antibody titer, complete blood cell count, differential leukocyte count, electrolytes, glucose, renal and liver function tests, and percent of $CD4^+$, $CD8^+$, $CD8^+$ $CD3^+$, $CD16^+$, $CD56^+$ cells in peripheral blood mononuclear cells (PBMCs).

Provirus load measurement and anti-HTLV-1 antibody titers

To assay the HTLV-1 provirus load, we carried out a quantitative polymerase chain reaction (PCR) method using ABI Prism 7700 (PE-Applied Biosystems) with 100 ng of genomic DNA (roughly equivalent to 10^4 cells) extracted from PBMCs using a QIAamp blood kit (Qiagen), according to the manufacturer's instructions (Nagai *et al*, 1998). Using β-actin as an internal control, the amount of HTLV-1 provirus DNA was calculated by the following formula: copy number of HTLV-1 *tax* per 1×10^4 PBMCs = [(copy number of *tax*)/(copy number of β-actin/2)] $\times$ 10^4. All samples were tested in triplicate. The lower limit of detection was one copy of HTLV-1 *tax* per 10^4 PBMCs. Serum antibody titers to HTLV-1 were determined by a particle agglutination method (Serodia-HTLV-1; Fujirebio). Namely, the antibody titers were achieved by performing a serial dilution of the patient serum and noting the highest dilution at which agglutination is still present.

Laboratory methods

Complete blood cell count, differential leukocyte count, electrolytes, glucose, renal and liver function tests, and the percentages of $CD4^+$, $CD8^+$, $CD8^+$ $CD3^+$, $CD16^+$, $CD56^+$ cells in PBMCs were measured on all fresh samples at the Kagoshima University Hospital Clinical Laboratory. Peripheral blood smears were obtained by smearing one drop of fresh blood onto a glass slide. All the slides were fixed by methanol and stained with Giemsa, and read by observers who were blinded to clinical information. The identification of flower cell (ATL cell)–like abnormal lymphocytes (Ably) followed the criteria by Sacher *et al* (1999). Namely, we classified the cells as Ably when they fulfilled the following criteria: the absence of azurophil granules; the presence of nuclear folding or lobulation; and at least two of the following characteristics: nuclear chromatin condensation, nuclear to cytoplasmic ratio of >80%, and/or cell size >1.5 times that of small lymphocytes. The number of abnormal lymphocytes and atypical lymphocytes in Table 5 were calculated as follows: (1) a trained medical technologist blind to subject HTLV serostatus performed three 100–white cell differential counts on a total of 300 leukocytes, then percentage of Ably or atypical lymphocytes was obtained. (2) The findings obtained by a trained medical technologist were reviewed by a board-certified hematologist to confirm the findings. (3) Using percentage of Ably or atypical lymphocytes and absolute WBC counts, the number of abnormal lymphocytes and atypical lymphocytes were calculated.

Restriction fragment length polymorphism (RFLP) analysis of the HTLV-1 tax gene

To identify the HTLV-1 *tax* gene subgroup (*tax* subgroup A or B), we carried out a PCR-RFLP analysis as previously described. (Furukawa *et al*, 2000). For RFLP analysis, 4 μl of the PCR product was digested with 5 U of *AccII* (Takara, Tokyo, Japan) in a 10-μl volume at 37°C for 1 h followed by electrophoresis on 2% Nusieve agarose gel. Positive and negative controls of known samples of *tax* gene subgroups A and B, confirmed by direct sequencing analysis, were included in all the experiments.

HLA typing

PCR sequence-specific primer reactions were performed to detect HLA-A*02 and HLA-Cw*08 as previously described (Bunce *et al*, 1995; Olerup and Zetterquist, 1992).

Receiver operator characteristic (ROC) curve analysis

Receiver operator characteristic (ROC) curve was constructed by plotting sensitivity against the false-positive rate (1-specificity) over a range of values of either the odds of HAM/TSP or the HTLV-1 provirus load or the anti-HTLV-1 antibody titers. These curves were constructed with data from our previously reported Kagoshima cohort, which consisted of 222 patients with HAM/TSP and 184 HCs (Vine *et al*, 2002). The area under the curve (AUC) of the ROC was used to estimate the predictive value of each parameter. The AUC is classified as low if the area is between 0.5 and 0.7; as moderate, if between 0.7 and 0.9; and as high, if greater than 0.9. The cut-off value to differentiate HAM/TSP and HCs was also determined from the ROC curve.

Statistical analysis

The chi-squared test, the Mann-Whitney U test, and the odds ratio were used for statistical analysis. Significance was considered at $P < 0.05$.

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プロバイオティクスの臨床応用

HTLV-1-associated myelopathy (HAM)

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はじめに

HTLV-1(ヒトT細胞白血病ウイルス1型)は，主にCD4陽性Tリンパ球に感染するヒトレトロウイルスの一種である．HTLV-1関連脊髄症(HAM)は，HTLV-1無症候性感染者(キャリアー)の一部から発症する慢性炎症性の膀胱直腸障害，感覚障害を伴う痙性脊髄麻痺であり，1986年に納，井形により新しい疾患単位として提唱された[1]．後にカリブ海沿岸を中心に報告された抗HTLV-I抗体陽性の熱帯性痙性脊髄麻痺(TSP)と同一の疾患であることが確認されている[2]．

1999年時点の報告では，HTLV-1キャリアー数は世界で220万人，日本で120万人であり，HAM患者の実数は世界で3,000人＋α，日本で1,422人と報告された．外国の文献ではHTLV-Iキャリアーが1100万～2000万人とも推測されている[3]．HAM患者は，HTLV-1キャリアーの多い地域に広く分布しており，日本(特に九州，沖縄を含む西日本，東北，北海道の一部)のほか，世界的にはカリブ諸島，アフリカ，イラン北東部，ヨーロッパの一部(ほとんどは感染地域からの移民)などに多くみられる．わが国におけるHAMの年間生涯発症率は無症候性キャリアー全体の0.23%である．

