究は新規薬剤を用いた治療の、日本人における有効性・安全性の検証を目的とする。

B．研究方法

国立国際医療研究センター エイズ治療・研究開発センターにおいて新規抗HIV薬を含む治療を行ったHIV感染者の治療成績を、診療録を用いて後方視的に解析する。

（倫理面への配慮）

研究結果の公表にあたっては個人を特定できる情報を含めない。

C．研究結果

本年度は、ラルテグラビルRALと（リトナビルにより増強した）ダルナビルDRVrの併用による核酸系逆転写酵素阻害薬回避NRTI-sparingレジメンの長期有効性に関する検討を行った。

2009年1月の第1例以降、当院でRAL+DRVrの併用療法（他の薬剤を含まない）を開始され24週以上経過観察可能であったのは解析時点（2012年2月）で23例（男性21例、女性2例）であった（臨床試験参加例・計画的治療中断例は除く）。うち7例はこの組み合わせによる治療が開始された時点で良好なHIV抑制（<100 copies/mL）を達成していない症例であり、16例は良好なHIV抑制を達成した状態での変更例であった。この組み合わせが選択された理由としては、腎障害、NRTIの長期毒性、NRTIと関連した重篤な有害事象（アレルギー等）が主たる部分を占めた（表1）。

変更時点で良好なHIV抑制を達成していない7例の観察期間は140週～46週であった。観察期間中に明らかな治療失敗や薬剤耐性変異出現は認められなかったが、200 copies/mL未満の低力価のHIV血症が5例で観察された。

良好なHIV抑制が得られた状態で変更された16例の観察期間は144週～33週であった。通院自己中断1例、RALアレルギーによる治療中断1例が治療中止に至った。残る14例のうち8例で少なくとも1回のHIV血症が確認された。うち7例ではHIV血症は

200 copies/mL未満と低力価であり新規耐性変異は確認されていないが、1例で200 copies/mLを超えるHIV血症とともに新規のRAL関連変異の出現が確認されたことから、薬剤変更が予定されている。

テノホビルを含む組み合わせからの変更例のうち、変更前後で腎尿細管マーカー（尿中β-2マイクログロブリン）が測定されていた4症例においては、変更後に測定値の改善が認められた（図1）。

D．考察

現在標準的と考えられている抗HIV療法は1剤の「キードラッグ」（NNRTI、PIあるいはインテグラーゼ阻害薬INI）と2剤の「バックボーン」（NRTI）から構成されるが、NRTIの長期毒性や副作用不耐、蓄積耐性のため、適切な2剤のNRTIを選択できない症例が一部存在する。NRTI-sparingレジメンのうち、今回検討対象としたRALとDRVrの併用レジメンに関しては初回治療例を対象とした前向き試験が海外で進行中であるが、中間報告の時点で、治療前のHIV-RNA量が高い（10^5 copies/mL以上の）症例においてRAL耐性変異が出現する可能性が示唆されている。また実際にNRTI回避の必要性が高いのは初回治療例よりむしろNRTI不耐例であるが、治療歴を有する症例におけるこの組み合わせの有効性に関する知見は症例報告レベルのものに留まる。

今回の検討対象のうち変更時点で良好なHIV抑制を達成していなかった7例のうち5例、変更時点で良好なHIV抑制が得られていた16例のうち11例で、過去のHIV-RNA量の最高値が10^5 copies/mLを超え

