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Rapidly Progressive Human Herpesvirus 8-associated Solid Anaplastic Lymphoma in a Patient with AIDS—Associated Kaposi Sarcoma

YOSHIHIKO YAMAMOTO[a], KATSUJI TERUYA[a], HARUTAKA KATANO[b], HITOSHI NIINO[c], AKIRA YASUOKA[a], SATOSHI KIMURA[a] and SHINICHI OKA[a,*]

[a]*AIDS Clinical Center, International Medical Center of Japan, Toyama 1-21-1, Toyama, Shinjuku, Tokyo 162-8655, Japan;* [b]*Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan;* [c]*Department of Pathology, International Medical Center of Japan, Tokyo, Japan*

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We report a case of rapidly progressive solid lymphoma with anaplastic large cell morphology, followed by systemic Kaposi sarcoma in an adult patient with AIDS. The lymphoma cells expressed human herpesvirus 8 (HHV-8)-encoded latent and lytic proteins and Epstein–Barr virus -encoded small RNA, suggesting that this case could be categorized into HHV-8-associated solid lymphoma, a recently identified disease entity.

Keywords: HHV 8; HHV 8-associated lymphoma; Rapid progression; AIDS; Kaposi sarcoma

INTRODUCTION

AIDS-associated lymphoma is usually classified histologically into diffuse large B cell lymphoma, small non-cleaved cell and Burkitt-like lymphoma, and is associated with Epstein–Barr virus (EBV) infection. However, primary effusion lymphoma (PEL, body-cavity-based lymphoma), a rare effusion lymphoma occurring in body-cavities of AIDS patients, is associated with human herpesvirus 8 (HHV-8) infection [1]. Recent studies reported that some cases of solid lymphomas in AIDS patients were also associated with HHV-8 infection [2,3]. In those cases, lymphoma cells expressed CD30 and showed an anaplastic large cell lymphoma (ALCL)-like morphology or plasmablastic lymphoma. This type of lymphoma, designated "HHV-8-associated solid lymphoma", is often complicate by other HHV-8-associated diseases such as Kaposi sarcoma (KS), PEL and multicentric Castleman disease (MCD). In the present study, we describe a case of rapid progressive HHV-8-associated solid lymphoma with anaplastic large cell morphology followed by systemic KS within a short clinical course of 3 months.

CASE REPORT

A 30-year-old Caucasian male with HIV-1 infection was admitted to our hospital on June 25, 2000, because of loss of consciousness. The past history included homosexual behavior and intravenous drug use over the past 10 years. He was diagnosed with HIV-1 infection in 1994, but he did not take any medical check-up thereafter. He had not contracted any opportunistic infections until he found red spots on the skin of the arms, chest and abdomen at the beginning of May 2000. He visited a local hospital complaining of increased number and rapid enlargement of skin lesions, and was later diagnosed as KS based on examination of skin biopsy. The CD4+ lymphocyte count was 75/μl and HIV-1 plasma viral load was 431,992 copies/ml on May 26, 2000. The patient was discharged after commencement of highly active antiretroviral therapy (HAART) containing 80 mg/day stavudine, 300 mg/day lamivudine, 800 mg/day saquinavir and 600 mg/day ritonavir on June 13, 2000. Eleven days later, he started to develop severe edema of the foot, nausea, hematocezia, speech disturbance, and loss of consciousness. The patient was transferred to our hospital on June 25, 2000. On admission, KS lesions, each measuring 1–2 cm in

*Corresponding author. Tel./Fax: +81-3-5273-5193. E-mail: oka@imcj.hosp.go.jp

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diameter, were noted on the whole body. Serum anti-HHV-8 antibody examined by enzyme immunoassay (EIA) and immunofluorescence assay (IFA) were both negative [4]. KS lesions were identified in the esophagus, stomach, rectum and colon, as well as a nodular lesion measuring 2 cm in diameter in the cecum by upper gastrointestinal and colon endoscopic examination. He could not continue HAART because of loss of consciousness. Liposomal doxorubicin (20 mg/m^2) was administered intravenously on July 4 and 18 for treatment of systemic KS. During the treatment, right axillary and left cervical lymph nodes grew to 2 and 3–5 cm in diameter, respectively. Examination of biopsy material from these lymph nodes on July 18 revealed large cell lymphoma affecting the cervical lymph nodes. The patient died 6 days after the induction of the CHOP regimen, which consisted of prednisolone (120 mg), doxorubicin (30 mg, reduced by 30% due to the associated thrombocytopenia), vincristine (2 mg, reduced by 71.4%) and cyclophosphamide (750 mg, reduced by 50%).