HAM患者の男女比は1：2.3と女性に多く，平均発症年齢は45.1±16.5歳である．主な感染経路は母乳による母子垂直感染で，ほかに輸血，夫婦間伝播(ほとんど男性から女性)などがある．HAMの主症状として下肢の痙性による運動障害，頻尿，残尿，尿失禁といった排尿障害，レベルを伴わないジンジン感などの感覚障害，自律神経障害として，頑固な便秘，皮膚乾燥，障害を受けた脊髄レベル以下の発汗低下が認められる．検査所見で，血清抗HTLV-1抗体価高値，血清IgE低値，NK活性低下[4,5]，末梢血中HTLV-1プロウイルス量高値[6]，髄液中抗HTLV-1抗体陽性を認めるほか，髄液ネオプテリン値高値，髄液内IgG産生亢進などもみられる．ATL細胞様の異型リンパ球を末梢血，髄液中に認めることもある．通常，脊髄MRIの異常を認めないが，慢性期では胸髄萎縮を，急速進行群(発症後2年で運動障害度が3段階進行する)では脊髄腫脹を認めることがある[7]．プロウイルス量と疾患の活動性にはある程度の相関があり，約半数以上が治療後も進行する[8]．

初期治療として，ステロイド療法，天然型インターフェロン-α製剤が使われる．症状が固定した慢性期の症例に対しては，経口ステロイド剤の少量持続療法やビタミンC大量療法(1.5～3g/日)，erythromycin(600mg/日)やsalazosulfapyridineが使われる．しかし，シェーグレン症候群，呼吸器障害，関節症，筋炎，成人T細胞性白血病などの合併症があること，ステロイド抵抗性の症例が20%ほど存在することなどの問題点があり，

表1　HAM患者10例に対するLcS投与前後の臨床効果

症例	年齢（歳）	性別	罹病期間（年）	末梢血抗HTLV-I抗体価（PA法）	末梢血HTLV-Iプロウイルス量（投与前）	末梢血HTLV-Iプロウイルス量（投与後）	併用薬剤	便秘の改善	痙性スコア（投与前）	痙性スコア（投与後）	運動障害（投与前）	運動障害（投与後）	排尿障害スコア（投与前）	排尿障害スコア（投与後）	全体の評価
HAM1	34	女性	19	×131,072	1,757	1,397	VC 375mg/日	有	2	1	4	3	2	0	有効
HAM2	62	男性	14	×32,768	634	777	PSL 5mg/日 VC 375mg/日	有	＋1	0	6	6	3	0	有効
HAM3	50	女性	17	×2,048	907	779	なし	有	2	1	5	4	3	0	有効
HAM4	45	女性	15	×16,384	2,942	471	PSL 5mg/日 VC 375mg/日	有	＋1	0	5	3	6	3	著効
HAM5	60	女性	7	×8,192	204	194	PSL 5mg/日 VC 375mg/日	有	3	4	4	4	1	0	やや有効
HAM6	47	男性	18	×2,048	716	849	PSL 10mg/日 VC 375mg/日	有	2	1	6	6	5	4	やや有効
HAM7	46	男性	24	×16,384	278	361	なし	有	3	1	4	3	2	0	有効
HAM8	55	女性	10	×65,536	2,882	524	なし	有	2	1	4	3	6	3	有効
HAM9	41	女性	17	×65,536	1,073	1,263	なし	有	3	0	2	2	3	1	有効
HAM10	57	女性	13	×32,768	245	387	なし	無	2	1	4	4	2	1	やや有効

HTLV-Iプロウイルス量：コピー/10^4末梢血リンパ球
VC：ビタミンC，　PSL：プレドニゾロン

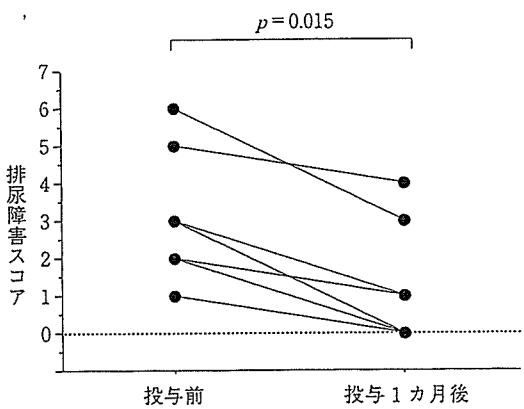

図1　*Lactobacillus casei* Shirota株
投与前後の排尿障害スコアの変化

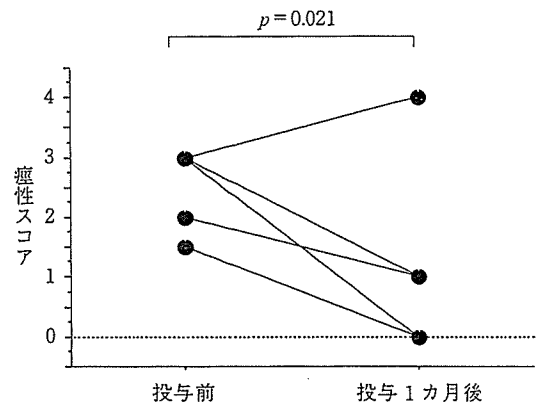

図2　*Lactobacillus casei* Shirota株の
投与前後の痙性スコア

いまだ根治療法がなく，特に病期が進んだ慢性期の症例に対する有効な治療法が確立されていないのが現状である[9]．

Lactobacillus casei Shirota株(LcS)は人の腸内から分離され，葉酸を利用して増殖する乳酸菌である．長い間，発酵乳を産生させるのに使われてきたが[10]，LcSにはNK細胞活性を増加させる作用が報告されている．そこで，健常人に比較してNK活性が低いと報告されている[4,5]HAM患者に対するLcSの治療効果を検討した．

対象と方法

鹿児島大学臨床倫理委員会の承認を受け，インフォームドコンセントを得たHAM患者10例(男性3例，女性7例)を対象とした．対象患者の平均年齢は49.7±8.8歳，平均罹病期間15.4±4.8(10～20)年である．対象患者にLcS400億個を含む乳製品(ヤクルト400®)を1日2本，4週間飲用