表1

背景			変更前の経過			変更後の経過				
治療状況	年齢	主な変更理由	HIV-RNA 経過中の最高値	HIV-RNA 変更時	変更前のHIV抑制期間（月）	観察期間（週）	継続状況	detectable HIV viremia 観察期間中の回数	detectable HIV viremia HIV-RNA最高値	detectable HIV viremia 初めて観察された時期
HIV抑制前の変更	60s	腎不全	$9.9x10^5$	$8.8x10^5$	0	140	（継続中）	10	$1.0x10^2$	28 wk
	50s	NRTI重症薬疹	$5.6x10^6$	$5.5x10^5$	0	132	（継続中）	-	-	-
	40s	腎不全	$3.5x10^4$	$9.7x10^3$	0	114	（継続中）	2	$1.3x10^2$	77 wk
	50s	NRTI不耐	$2.7x10^5$	$1.2x10^2$	0	93	（継続中）	7	$8.3x10^1$	32 wk
	50s	腎障害	$8.6x10^4$	$8.6x10^4$	0	79	（継続中）	5	$8.5x10^1$	35 wk
	40s	NRTI不耐	$3.7x10^5$	$4.6x10^3$	0	65	（継続中）	3	$1.1x10^2$	42 wk
	40s	TDF不耐	$2.8x10^7$	$1.6x10^2$	0	46	（継続中）	-	-	-
HIV抑制下での変更	40s	腎障害	$3.5x10^4$	< 40	16	8	自己中断	2	$7.0x10^1$	3 wk
	70s	腎障害	$2.5x10^5$	< 40	46	144	（継続中）	-	-	-
	60s	腎障害	$3.5x10^4$	< 40	36	3	副作用中止	-	-	-
	60s	腎障害	$4.6x10^5$	< 40	3	105	（継続中）	5	$6.7x10^1$	7 wk
	50s	腎不全	$2.6x10^5$	< 40	15	105	（継続中）	1	$6.0x10^1$	72 wk
	50s	腎障害	$2.7x10^5$	< 40	14	89	（継続中）	-	-	-
	40s	TDF不耐	$6.8x10^4$	< 40	45	82	RAL耐性	2	$3.2x10^2$	80 wk
	60s	腎不全	（不明）	< 40	（不明）	83	（継続中）	1	$5.8x10^1$	60 wk
	50s	HIV抑制強化	$2.1x10^5$	< 40	15	76	（継続中）	-	-	-
	30s	NRTI長期毒性	$1.5x10^5$	< 40	51	77	（継続中）	1	$4.8x10^1$	64 wk
	60s	NRTI長期毒性	$3.7x10^4$	< 40	144	75	（継続中）	-	-	-
	30s	腎障害	$1.6x10^5$	< 40	66	73	（継続中）	-	-	-
	60s	NRTI不耐	$8.8x10^5$	< 40	41	61	（継続中）	1	$4.3x10^1$	18 wk
	50s	腎障害	$1.3x10^6$	$5.9x10^1$	0	51	（継続中）	1	$8.4x10^1$	7 wk
	30s	NRTI長期毒性	$5.1x10^5$	< 40	137	46	（継続中）	-	-	-
	60s	腎障害	$5.6x10^5$	< 40	68	33	（継続中）	-	-	-

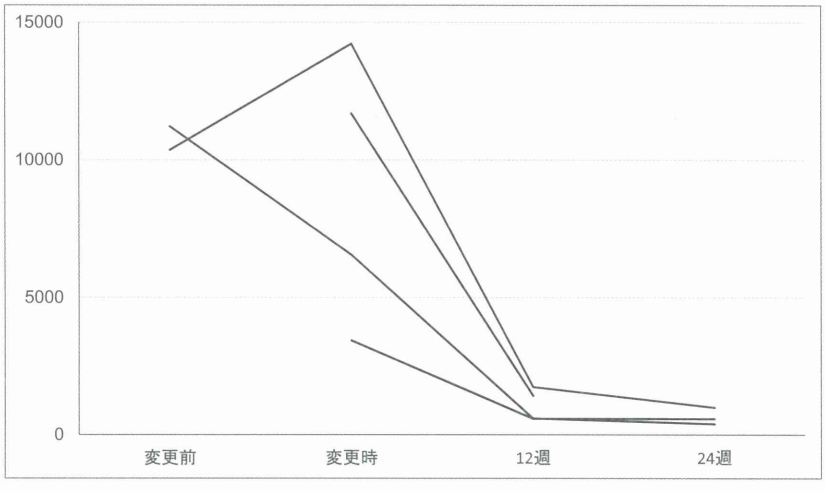

図1：変更前後の尿中β-2マイクログロブリンの推移

ていた。RALとDRVrの併用に変更されてからのHIV抑制は概ね良好であったが、一部の症例において低力価のHIV血症が時折みられた。このHIV血症の頻度およびHIV-RNA量の最高値は、変更時点で良好なHIV抑制を達成していなかった例でより多い傾向があり、治療開始時点でのHIV抑制の程度がその後のHIV抑制維持と関連している可能性を示唆した。

2011年12月時点では新規耐性変異は確認されていなかったが、2012年2月時点で1例に新規RAL耐性変異が確認された。この症例はRALとDRVrの組み合わせに変更後も検出感度未満の血中HIV-RNA量を維持していたが、80週の時点で320 copies/mLとやや高い血中HIV-RNA量が初めて確認され、その後の受診の際にRAL耐性変異が確認されている。本症例は変更前後を通じて服薬アドヒアランスも保たれていたと考えられる症例であり、このHIV血症の原因は不明であるが、いずれにしても200 copies/mLを超えるHIV-RNA量が確認された場合には遅滞なく耐性検査を行うことが必要であると考えられた。

血中HIV-RNA定量法として現在広く用いられているTaqMan法では低力価のHIV血症がしばしばみられることから、主要国ガイドラインにおいては200 copies/mL未満のHIV血症を治療失敗とは解釈していない。今回の検討対象者のうち期間中にHIV-RNA量が200 copies/mL以上となったのは前述の1例のみであり、その他の例では新規RAL耐性変異は確認されていない。しかしRALに関しては、（標準的な2NRTIとRALの組み合わせにおいて）100 copies/mL程度のHIV血症にもかかわらず耐性変異が出現したとする症例報告があり、我々の施設においても同様の症例は確認されている。これはRALのgenetic barrier（耐性変異出現に対する抵抗性）の低さによるものと考えられ、低力価のHIV血症がみられる症例では引き続きRAL耐性変異に関する厳重な経過観察が必要である。