Autopsy was performed 14 h postmortem. Lymphoma cells were found not only in the cervical, mediastinal and inguinal lymph nodes, but also in the spleen, tonsils, gastrointestinal mucosa, lungs, adrenal glands and bone marrow. No lymphoma cells were detected in the peripheral blood during the entire clinical course. The lymphoma cells showed anaplastic large blastic cell morphology with occasional horseshoe shape or multiple nuclei and large cytoplasm (Fig. 1A). Immunohistochemical staining showed that these cells were CD30-positive (Ki-1) (BerH2, Dako, Kyoto, Japan), CD43-positive (MT1, Novocastra Laboratories, Newcastle upon Tyne, UK), CD45RO-positive (UCHL-1, Dako), CD45-positive (LCA) (PD7/26 and 2B11, Dako), CD4-negative (1F6, Novocastra Laboratories), CD5-negative (4C7, Novocastra Laboratories), CD8-negative (4B11, Novocastra Laboratories), CD15-ngative (MCS-1, Nichirei, Tokyo, Japan) and CD20-negative (L26, DAKO). Almost all lymphoma cells expressed HHV-8-encoded latency-associated nuclear antigen (LANA) in the nuclei, exhibiting a dot-like pattern (Fig. 1B) [5]. A small portion of lymphoma cells also expressed HHV-8-encoded ORF59 (lytic protein) in the nuclei, exhibiting a diffuse staining pattern (Fig. 1C) [5]. EBV-encoded small RNA-1 (EBER-1) was positive in some lymphoma cells by *in situ* hybridization (Fig. 1D). KS was found in skin and perilymph nodal soft tissue of the inguinal region. Microscopic examination of KS lesions showed proliferation of spindle-shaped cells in the dermis with enlarged vascular space in hematoxylin and eosin stained sections (Fig. 1E), and the expression of LANA in nuclei of the spindle-shaped cells, appearing as a dot-like staining pattern, in immunohistochemically stained sections (Fig. 1F) [5].

DISCUSSION

In the present study, we describe an aggressive CD30-positve solid lymphoma in a patient with AIDS-KS. HHV-8 gene products were detected in both the lymphoma and KS of this case. The lymphoma has an undeterminant phenotype and takes an ALCL-like morphology. In addition, neither effusion lymphoma nor lymphocytic leukemia was detected in this patient. These features of this lymphoma correspond to those of HHV-8-associated solid lymphoma, a new recently proposed disease entity [2]. In a recent classification of lymphoma, HHV-8-associated solid lymphoma is categorized into diffuse large B cell lymphoma and anaplastic variant [6]. A few reports have described "CD30-positive ALCL" in AIDS patients [7–9]. Since these "ALCL" cases contained both T cell and B cell lineages, the criteria of "CD30-positive ALCL" in those papers were confusing and included both true ALCL and HHV-8-assoicated solid lymphoma. Although the linage (T or B cell) was not delineated in our case, all features noted in this case are compatible with the criteria of HHV-8-assoicated solid lymphoma. This is the first clinical case report of rapid progressive HHV-8-assoicated solid lymphoma with systemic KS and without effusion lymphoma.

The underlying mechanism(s) of lymphomagenesis of HHV-8 is not clear at present. It is known that EBV infection is common among HHV-8-associated solid and

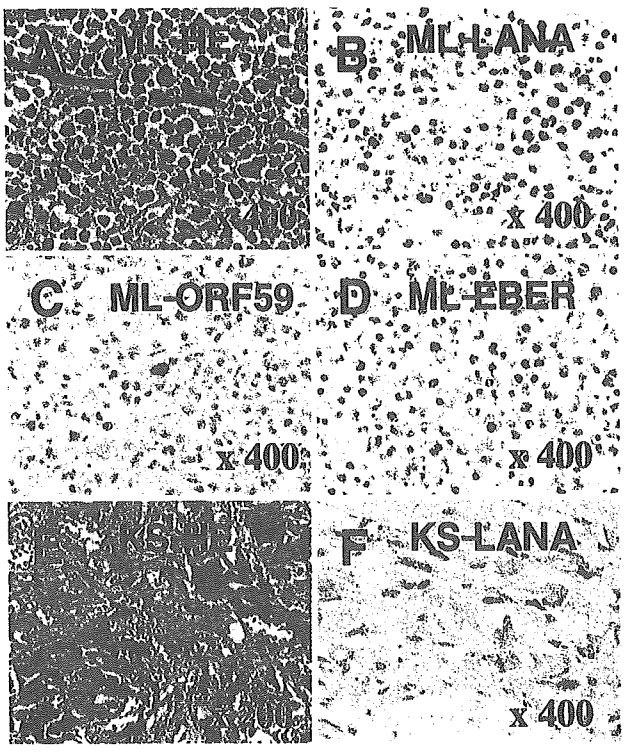

FIGURE 1 Immunohistochemistry and *in situ* hybridization of malignant lymphoma and Kaposi sarcoma. Specimens were obtained at autopsy. (A) Hematoxylin and eosin (H and E) staining of malignant lymphoma. (B) Expression of HHV-8-encoded LANA in lymphoma cells. (C) Expression of the HHV-8-encoded ORF59 (lytic protein) in lymphoma cells. (D) Epstein–Barr virus-encoded small RNA-1 (EBER) in lymphoma cells by *in situ* hybridization. (E) H and E staining of Kaposi sarcoma. (F) Expression of HHV-8-encoded LANA in spindle-shaped KS cells.