表2　*Lactobacillus casei* Shirota 株投与前後のリンパ球サブセット，NK 細胞活性および HTLV-I プロウイルス量の変化

サブセット	投与前		投与後		p^*	
	絶対数 $\times 10^2/mm^3$	頻度 %	絶対数 $\times 10^2/mm^3$	頻度 %	絶対数	頻度
$CD4^+$細胞	5.79±4.54	26.38±12.71	5.96±3.16	27.13±11.09	0.674	0.575
$CD8^{high+}$細胞	3.89±1.61	20.47±6.93	4.72±2.02	22.60±9.01	0.208	0.327
$CD8^{high+}$ナイーブ細胞	1.13±1.71	5.24±7.11	0.93±1.06	4.25±4.08	1.000	0.674
$CD8^{high+}$メモリー細胞	4.84±2.60	24.05±6.44	5.25±2.13	24.47±7.37	0.401	0.889
$CD8^{high+}$エフェクター細胞	7.56±4.46	37.35±10.81	7.57±2.56	36.10±9.93	1.000	0.674
$CD8^{high+}$エフェクター・メモリー細胞	5.99±2.36	33.36±14.54	7.21±3.47	34.07±13.78	0.263	0.889
$CXCR3^+$細胞	4.38±2.38	21.07±4.28	3.93±1.16	18.78±6.57	0.735	0.398
CD4 陽性 $CXCR3^+$細胞	2.03±2.13	8.26±5.06	1.60±0.75	7.46±3.28	0.866	0.612
$\gamma\delta T^+$細胞	0.40±0.25	2.28±1.46	0.47±0.34	2.35±1.83	0.208	0.674
$NKG2A^+$細胞	0.72±0.43	3.68±1.84	0.74±0.37	3.84±2.39	0.674	0.889
$CD16^+CD56^+/CD3^-$細胞	3.14±1.32	18.63±11.33	3.38±1.73	16.13±8.81	0.575	0.635
NK 細胞活性(%)	26.54±16.13		39.43±15.48		0.015	
HTLV-1 プロウイルス量	867.38±874.62		641.75±343.12		0.401	

HAM 患者 10 症例(NK 細胞活性のみ 9 症例)のまとめ(mean±SD)
*：ウィルコクソン符号付順位和検定

してもらい，投与前後で臨床および検査所見を比較検討した．全体の臨床評価は納の運動障害度スコア(0～13 段階)，排尿障害スコア(頻尿，残尿，尿失禁それぞれ 0～3 の合計)，modified Ashworth scale(MAS)による痙性スコア(0～4)[11]，10m 歩行時間，日常生活動作，下肢の筋力などを用いた．運動障害スコアは 0：正常，1：走るスピードが遅い，2：歩行異常，3：駈足不能，4：階段昇降に手すり要，5：片手による伝い歩き，6：片手による伝い歩き不能，7：両手による伝い歩き 5m以上 10m 以内なら可，8：両手による伝い歩き 5m 以内なら可，9：4 つばい移動可，10：4 つばい移動不能，いざり等移動可，11：自力で移動不能，寝返り可，12：寝返り不能，13：足の指も動かせない，の 13 段階で評価した．排尿障害スコアは，頻尿，残尿，尿失禁を 0：正常，1：わずかに存在，2：明らかに存在，3：著明に存在，として評価し，特に残尿については 2：圧迫排尿状態，3：自己導尿とした．検査は，HTLV-I プロウイルス量，抗 HTLV-I 抗体価(PA 法)，リンパ球サブセット($CD4^+$，$CD8^+$，$CD56^+CD16^+/CD3^-$：NK 細胞，NKG2A，CXCR3，$\gamma\delta T$ 細胞の頻度および絶対数)，NK 細胞活性を投与前後で比較した．

結　果

LcS 4 週間の投与により，検討した HAM10 例全例において排尿障害スコアの改善($p=0.0085$)が，9 例で下肢痙性の改善が認められ，半数の 5 例で運動障害度が改善した．全体の評価は 10 例中 7 例で有効から著効を示した．また，10 例中 9 例で排便障害が改善した(表 1，図 1，図 2)．飲用前後で抗 HTLV-I 抗体価，HTLV-I プロウイルス DNA 量，リンパ球サブセットに有意な変化はなかったが，NK 細胞活性が有意に増加($p=0.015$)し(表 2)，これは投与前の NK 細胞活性が低い症例ほど，増加幅が大きい傾向であった．

考　案

今回の検討で LcS を HAM 患者に 4 週間投与することで，HAM の特徴的な臨床症状である排尿障害，運動障害，下肢の痙性を有意に改善できることが明らかになった．検査所見で LcS 治療後 HAM 患者の NK 細胞活性は増加したのに対

し，リンパ球サブセット同様 HTLV-I プロウイルス量には有意な変化がみられなかった．以前，われわれは天然型インターフェロン-α製剤による治療後に $CD8^{high+}$ 細胞内のメモリー細胞（$CD45RA^{-}CD27^{+}$），Th1 細胞の指標である $CXCR3^{+}$ 細胞数や HTLV-I プロウイルス量が有意に減少したことを報告した[12]．Th1 タイプの T 細胞やプロウイルス量の減少がインターフェロン-α の治療効果に関連した可能性があるのに対し，LcS 投与後の HAM の臨床症状改善には NK 細胞活性の回復が関連している可能性がある．今回の報告は，以前 Nagao ら[13]が健常人を対象に LcS 400 億個を含む乳飲料を投与した後，末梢血中の NK 細胞数，$CD4^{+}$T 細胞数，$CD8^{+}$T 細胞数，マイトジェンに対する T 細胞反応が変化しなかった一方で，NK 細胞活性のみが増加したという報告と矛盾しない結果である．また，治療前の NK 細胞活性が低い人ほど投与後よく増加し，同時に臨床症状が改善していたことも，HAM の臨床改善に NK 細胞活性の回復が密接に関与していることを示唆している．この LcS の治療効果は治療前後における少量（維持量）のステロイド投与の有無とは関係なかった．一般的に LcS のようなプロバイオティクスは，腸内菌叢のバランスを変え，有害な菌の増殖を抑え，食物の消化を促進することで免疫機能を高め，生体防御を高めていることが知られており，実際に LcS が NK 細胞や NK 細胞活性[14]，細胞障害性 T 細胞[15]を活性化することで，単純ヘルペスウイルス[16]やインフルエンザウイルス[17]の増殖を抑制することが報告されている．