RALを含むNRTI-sparingレジメンの併用薬としては、今回の検討対象としたDRVr以外に、NNRTIであるETRやCCR5受容体アンタゴニストであるMVCも候補となる。低力価のHIV血症を呈する例に対してこれらの薬剤を追加することによりHIV血症の頻度を低下させることができる可能性はあるが、第3の薬剤の追加は治療費用増加をもたらすのみならず、服薬アドヒアランスの低下からかえって治療効果を低下させる可能性もあり、その必要性についても今後検討してゆく必要がある。

E．結論

NRTI不耐例、特に前治療により良好なHIV抑制が得られている症例において、RALとDRVrの併用によるNRTI-sparingレジメンは有効な治療選択枝となり得るが、長期有効性に関しては不明な部分もあり、低力価HIV血症を呈する例においては厳重な経過観察が必要である。

F．研究発表

1．論文発表

なし

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G．知的財産権の出願・登録状況（予定を含む）

なし

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平成 23 年度研究業績一覧

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PLoS NEGLECTED TROPICAL DISEASES

Amebiasis in HIV-1-Infected Japanese Men: Clinical Features and Response to Therapy

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Abstract

Invasive amebic diseases caused by *Entamoeba histolytica* are increasing among men who have sex with men and co-infection of ameba and HIV-1 is an emerging problem in developed East Asian countries. To characterize the clinical and epidemiological features of invasive amebiasis in HIV-1 patients, the medical records of 170 co-infected cases were analyzed retrospectively, and *E. histolytica* genotype was assayed in 14 cases. In this series of HIV-1-infected patients, clinical presentation of invasive amebiasis was similar to that described in the normal host. High fever, leukocytosis and high CRP were associated with extraluminal amebic diseases. Two cases died from amebic colitis (resulting in intestinal perforation in one and gastrointestinal bleeding in one), and three cases died from causes unrelated to amebiasis. Treatment with metronidazole or tinidazole was successful in the other 165 cases. Luminal treatment was provided to 83 patients following metronidazole or tinidazole treatment. However, amebiasis recurred in 6 of these, a frequency similar to that seen in patients who did not receive luminal treatment. Recurrence was more frequent in HCV-antibody positive individuals and those who acquired syphilis during the follow-up period. Various genotypes of *E. histolytica* were identified in 14 patients but there was no correlation between genotype and clinical features. The outcome of metronidazole and tinidazole treatment of uncomplicated amebiasis was excellent even in HIV-1-infected individuals. Luminal treatment following metronidazole or tinidazole treatment does not reduce recurrence of amebiasis in high risk populations probably due to amebic re-infection.

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Introduction

Invasive amebiasis (IA) caused by *Entamoeba histolytica* is the second most common cause of mortality associated with parasitic infections worldwide, accounting for 40,000 to 100,000 deaths annually [1]. Amebiasis is transmitted by ingestion of food or water containing the cyst form of *E. histolytica*, which is prevalent in developing countries in Central and South America, Asia, and Africa. In the developed countries, most cases arise in travelers and immigrants from such endemic areas [2]. Recently, however, three developed East Asian countries (Japan, Taiwan, and South Korea) reported increased risk for amebiasis among men who have sex with men (MSM) due to oral-anal sexual contact [3–12]. The annual incidence of human immunodeficiency virus type 1 (HIV-1) infection is also increasing among MSM in these countries [13-17], resulting in growing concern on IA in HIV-1-infected MSM [6,9–12,18]. The recommended treatment for IA is metronidazole (750 mg t. i. d. for 10 days) or tinidazole (2 g q. d. for 3 days), followed by a luminal agent (paromomycin 500 mg t. i. d. for 10 days or diloxanide furoate 500 mg t. i. d. for 10 days) to eliminate intestinal colonization [18,19]. A previous report described no difference in the response to metronidazole or tinidazole treatment between HIV-1-positive and –negative IA patients [20]. However, the efficacy of luminal treatment in preventing recurrence, which can arise by relapse or re-infection, has not yet been assessed rigorously. In this study, we retrospectively analyzed 170 HIV-1-infected Japanese patients with IA, together with genomic typing of *E. histolytica* in 14 of these patients, and delineated the clinical features of IA in HIV-1-infected individuals and the efficacy of metronidazole, tinidazole and luminal treatment.

Methods

Ethics statement

The Institutional Review Board of National Center for Global Health and Medicine (Tokyo, Japan) approved this study. All patients who provided clinical samples for genotyping of *E. histolytica* gave written informed consent.

Case review

The medical records of HIV-1-infected cases diagnosed with IA at the AIDS Clinical Center, National Center for Global Health and Medicine, between April 1997 and March 2010, were reviewed. The diagnosis of IA was made when one of the following criteria was satisfied; 1) identification of and/or positive PCR (methods; see below) in clinical specimens (stool or punctuate-exudate) for erythrophagocytic trophozoites in patients with IA-

Author Summary

Amebiasis is usually transmitted by ingestion of contaminated food or water in developing countries. Recently, however, increased risk for amebiasis among men who have sex with men (MSM) due to oral-anal sexual contact was reported in developed countries, resulting in growing concern on amebiasis in HIV-1-infected MSM. The recommended treatment of amebiasis is metronidazole or tinidazole, followed by a luminal agent to eliminate intestinal cyst colonization. However, the efficacy of luminal treatment in preventing recurrence has not been assessed yet. In this study, we analyzed the medical records of 170 patients with amebiasis and HIV-1 co-infection. Treatment with metronidazole or tinidazole was excellent whereas luminal treatment did not reduce the frequency of recurrence of amebiasis. Recurrence was more frequent in those MSM with signs of sexual activity such as syphilis infection. Luminal treatment following metronidazole or tinidazole treatment does not reduce recurrence of amebiasis in high risk populations.

related symptoms, e.g., fever and liver abscess, or tenesmus and diarrhea, 2) high serum titer (>1:100) for antibody against *E. histolytica* in patients with IA-related symptoms in whom microbiological cultures or histological examination of clinical specimens did not identify any pathogen, and who showed improvement of IA symptoms following metronidazole or tinidazole monotherapy [10–12]. The medical records were surveyed for patients' characteristics, presenting forms of clinical IA [e.g., colitis, amebic liver abscess (ALA), and perianal abscess], HIV-1-induced immunocompromised status, and symptoms, laboratory data and serological markers of other sexually-transmitted diseases (STD) including syphilis, hepatitis B and C viruses (HBV and HCV). After completion of treatment for IA, the medical records were followed-up until March 2010, excluding those cases found to have died or lost to follow-up.

Genotyping of *E. histolytica*

To determine the strains of *E. histolytica* among HIV-1-infected Japanese patients, genotyping of *E. histolytica* was performed in patients who were PCR positive. The PCR method was used for the first time in our clinic for the diagnosis of amebiasis in December 2008, and since then 14 patients had been diagnosed as IA based on a positive PCR. For the PCR, DNAs were extracted from various biological specimens (e.g., stool, colon wash and punctuate-exudate) by using QIAamp DNA stool Mini Kit (Qiagen, Valencia, CA). Polymerase chain reactions were performed with specific sets of primers designed to target each of 6 loci (D-A, S-Q, R-R, A-L, S^{TGA}-D, and N-K) of tRNA-linked polymorphic short tandem repeats (STR), as described previously [21]. The PCR product was sequenced by ABI 3130XL Genetic Analyzer (Applied Biosystem, Foster city, CA) in both forward and reverse directions. Phylogenetic analysis and genotyping were performed as described previously [22].

Statistical analysis

Differences in patients' characteristics and clinical features were examined using the chi-square test or nonparametric test. The cumulative risk for recurrence was analyzed by the Kaplan-Meier method, and differences were tested by the log-rank test. The Cox proportional hazards model was used to assess the impact of luminal treatment on the recurrence rate after adjustment for other factors. The hazard ratio and 95% confidence interval were calculated. *P* values less than 0.05 were considered to denote statistical significance. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL).

Results

Clinical data and response to treatment

IA was diagnosed in 170 HIV-1-infected cases between April 1997 and March 2010 (including amebic colitis, n = 102; ALA, n = 63; and perianal abscess, n = 5, Table 1). Thirty-three patients had two of the above three clinical forms of IA. All patients were males and 164/170 (96.5%) were MSM. High rates of positive TPHA (*Treponema pallidum* hemagglutination assay) (71.2%) and HBV exposure (HBs antigen-positive, HBs antibody-positive, or HBc antibody-positive) (60.0%) were observed. No significant differences were seen in CD4 counts, HIV-1 loads, coexisting AIDS definite disease and the proportion of patients treated with antiretrovirals, suggesting that HIV-induced immunocompromised status did not have an impact on the clinical presentation of amebic infection, in agreement with previous data [12]. In cases of amebic colitis (n = 102), diarrhea (69.7%) was the most common symptom followed by dysentery (55.9%) (Table 2). Fever (>37.5°C) was seen in only 20 patients (19.6%), including 5 cases with perforative peritonitis. In cases with ALA (n = 63), fever (95.2%) was the most common symptom followed by abdominal pain (55.6%). Diarrhea (46.0%) and dysentery (19.0%) were only seen in less than half of ALA cases. Single abscess (72.6%) was identified in most cases. Liver abscesses were seen more frequently in the right lobe (70.5%) than the left (9.8%). Nine patients (14.3%) had pleuritis (considered a co-existing disease), as well as abscesses in the right lobe, and 7 of these presented chest pain. Comparison of physical and laboratory data showed higher peak body temperature (BT), leukocyte count and C reactive protein (CRP) in ALA cases (Table 2) and perforative peritonitis cases (data not shown) compared with colitis cases, indicating that high fever, leukocytosis and high CRP could be the signs of extraluminal amebiasis. It is reported that high fever and leukocytosis are also common in ALA patients free of HIV-1 infection, though both parameters were unusually associated with simple amebic colitis [23]. In ALA cases, however, leukocyte count correlated positively with CD4 count (data not shown in tables: Pearson product-moment correlation coefficient 0.36, p value 0.004) and negatively with HIV-RNA load (Pearson product-moment correlation coefficient -0.28, p value 0.03), but CRP correlated neither with CD4 count nor HIV-RNA load (CRP-CD4, $p = 0.81$, CRP-HIV-RNA, $p = 0.32$). There were also no correlations between CD4 count, HIV-RNA load, BT, leukocyte count or CRP and abscess size or number.