LcS 投与後の NK 細胞活性増強の機構は不明であるが，いくつかの研究では，特に初期免疫に関与する免疫細胞の食細胞活性に対する効果が指摘されている．つまり，LcS に刺激された食細胞が，抗原提示やサイトカイン合成により獲得免疫を効率よく誘導させる役割を担っている可能性がある．実際に動物実験では，LcS がパイエル板の M 細胞に取り込まれ，腸内リンパ組織に分解されて，食細胞の刺激，サイトカイン合成，IgA 分泌，NK 細胞活性の増加などを引き起こすことが報告されている[18]．HAM 患者では NK 抑制性受容体である NKG2A 陽性細胞やγδT 細胞の末梢血単球における発現頻度が低いことが報告されているが[19]，これらの細胞群は LcS 投与前後では有意な変動を示さなかったことより，NK 細胞活性増加の調整が，NK 細胞における活性化受容体や抑制性受容体の発現レベルの変化以外の機構で行われている可能性が考えられた[20]．今後，LcS の HTLV-I に対する作用機序を検討する必要がある．

おわりに

今回のわれわれの検討で，1 日 800 億個の LcS の投与が HAM 患者の臨床症状改善に対して有効であり，さらに安価で安全であるため，HAM の維持療法として有望である可能性が示唆された．LcS が HTLV-1 キャリアーから HAM，および成人 T 細胞性白血病（ATL）の発症の予防に役立つかどうかについても検証したい．

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Zidovudine plus lamivudine in Human T-Lymphotropic Virus type-I-associated myelopathy: a randomised trial

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Abstract

Background: No therapies have been proven to persistently improve the outcome of HTLV-I-associated myelopathy. Clinical benefit has been reported with zidovudine and with lamivudine in observational studies. We therefore conducted a randomised, double blind, placebo controlled study of six months combination therapy with these nucleoside analogues in sixteen patients.

Results: Primary outcomes were change in HTLV-I proviral load in PBMCs and clinical measures. Secondary endpoints were changes in T-cell subsets and markers of activation and proliferation.

Six patients discontinued zidovudine. No significant changes in pain, bladder function, disability score, gait, proviral load or markers of T-cell activation or proliferation were seen between the two arms. Active therapy was associated with an unexplained decrease in CD8 and non-T lymphocyte counts.

Conclusion: Failure to detect clinical improvement may have been due irreversible nerve damage in these patients with a long clinical history and future studies should target patients presenting earlier. The lack of virological effect but may reflect a lack of activity of these nucleoside analogues against HTLV-I RT *in vivo*, inadequate intracellular concentrations of the active moiety or the contribution of new cell infection to maintaining proviral load at this stage of infection may be relatively small masking the effects of RT inhibition.

Background

The first identified human retrovirus, Human T-cell Lymphotropic Virus Type I (HTLV-I) [1], was initially associated with Adult T-cell Leukaemia/Lymphoma[2] before becoming causally associated with a chronic inflammatory myelopathy (HTLV-I associated myelopathy, HAM)[3,4] in a minority of carriers. In Japan it has been estimated that the lifetime risk of HAM among the one million infected persons is 0.25%[5] whilst in England, the estimated 22,000 carriers, of mainly African descent, have a 3% lifetime risk[6].

The onset of HAM is usually sub-acute and the initial presenting symptoms may relate to the urinary bladder, bowels or sexual function as well as gait disturbance or back pain. Diagnosis is often delayed until the more characteristic constellation of combined symptoms appears some months or years later. The most rapid progression is usually seen within the first two years with the neurological deficit varying from mild gait impairment to paraplegia. Thereafter some patients remain relatively stable whilst slow deterioration compounded by physiological aging is observed in others. In our experience approximately half of all patients become wheel-chair dependant and although many patients with HAM have a normal life span, premature deaths directly related to HAM occur.

The pathogenesis of HAM is not fully understood but several observations point to potential therapeutic approaches. An association between proviral load and disease has been repeatedly found with proviral load, as measured by HTLV-I DNA copies in peripheral blood mononuclear cells (PBMCs), about ten fold higher in patients with HAM than in asymptomatic carriers[7,8] and the risk of disease increases exponentially if the proviral load is greater than one HTLV-I DNA copy per 100 PBMCs[9]. Thus measures to reduce viral burden could potentially reduce the risk, or modify the course of the disease. Neuropathological examination reveals a perivascular lymphocytic infiltration in the spinal cord that is composed predominantly of CD4 positive T-lymphocytes (CD4 cells) in early disease and CD8 positive T- lymphocytes (CD8 cells) in later disease followed by a less cellular, atrophic phase[10,11]. The same cell types can be found in the cerebrospinal fluid of patients with HAM[12]. Like other cytotoxic T-lymphocytes (CTL) HTLV-I specific CTL have been shown to release potentially neurotoxic cytokines such as interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α)[13]. In the periphery HTLV-I-specific CD4 T cells differ much more in frequency between patients with HAM and asymptomatic carriers than do anti-HTLV CTL and these also secrete IFN-γ and TNF-α [14]. Thus therapies, which might modulate the immune response such as corticosteroids and interferons, have also been considered.

The literature on the specific management of HAM consists mostly of small, uncontrolled studies and cohort data. Conflicting results have been obtained in studies of corticosteroids therapy[15]. Interferon-α has been shown to be of short-term benefit in some patients in a randomised study comparing three different dose regimens with evidence of a dose dependant response[16]. Some nucleoside analogues have been shown to inhibit HTLV-I reverse transcriptase. The thymidine analogue, zidovudine, which inhibits HTLV-I RT in vitro[17,18] and in a rabbit infection model[19], was reported to show no clinical benefit in a study of six patients with chronic disease[20] but associated with improved mobility is some patients in a second study[21]. The cytosine analogue, lamivudine, was reported to reduce HTLV-I proviral load in five patients with clinical improvement in one patient with early disease[22]. All three studies were small, open and uncontrolled. In the management of both HIV and hepatitis B virus treatment with nucleoside analogues fails because of the emergence of viral strains with reduced sensitivity to these compounds especially when the drugs are used singly.

We report here the results of a randomised, double blind, placebo controlled study of the combination of zidovudine and lamivudine to determine their safety and efficacy with medium term (six to twelve months) usage.