All patients were treated with metronidazole (750 mg t. i. d. for 10 days) for IA, with the exception of two who were treated with tinidazole (2 g q. d. for 3 days). Complete remission of all IA symptoms was observed in 165 patients including the two treated with tinidazole. Five cases died within six months after diagnosis of IA; two from complications related to amebic colitis (one peritoneal perforation and one gastrointestinal bleeding), one from malignant lymphoma, one from *Pneumocystis jirovecii* pneumonia, and one from pulmonary thrombosis. The overall mortality rate was 3% in this study, which was comparable to those reported in non-HIV cases [2,23].

Recurrence after treatment

Luminal agents; paromomycin and diloxanide, are not approved in Japan, and they were not always available in our facility during the study period. After completion of IA treatment with metronidazole or tinidazole, luminal agents were administered when available. Consequently, 83 cases were treated with luminal

Table 1. Patient demographics, state of HIV, and serological markers.

	Colitis (n = 102)[1]	ALA (n = 63)[2]	Perianal abscess (n = 5)[3]	All (n = 170)	*P* value[4]
Age (years) [IQR]	38 [32–43]	37 [31–44]	45	38 [31–44]	0.58
Male sex (%)	102 (100)	63 (100)	5 (100)	170 (100)	–
Homosexual (%)	96 (94.1)	63 (100)	5 (100)	164 (96.5)	0.053
Past History of amebiasis (%)	16 (15.7)	9 (14.3)	1 (20.0)	26 (15.3)	0.81
CD4 count (/μl)	262 [98–398]	271 [123–411]	58	269 [107–403]	0.84
HIV-RNA (log copies/ml)	4.60 [3.89–5.32]	4.66 [3.91–5.11]	5.04	4.66 [3.93–5.28]	0.70
AIDS (%)	18 (17.6)	8 (12.7)	2 (40.0)	28 (16.5)	0.40
ART initiated (%)	18 (17.6)	11 (17.5)	1 (20.0)	30 (17.6)	0.98
TPHA test positive (%)	77 (75.5)	40 (63.5)	4 (80.0)	121 (71.2)	0.10
HBV exposure (%)	59 (57.8)	41 (65.1)	2 (40.0)	102 (60.0)	0.36
HCV Antibody positive (%)	3 (2.9)	3 (4.8)	0 (0)	6 (3.5)	0.42

Data are median [interquartile range: IQR] or number (percentage) of patients.
[1]5 cases of perforative peritonitis are included as co-existing diseases. Four cases were diagnosed coincidentally by colonoscopy in asymptomatic patients.
[2]31 cases of colitis, 1 case of perianal abscess, 9 cases of pleuritis, and 2 cases of peritonitis are included as co-existing diseases.
[3]1 case of colitis is included as co-existing diseases.
[4]Chi-square test or non-parametric test was performed for data of colitis and ALA.
UD: undetectable, ART: anti-retroviral therapy, TPHA test: *Treponema pallidum* Hemagglutination Assay test, HBV exposure: HBsAg-positive or HBsAb-positive, and/or HBc-Ab positive.
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agents; 38 cases with promomycin (500 mg t. i. d. for 10 days) and 45 cases with diloxanide furoate (500 mg t. i. d. for 10 days). No significant differences were seen in patients' characteristics, including HIV-1-induced immunocompromised status, serological markers of other STD, and clinical forms and severity of amebiasis between the 83 cases with luminal treatment and 82 cases who did not receive such treatment (Table S1). The median follow-up period after completion of metronidazole or tinidazole treatment was 50 months (inter quartile range: 19–85) in those who received luminal treatment, and 43 months (inter quartile range: 23–98) in those without.

Table 2. Clinical features of amoebic colitis and ALA.

	Colitis (n = 102)	ALA (n = 63)	*P* value
Symptoms			
Diarrhea (%)	71/102 (69.6)	29/63 (46.0)	0.003
Dysentery (%)	57/102 (55.9)	12/63 (19.0)	<0.001
Abdominal pain (%)	23/102 (22.5)	35/63 (55.6)	<0.001
Chest pain (%)	0/102 (0.0)	7/63 (11.1)	<0.001
Peak BT (°C) [IQR][3]	36.8 [36.5–37.4]	39.0 [38.8–39.5]	<0.001
WBC (/μ l) [IQR][3]	5,830 [4490–7580]	11,760 [9460–15170]	<0.001
CRP (mg/dl) [IQR][3]	0.62 [0.16–3.02]	19.15 [10.53–24.75]	<0.001
Frequency of diarrhea[1]			
≤ 5 times/day (%)	63/101 (62.4)	–	
6–10 times (%)	26/101 (25.7)	–	
≥ 11 times (%)	12/101 (11.9)	–	
Size of abscess (mm)	–	59 (10–180)	
Location of abscess[2]			
Right lobe only	–	43/61 (70.5)	
Left lobe only	–	6/61 (9.8)	
Both lobes	–	12/61 (19.7)	
Number of abscesses[1]			
Single (%)	–	45/62 (72.6)	
Multiple (%)	–	17/62 (27.4)	

[1]Data of one case were not available.
[2]Data of two cases were not available.
[3]Data are median [interquartile range: IQR] or number (percentage) of patients.
BT: body temperature, WBC: White Blood Cell counts, CRP: C reactive protein.
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Within the 12-month post-metronidazole treatment period, recurrence of IA was noted in only two patients who did not receive luminal treatment, suggesting reactivation of residual cysts of *E. histolytica* (Figure 1). However, during the entire follow-up period, six in each group experienced recurrence of IA, with no significant difference in the recurrence frequency by the log-rank chi-square test. Multivariate analysis showed that recurrence did not correlate with past history of IA, CD4 count, TPHA, HBV exposure (HBs antigen-positive or HBs antibody-positive), or the presence of extraluminal IA disease (Table 3). However, a positive HCV antibody was significantly associated with IA recurrence. Recurrence also tended to occur in those who acquired new syphilis infection during the follow-up period, though the difference did not reach statistical significance.

Genotypes of *E. histolytica*

Genotyping of *E. histolytica* was performed in samples obtained from 14 patients between December 2009 and March 2010 (colitis, n = 8; ALA, n = 4; colitis and ALA, n = 1; and perianal abscess, n = 1; Table S2). Eleven different genotypes were recognized, including five genotypes (J8, J12, J13, J20, and J23) identified previously in Japan [22], and six newly recognized genotypes (J24-J29). There was no significant relation between *E. histolytica* genotype and clinical presentation.

Discussion

In the present study, retrospective analysis of the medical records of 170 patients with HIV-1-infection and IA showed no