Results

Sixteen patients were recruited to the study, twelve in London and four in Kagoshima. Eight were randomly assigned to each study arm. Baseline demographics, clinical and laboratory characteristics of the participants are shown in Table 1. Treatment was initiated on the day of randomisation in seven participants, within two weeks for seven participants and after two weeks for two participants. All participants were followed up to week 48.

Adherence

Mean red cell volume rose during the first 24 weeks of the study in the active arm. At week 24, 100% of patients in the active arm and 0% in the placebo arm had a change in MCV from baseline greater than 3 within-person SDs. During the second 24 weeks of the study a similar rise in MCV was seen in the placebo arm at week 48, 75% of patients in each arm had a change in MCV from baseline greater than 3 within-person SDs. There was good consistency between lamivudine and zidovudine returned tablet counts but the average number of tablets returned in the previous month rose from 9.5% at the end of the 3rd month to 25.5% at the end of the 6th month. There was little detectable difference in returned tablet counts between the two arms or between the first, randomised and second, open phases of the study.

Table 1: Baseline characteristics

	Placebo (n = 8)	Active (n = 8)	Total (n = 16)
Demographics			
Country [n(%)]			
England	6 (75)	6 (75)	12 (75)
Japan	2 (25)	2 (25)	4 (25)
Gender [n(%)]			
Male	4 (50)	1 (13)	5 (31)
Ethnic origin [n(%)]			
Afro-Caribbean	5 (63)	5 (63)	10 (63)
Indian	1 (13)	0 (0)	1 (6)
Japanese	2 (25)	2 (25)	4 (25)
Persian	0 (0)	1 (13)	1 (6)
Age at randomisation			
mean years (SD)	61.0 (10.8)	53.9 (15.5)	57.4 (13.4)
Likely mode of infection[a] [n(%)]			
Mother to child	3 (30)	5 (33)	8 (32)
Blood transfusion	1 (10)	2 (13)	3 (12)
Sexual intercourse	2 (20)	6 (40)	8 (32)
Unknown	4 (40)	2 (13)	6 (24)
Clinical Characteristics			
Pain score: scale 0–10			
median (range)	2.2 (0–5)	2.6 (0–8)	2.6 (0–8)
Osame's score [n(%)]			
0 – 4 (Unaided walk)	3 (38)	3 (38)	6 (38)
5 – 8 (Needs aid to walk)	4 (50)	3 (38)	7 (44)
9 – 13 (Unable to walk)	1 (13)	2 (25)	3 (19)
Time to walk 13 m in seconds [mean (SD)]			
0 – 4 (Unaided walk)	19 (13)	13 (2)	16 (9)
5 – 8 (Needs aid to walk)	82[b] (65)	112 (112)	95[b] (81)
Bladder function[c] [median (range)]			
Daytime frequency	5 (4–8)	4.5 (2–8)	5 (2–8)
Nocturia	4 (3.5–4)	2 (0.5–4)	4 (0.5–4)
Duration of symptoms in years			
median (range)	10.5 (5 – 19)	9 (1–18)	9 (1–19)
Laboratory Measurements*			
HTLV-I proviral load[d] (log_{10} copies/10^5 PBMCs)			
mean (SD)	3.57 (0.44)	3.76 (0.39)	3.67 (0.41)
Total lymphocytes[e] (10^6/L)	1600 (1250–2600)	1800 (1250–3000)	1775 (1250–3000)
CD3 lymphocytes[e] (10^6/L)	1165(488–2018)	1240(430–2144)	1190(430–2144)
CD3[e] %	71 (35–81)	69 (34–90)	71 (34–90)
CD4[e] lymphocytes (10^6/L)	802(340–1670)	801(334–1375)	801(334–1670)
CD4[e] %	39 (25–67)	44 (27–59)	44 (25–67)
CD8[e] lymphocytes (10^6/L)	375 (149–941)	185 (96–840)	362 (96–941)
CD8[e] %	27 (11–36)	10 (6–46)	21 (6–46)
CD25[d] %	3 (2–26)	7 (2–33)	3 (2–33)
CD69[d] %	8 (4–10)	7 (4–14)	7 (4–14)
CD71[d] %	9 (3–12)	7 (3–18)	8 (3–18)
HLA-DR%	20 (13–40)	9 (5–16)	15 (5–40)

*median (range) unless otherwise specified

[a] Total exceeds 16 because more than one possible mode of infection was documented in some patients

[b] One patient could only walk half distance. For the analysis their timed walk was doubled

[c] One patient in each group is excluded from the analysis because they had an indwelling urinary catheter.
A further 5 patients (3 in placebo group, 2 in active group) have missing nightly frequencies. Values given as average daily/nightly urinary frequency between screening and week 0

[d] Baseline data only available on n = 6 in placebo group and n = 7 in active group

[e] Baseline data only available on n = 7 in placebo group and n = 7 in active group

Safety

During the first 24 weeks of the study no participants in the placebo arm discontinued therapy. In the active arm one participant, whose disease had progressed from first symptom to bed-bound in the 9 months prior to study entry, was un-blinded at week 8 because of continuing deterioration. Interferon-α was added to the active compounds until week 20 when zidovudine was discontinued following the development of autoimmune haemolytic anaemia. During the second 24-week period of open therapy 5 participants previously on placebo discontinued zidovudine, one within 4 weeks, with gastrointestinal symptoms, the remainder after 16 – 20 weeks of therapy. Two had anaemia, one necessitating blood transfusion, one complained of drowsiness and paraesthesia and one of lethargy. One participant from the active therapy arm elected to discontinue therapy at week 40 when antibiotics were prescribed out of study. No side affects attributable to lamivudine were reported. There were no significant biochemical abnormalities.

Clinical efficacy

No significant changes in pain score, urinary frequency or nocturia were seen between the active arm and the placebo arm during the study (Table 2) nor within the placebo arm when comparing the first 24 weeks on placebo with the second 24 weeks on active therapy. Similarly there was no consistent pattern of change in disability. Timed walks remained relatively constant throughout the study in most participants except for two in the placebo arm whose timed walks improved between the two baseline assessments and week 4 (Figure 1).