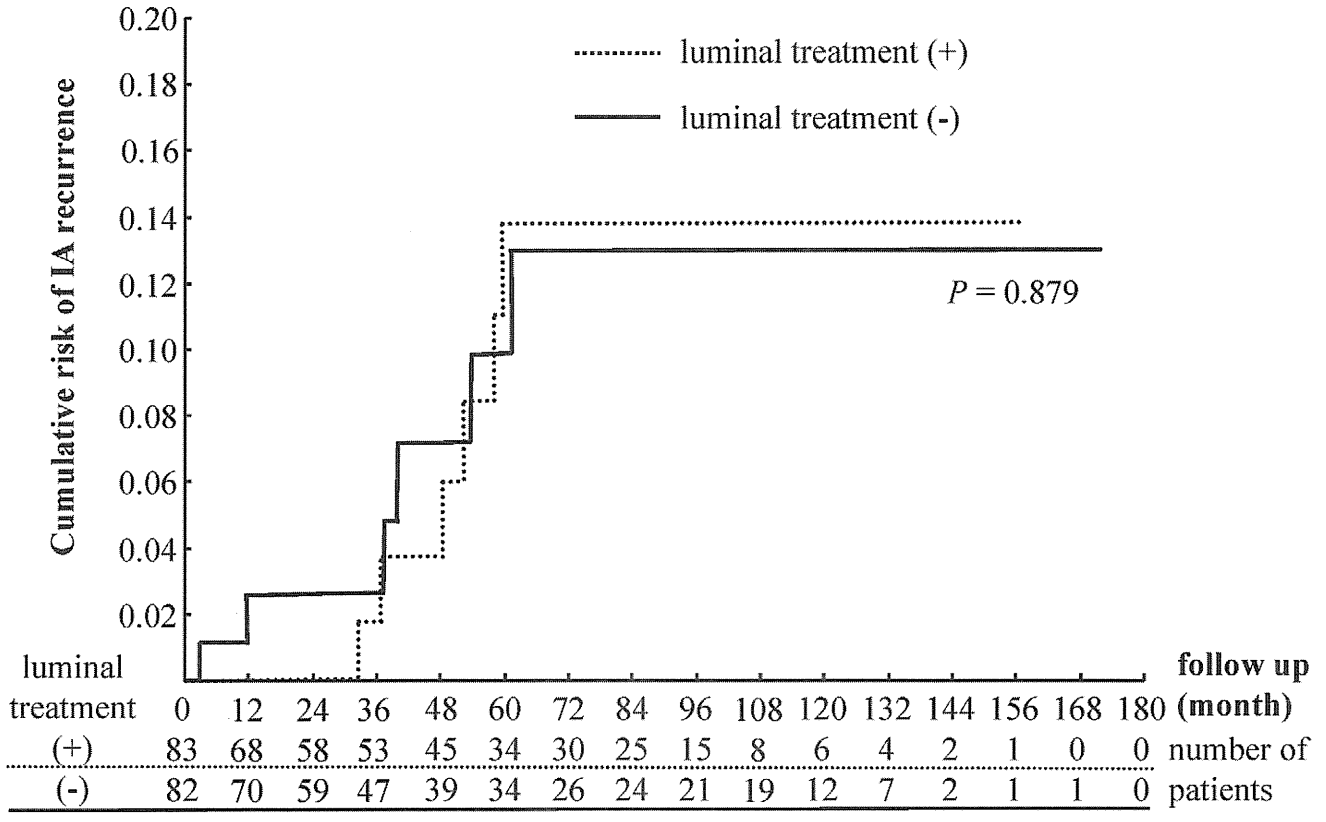

Figure 1. Kaplan-Meier estimates of time to IA recurrence. Cumulative probability of IA recurrence after completion of metronidazole or tinidazole treatment with or without subsequent luminal treatment.
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impact for HIV-1-induced immunocompromised status on the clinical forms of amebiasis. The physical and laboratory findings showed that high fever, leukocytosis and high CRP correlated with extraluminal diseases of amebiasis. In ALA cases, however, leukocyte count correlated positively with CD4 count and negatively with HIV-RNA load, indicating that CRP is more sensitive marker for the detection of the extraluminal diseases in advanced immunocompromised patients.

Only five patients died after the diagnosis of IA; two from IA complications and three from other causes. The results indicate excellent outcome for HIV-1-infected individuals with uncomplicated amebiasis treated with metronidazole or tinidazole, in agreement with previous reports on HIV and non-HIV cases [2,11,12,20,23]. Based on conventional wisdom and written opinion, adequate management of IA should include treatment with a luminal agent following metronidazole or tinidazole treatment, in order to eradicate residual cysts of *E. histolytica* due to the high rate (40–60%) of luminal colonization [2,23–27]. On the other hand, the results of longitudinal observational studies indicated that asymptomatic cyst carriers rarely develop IA, and

Table 3. Multivariate analyses for factors associated with frequency of recurrence.

	No recurrence (n = 153)[1]	Recurrence (n = 12)	Hazard ratio (95.0% CI)	*P* value
Past history of IA[2] (%)	24 (15.7)	2 (16.7)	0.914 (0.186–4.478)	0.911
CD4 counts <200[2] (%)	57 (37.3)	3 (25.0)	0.385 (0.101–1.470)	0.162
TPHA test positive[2] (%)	108 (70.6)	10 (83.3)	2.435 (0.501–11.827)	0.270
HBV exposure[2] (%)	92 (60.1)	7 (58.3)	1.248 (0.364–4.277)	0.725
HCV Antibody positive[2] (%)	3 (2.0)	2 (16.7)	7.664 (1.369–42.890)	0.020
Extraluminal disease[2] (%)	66 (43.1)	4 (33.3)	0.559 (0.163–1.921)	0.356
No luminal agent (%)	76 (49.7)	6 (50.0)	1.070 (0.322–3.559)	0.912
Syphilis during follow-up period (%)	33 (21.6)	7 (58.3)	3.332 (0.961–11.547)	0.059

[1]Five patients died within 6 months from disease onset and their data were excluded from analysis.
[2]Status at diagnosis of IA.
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that cyst form ameba often disappears spontaneously without any treatment [28,29]. There is controversy about the need for cyst eradication following metronidazole or tinidazole treatment, especially in endemic areas where re-infection is frequent. In this study, recurrence of IA within the first year of metronidazole treatment was noted in only two patients of 82 patients who did not receive luminal therapy. Moreover, long-term follow-up indicated IA recurrence also in those who received luminal agents, and the benefits obtained from luminal treatment seemed to have disappeared. IA recurred more frequently in those with HCV infection, which was recently reported to be transmittable sexually among MSM [30], and in those who acquired new syphilis infection during the follow-up period, suggesting that sexually active MSM tend to experience IA recurrence due to re-acquisition of new *E. histolytica* infection. HBV exposure and positive TPHA at IA diagnosis did not correlate with IA recurrence probably because the high prevalence of these two parameters in this study masked the difference between recurrence and non-recurrence cases. Educational approach for safer sex may be more appropriate rather than luminal treatment to prevent IA recurrence after treatment.