Laboratory markers of efficacy: The median (IQR) HTLV-I proviral load at baseline was 4.2 (2.6–7.5) copies per 100 PBMCs (mean (SD) 3.7 (0.4) $\log_{10}$/100,000 PBMCs). No significant change in proviral load was seen during the first 24 weeks of the study in the active arm compared with the placebo arm (mean difference (SE): 0.02 (0.17), p = 0.92) (Table 3) nor within the placebo arm comparing the first 24 weeks of placebo therapy with the second 24 weeks of active therapy (mean difference (SE): 0.12 (0.16), p = 0.50) (Table 4).

At baseline, the total lymphocyte, CD3, CD4 and CD8 cell counts were normal. During the first 24 weeks the mean total lymphocyte counts (AUCMB) increased by 148 × 10^6/L in the placebo arm but decreased by 159 × 10^6/L in the active arm, a mean difference of 307 × 10^6/L (SE = 158, p = 0.07) (Table 3). This trend was confirmed when comparing the lymphocyte counts in participants on placebo compared with their counts when taking active therapy, with the initial increase of 148 × 10^6/L followed by a decrease of 71 × 10^6/L (mean difference (SE): 219 (73), p = 0.02) (Table 4). Lymphocyte subset analysis of the first, randomised therapy phase of the study, showed an increase in CD4 in both arms and an increase in CD8 cells in the placebo arm but a decrease in the active arm (Table 3). These changes were also seen when comparing the first 24 weeks of placebo treatment with the subsequent 24 weeks of active treatment in the placebo arm (Table 4). However the major impact on the absolute lymphocyte count appears mainly among non-T-lymphocytes with a mean rise of 50 × 10^6/L non CD3 lymphocytes during the first 24 weeks in the placebo arm compared with a mean decrease of 127 × 10^6/L from baseline in the active arm, a difference of 177 × 10^6/L (SE = 31, p < 0.001). A similar trend was seen by comparing active with placebo therapy in the placebo arm, although not statistically significant.

At baseline CD69 was expressed on average on 8%, CD71 on 8% and CD25 on 9% of lymphocytes. Compared with placebo no significant changes were seen in these markers of activation (CD69) and proliferation (CD71) nor in the expression of the IL-2 receptor (CD25) (Tables 3 and 4) during treatment with zidovudine plus lamivudine.

Table 2: Clinical outcome measures during randomised treatment phase presented as average [mean (SE)] changes from baseline during weeks 0 – 24 as measured by AUCMB

	Placebo (n = 8)	Active (n = 8)	p
Osame's score	+0.19 (0.19)	+0.18 (0.34)	0.99
Pain score	+0.43 (1.13)	+0.41 (0.66)	0.99
Bladder function			
Daytime frequency[a]	-0.11 (0.38)	-0.19 (0.62)	0.93
Nocturia[b]	-0.18 (0.55)	-0.81 (0.47)	0.41

[a] One patient in each group is excluded from the analysis because they had an indwelling urinary catheter

[b] One patient in each group is excluded from the analysis because they had an indwelling urinary catheter. The four Japanese patients had no night urinary frequency data (2 in each group) and baseline frequency was unknown for another patient in the placebo group. All are excluded from the analysis.

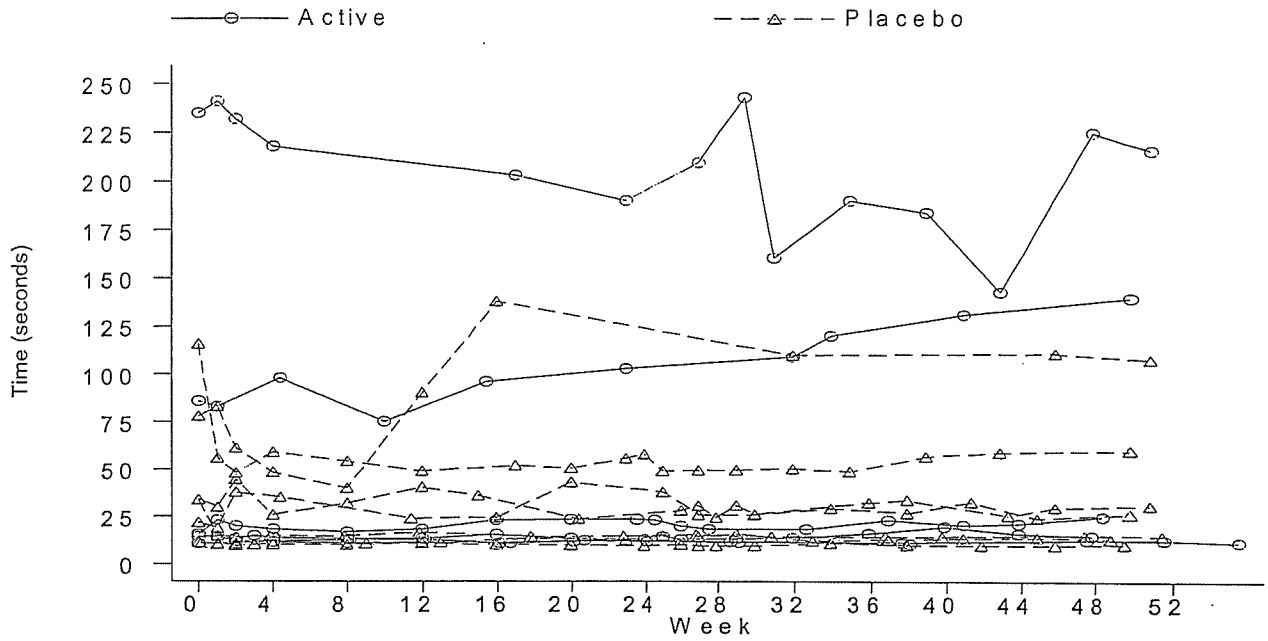

Figure 1
Changes in timed 13 meter walk observed during the study period.

Discussion

Since monotherapy with nucleoside analogues had been reported to reduce HTLV-I viral load, the inhibition of HTLV-I reverse transcriptase by two nucleoside analogues, zidovudine and lamivudine was thought to be more likely to cause a sustained decrease in HTLV-I viral burden than either drug alone. A decrease in HTLV-I viral DNA and thus a presumed decrease in viral antigen burden has been associated with a reduction in HTLV-I specific CTL lymphocyte activity[22].

Since this activity is postulated to contribute to HAM pathogenesis anti-HTLV therapy might in turn delay progression or cause clinical improvement.

Table 3: Laboratory outcome measures during randomised treatment phase presented as average [mean (SE)] changes from baseline during weeks 0 – 24 as measured by AUCMB

	Placebo	Active	p
HTLV-I proviral load[a] (log_{10} copies/10^5 PBMCs)	+0.03 (0.10)	+0.05 (0.13)	0.92
Total Lymphocytes[b] (10^6/L)	+148.2 (58.0)	-159.3 (146.7)	0.07
CD3 Lymphocytes[b] (10^6/L)	+98.4 (63.4)	-23.2 (151.3)	0.47
CD3[b] %	+0.2 (2.8)	+4.3 (2.6)	0.14
CD4[b] Lymphocytes (10^6/L)	+32.6 (33.1)	+10.4 (98.7)	0.55
CD4[b] %	-1.2 (0.9)	+4.7 (1.8)	0.01
CD8[b] Lymphocytes (10^6/L)	+65.8 (31.3)	-38.1 (60.7)	0.15
CD8[b] %	+1.4 (1.2)	+1.4 (1.9)	0.99
CD25[a] %	-0.8 (1.8)	+0.3 (0.8)	0.58
CD69[a] %	+0.4 (1.3)	+1.2 (1.8)	0.73
CD71[a]%	-0.3 (1.0)	-0.2 (1.9)	0.97
HLA-DR%	-4.85 (4.0)	+3.59 (2.4)	0.42

[a] Data only available on n = 6 in placebo group and n = 7 in active group due to missing baseline data
[b] Data only available on n = 7 in placebo group and n = 7 in active group due to missing baseline data

Table 4: Laboratory outcome measures for the placebo group presented as average [mean (SE)] changes from baseline for the time on active therapy (weeks 24 – 48) compared with the time on placebo (weeks 0–24). Baseline values are the average of weeks – 2 and 0 for 0 – 24 week analysis and the average of weeks 20 and 24 for 24 – 48 week analysis

	Weeks 0–24	Weeks 24–48	P
HTLV-I proviral load[a] (log_{10} copies/10^5 PBMCs)	+0.03 (0.10)	-0.09 (0.08)	0.50
Total Lymphocytes[b] (10^6/L)	+148.2 (58.0)	-71.1 (63.1)	0.02
CD3 Lymphocytes[b] (10^6/L)	+98.4 (63.4)	+7.0 (39.2)	0.28
CD3[b] %	+0.2 (2.8)	+2.8 (2.0)	0.22
CD4[b] Lymphocytes (10^6/L)	+32.6 (33.1)	+30.4 (46.9)	0.97
CD4[b] %	-1.2 (0.9)	+3.6 (1.9)	0.08
CD8[b] Lymphocytes (10^6/L)	+65.8 (31.3)	-23.5 (13.7)	0.04
CD8[b] %	+1.4 (1.2)	-0.7 (1.1)	0.16
CD25[a] %	-0.8 (1.8)	-0.2 (1.3)	0.82
CD69[a] %	+0.4 (1.3)	-0.2 (3.0)	0.89
CD71[a]%	-0.3 (1.0)	+0.5 (1.3)	0.76
HLA-DR%	-4.85 (4.0)	+1.05 (2.3)	0.40

[a] Data only available on n = 6 due to missing baseline data
[b] Data only available on n = 7 due to missing baseline data

HAM is a rare disease in the UK with only 10 – 12 cases diagnosed annually[23]. A similar number of cases are seen in Kagoshima, Japan where the prevalence of infection is much higher. There is no licensed or clinically proven effective therapy for HAM in the UK. However short courses of interferon-α were made available by the Japanese government for their patients with HAM coincident with the start of the study and this affected recruitment in Japan.

Although the basic study design was double-blind and placebo-controlled a second open-therapy phase was incorporated, with all study participants offered open therapy for a further six months, to maximise the likelihood of detecting significant clinical and laboratory changes. The study was not un-blinded until the last participant had completed twelve months of therapy. In this way the safety of zidovudine plus lamivudine could be studied for a total of twelve months exposure whilst those originally randomised to the placebo arm acted as their own control for the second six months of active compound in a non-randomised comparison.

The primary outcome measures of the study were the clinical and virological effects of zidovudine in combination with lamivudine. The failure to detect any clinical improvement after up to 12 months of therapy may have been due to the long history of HAM among the participants who had an average duration of symptoms of 10.1 years. After this prolonged time the neurological damage may have been irreversible. However, the stability of the HTLV-I viral DNA load, which was expected to fall during the study, leaves the possibility that agents that effectively reduced viral burden, might result in clinical improvement even in longstanding disease.

The persistence of high viral load during therapy could be due to a lack of activity of these nucleoside analogues against HTLV-I RT, inadequate intracellular concentrations of the active triphosphate metabolite, or it may be that reverse transcription is not important in maintaining HTLV-I proviral load at this stage of infection. Thus, whilst a reduction in HTLV-I viral DNA during therapy with lamivudine has been documented in vivo, there are now conflicting reports of its activity against HTLV-I RT in vitro [24-26]. Conversely the in vitro inhibition of HTLV-I RT by zidovudine has been confirmed[27]. Second, the intracellular concentrations of the nucleoside analogues were not measured in this study but HTLV-I Tax, which is expressed within hours by the majority of HTLV-I infected cells in unstimulated culture conditions, is known to upregulate MDR-1[28]. Finally, the importance of HTLV-I RT in maintaining HTLV-I proviral load remains uncertain. Several paths of evidence point to continuing viral protein expression[29] and HTLV-I has been shown to spread directly from cell to cell, in ex vivo experiments, through the formation of a virus induced synapse[30]. Whether cell-to-cell infection within the host is predominantly through virion production, binding and fusion or directly through the viral synapse the reverse transcription of HTLV-I RNA is required to establish new infection. However HTLV-I infection is associated with proliferation of the infected cells, a mechanism that contributes to the total proviral load without the need of reverse transcription.