Eleven genetic strains of *E. histolytica* were identified in this study and none of them had been reported so far from geographic areas other than Japan [21,22,31,32], indicating that diverse Japan-specific isolates of *E. histolytica* are already prevalent among MSM in Japan. In fact, the *E. histolytica* seropositivity rate in HIV-1-infected MSM in our clinic was as high as 17.9% in 2009 (unpublished data), which is comparable with the seropositivity rate in Japanese MSM reported more than 20 years ago [5]. Unfortunately, we could not compare the genotypes of *E. histolytica* between the incidences of the primary and recurrent IA within the same individuals due to the lack of appropriate stocked samples, which would have probably demonstrated acquisition of new infection.

Considered together, the results emphasize the difficulty of preventing IA recurrence without educational approach to prevent new amebic infection even after successful IA treatment in the high risk groups such as HIV-1-infected MSM. The spread of *E. histolytica* in MSM of other developed countries beyond Asia should be of great concern.

Supporting Information

Table S1 Patient demographics with and without luminal treatment.
(DOC)

Table S2 Genotyping data of 6 STR loci in 14 clinical samples.
(DOC)

Author Contributions

Conceived and designed the experiments: HG JT SO. Performed the experiments: KW AEdC TN. Analyzed the data: KW HG. Contributed reagents/materials/analysis tools: KW HG JT SO. Wrote the paper: KW HG.

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Impact of Small Body Weight on Tenofovir-Associated Renal Dysfunction in HIV-Infected Patients: A Retrospective Cohort Study of Japanese Patients

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Abstract

Background: Treatment with tenofovir is sometimes associated with renal dysfunction. Limited information is available on this side effect in patients with small body weight, although the use of tenofovir will spread rapidly in Asia and Africa, where patients are likely to be of smaller body weight.

Methods: In a single-center cohort, Japanese patients with HIV infection who started tenofovir-containing antiretroviral therapy were retrospectively analyzed. The incidence of tenofovir-associated renal dysfunction, defined as more than 25% decrement of estimated glomerular filtration rate (eGFR) from the baseline, was determined. The effects of small body weight and body mass index (BMI) on tenofovir-associated renal dysfunction, respectively, were estimated in univariate and multivariate Cox hazards models as the primary exposure. Other possible risk factors were evaluated by univariate analysis and those found significant were entered into the multivariate analysis.

Results: The median weight of 495 patients was 63 kg. Tenofovir-related renal dysfunction occurred in 97 (19.6%) patients (incidence: 10.5 per 100 person-years). Univariate analysis showed that the incidence of tenofovir-related renal dysfunction was significantly associated with smaller body weight and BMI, respectively (per 5 kg decrement, HR = 1.23; 95% CI, 1.10–1.37; $p<0.001$)(per 1 kg/m^2 decrement, HR = 1.14; 95% CI, 1.05–1.23; $p=0.001$). Old age, high baseline eGFR, low serum creatinine, low CD4 count, high HIV viral load, concurrent nephrotoxic drugs, hepatitis C infection, and current smoking were also associated with tenofovir-related renal dysfunction. Multivariate analysis identified small body weight as a significant risk (adjusted HR = 1.13; 95% CI, 1.01–1.27; $p=0.039$), while small BMI had marginal significance (adjusted HR = 1.07; 95% CI 1.00–1.16; $p=0.058$).

Conclusion: The incidence of tenofovir-associated renal dysfunction in Japanese patients was high. Small body weight was identified as an independent risk factor for tenofovir-associated renal dysfunction. Close monitoring of renal function is advocated for patients with small body weight treated with tenofovir.

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Introduction

Tenofovir disoproxil fumarate (TDF) is one of the most widely used nucleotide reverse transcriptase inhibitors (NRTI) for patients with HIV infection, with proven efficacy and safety [1–6]. However, TDF is known to cause renal proximal tubular dysfunction, and several case reports have been published with TDF-related Fanconi syndrome, diabetes insipidus, and acute tubular necrosis, which sometimes lead to acute renal failure [7–10]. Long-term TDF use also reduces glomerular filtration rate more than other NRTIs [11–14]. To date, the nephrotoxic effect of TDF is regarded as mild and tolerable. A recently published meta-analysis has reported that the use of TDF is associated with a statistically significant but only modest renal dysfunction, and recommended that TDF use should not be restricted even when regular monitoring of renal function and serum phosphate levels is impractical [15]. However, the TDF-related renal dysfunction has hardly been evaluated in patients with small body weight, who are potentially at higher risk for larger drug exposure and thus, more severe toxicity [16–19].

The 2010 WHO guideline on antiretroviral therapy for HIV infection in adults and adolescents, usually applied to resource-constrained settings, recommends TDF as one of the components of first line therapies (URL:http://whqlibdoc.who.int/publications/