Changes in the lymphocyte markers of activation and proliferation might have been expected with a reduction in HTLV-I proviral load. In the absence of such an effect the observed, statistically significant, changes in total, CD4, CD8 and non-CD3 lymphocytes are of interest albeit unexplained.

Conclusion

The data from this study do not provide evidence of improvement in the clinical state of patients with HAM in the medium term following treatment with the combination of zidovudine and lamivudine but the treatment was well tolerated with no unexpected side effects. New, controlled studies of both anti-viral and anti-inflammatory agents are urgently required for patients with this chronic, disabling disease. International co-operation will be necessary to accelerate progress.

Methods

Study design

Patients were screened two weeks prior to baseline and eligibility criteria checked. Recruitment was by the study physicians at each site. The eligibility form was submitted with a request for a trial number to the coordinating centre at the Medical Research Council. Trial numbers had been randomly allocated, stratified by site, to active or placebo and this code was held, sealed and secure, at the MRC. Trial therapy was stored in site pharmacies and labelled by trial name and trial number only. The trial coordination staff at the MRC issuing the trial number, the pharmacy, the clinician and the patient were all blinded to the allocation of study arm. Trial numbers were transmitted by facsimile to the study centres. Recruitment commenced 8 November 1999. Eligible patients were randomised to start 24 weeks treatment with zidovudine 300 mg plus lamivudine 150 mg twice daily (the active arm) or matching zidovudine and lamivudine placebo tablets (the placebo arm). This was followed by 24 weeks of open therapy with the active compounds for all study participants. Last follow up was completed 30 July 2002. The primary endpoints were virological – change in HTLV-I viral DNA from baseline to 24 weeks and clinical – changes in gait, disability, pain and bladder function. The secondary endpoints were immunological – changes in CD4, CD8, CD25, CD69 and CD71. The study, based on recruiting twelve patients in each arm and assuming a standard deviation of 0.78 $\log_{10}$ copies of HTLV-I DNA per 100 PBMCs, was powered to detect a difference in average change from baseline up to 24 weeks (as measured by area under the curve minus baseline – AUCMB) of 1 $\log_{10}$ copy with 90% power and 5% probability of type 1 error. Therapeutic safety was determined by monitoring standard haematological and biochemical parameters according to National Institute of Health, Division of AIDS criteria[31].

Subjects

Patients were eligible for the study if they had clinical evidence of HAM according to WHO criteria [32], had confirmed HTLV-I infection, were aged 18 – 75 years, not pregnant and willing to use appropriate contraception if female and sexually active. Patients were excluded if they had HIV infection, had previous exposure to zidovudine or lamivudine, had significant haematology, liver or renal test abnormalities or were unable to give informed consent. Patients were not eligible to enter the study until at least four weeks after concluding other specific treatment for HAM e.g. corticosteroids. The study was conducted at two sites, The HTLV clinic at St. Mary's Hospital, London, UK and the 3rd Department of Internal Medicine, University of Kagoshima, Kagoshima, Japan. At each site the local research ethics committee approved the study and written informed consent was obtained in the local language.

Clinical evaluation

Participants underwent a full neurological examination at weeks 0, 24 and 48 and if new symptoms were reported. Participants were reviewed monthly with additional visits at weeks 1, 2, 25 and 26. At each visit a fixed distance walk was timed and the degree of walking aid documented for patients able to walk. Disability was graded 1 – 13 as described by Osame [15]. Pain was quantified using a visual analogue scale and urinary bladder diary cards recording daytime frequency and nocturia were collected. Participants were provided with 70 tablets of each compound or matching placebo every four weeks and any remaining tablets were returned and counted to provide a measure of adherence.

Laboratory evaluation

Full blood counts, biochemical profile, quantitative HTLV-I viral DNA and lymphocyte phenotypic assays were performed at each study visit. The clinical investigators were blinded to the mean red cell volumes although these were available for the end of study analysis. Full blood counts were measured on a Coulter LH750 Analyzer (Beckman Coulter Inc, Fullerton, CA). The renal, liver and bone chemistry assays were performed on an AU2700 Olympus Analyser (Olympus Diagnostica Gmbh, Hamburg, Germany). HTLV-I viral DNA was quantified by real-time PCR as previously described. [33]. In addition to the standard T-lymphocyte markers CD3, CD4, CD8, markers of T-cell activation CD25, CD69 and proliferation CD71 were assayed described earlier [34].

Statistical analysis

Data from clinical record files were entered into Oracle databases and checked manually and by computer consistency checks. Analysis text files were created from the database and imported into STATA for statistical analysis. Baseline values of laboratory tests were calculated as the mean of screening and week 0 results. Week 0 data were taken as baseline for all clinical measurements. Time was measured from the start of trial treatment and for all measurements during follow-up data from the closest date to the target assessment week, within a window of ±

2 weeks, were used. HTLV-I proviral load values were converted to copies per 100,000 PBMCs and $\log_{10}$ transformed prior to analysis. Data were analysed as average change from baseline to a given time point as measured by AUCMB using the T-test. All analyses were performed as randomised regardless of changes to study treatment during follow up. The study was funded by the departments of the participants. GlaxoSmithKline generously provided zidovudine and lamivudine for the study and matching placebos.

Abbreviations

AUCMB Area under the curve minus baseline

CD Cluster of Differentiation

CTL Cytotoxic T- Lymphocyte

DNA Deoxyribonucleic acid

HAM HTLV-I-associated myelopathy

HTLV-I Human T-cell Lymphotropic Virus type I

IFN-γ Interferon-gamma

IL-2 Interleukin – 2

IQR Inter-Quartile Range

MCV Mean cell volume

MDR Multi-drug Resistance

PBMC Peripheral blood mononuclear cell

SD Standard Deviation

SE Standard Error

TNF-α Tumour necrosis factor – alpha

WHO World Health Organisation

Competing interests

Graham P Taylor has received honoraria for teaching and travel grants from GlaxoSmithKline plc. No other conflicts of interest are declared.

Authors' contributions

The study was conceived and designed by GPT, AB, JNW & CRMB; conducted by GPT, PG, YF, AB, AM, NN, PR, KU & MO, analysed by HG & AB and the manuscript was written by GPT, HG, AB, CRMB & JNW

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