effusion lymphomas. In our case, EBV was detected in a proportion of lymphoma cells, whereas, almost all lymphoma cells expressed HHV-8-encoded LANA. In addition, the undeterminant phenotype and morphological features of this case were different from EBV-associated diffuse large cell lymphoma or Burkitt type lymphoma. Moreover, the expression of ORF59 protein, a replication-associated protein of HHV-8, suggests that the replication of HHV-8 occurred in lymphoma cells. Therefore, we presume that HHV-8 infection, rather than EBV, is associated with the pathogenesis of this type of lymphoma. Although HHV-8-encoded proteins were detected in both KS and lymphoma, serum antibody against HHV-8 could not be detected. The lack of anti-HHV-8 antibody might be related to rapid progression. HHV-8-assoicated solid lymphoma is often complicated with other HHV-8-assoicated diseases, such as KS, PEL and MCD [2]. Poor prognosis is common among cases of HHV-8-associated solid lymphoma. In our case, KS spread rapidly in systemic cutaneous regions within 6 weeks. Lymphoma appeared as rapidly growing lymphadenopathy on the eyelid, neck and arms, 10 weeks after the onset of KS when the second dose of liposomal doxorubicin was administered for KS. These results suggest that the rapid progressive lymphoma does not respond to liposomal doxorubicin. Because administration of two doses of liposomal doxorubicin caused bone marrow suppression at the time of induction of anti-lymphoma therapy, we reduced the dose of CHOP regimen. Further studies are required to establish the most appropriate treatment for HHV-8-associated solid lymphoma.

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臨　床

わが国で初めて Artemether-Lumefantrine 合剤で治療した輸入熱帯熱マラリアの1症例

[1]国立国際医療センターエイズ治療研究開発センター，[2]国立国際医療センター研究所適正技術開発移転研究部，
*現所属：富山医科薬科大学感染症治療部
石崎有澄美[1]*　菊池　嘉[1]　畑生　俊光[2]
狩野　繁之[2]　安岡　彰[1]*　岡　慎一[1]

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Key words：　falciparum malaria, drug resistance, artemether-lumefantrine

序　文

昨今の海外渡航者，海外長期滞在者数の増加につれ，国内における輸入マラリア症例が増加している[1]．マラリアは早期診断，適切な治療で治癒可能な疾患である．現在，世界的にクロロキン耐性熱帯熱マラリア，また地域によってはメフロキン耐性熱帯熱マラリアも出現しており，治療には渡航地域に応じた適切な抗マラリア薬の選択が重要である．今回，我々は日本国内で初めて Artemether-Lumefantrine (Coartem, Novartis) を使用し，良好な経過をたどった輸入熱帯熱マラリア1症例を経験したので報告する．

症　例

患者：42歳女性．

主訴：発熱．

既往歴：10年前に熱帯熱マラリア．

現病歴：2000年5月から2002年3月30日までガーナに滞在していた．2002年3月30日，空路帰国途中から，全身倦怠感が出現した．3月31日に37.6度の発熱及び関節痛（肩，腰，背中）を認めた．4月1日には38.6度まで発熱し，頭痛が出現した．4月2日より悪寒，嘔気が出現し，熱は39.2度まで上昇した．近医受診し熱帯熱マラリアを疑われ，同日当科紹介受診となった．尚，マラリア予防薬はガーナ滞在当初，1カ月間服用したが嘔気などの副作用のためその後は中止していた．また帰国3週間前の末梢血塗抹検査ではマラリア原虫は陰性であった．

入院時症状：全身倦怠感あり，頭痛あり，咳・痰なし，鼻汁なし，軽度咽頭痛あり．

入院時現症：身長159.3cm，体重47.7kg，体温39.4度，血圧114/84mmHg，心拍数115/分，整，SpO_2 99%(室内)，意識清明，貧血・黄疸なし，口腔粘膜白苔なし，扁桃発赤・腫脹なし，頚部腫瘤なし，呼吸音正常，心音正常，腹部平坦・軟，腸音正常，自発痛・圧痛なし，腹膜刺激症状なし，肝：右季肋部にて1.5横指触知，脾触知せず，頚部リンパ節腫脹左右に3～5mm程度を数個ずつ触知，腋窩・鼠径リンパ節腫脹なし，四肢浮腫なし，皮膚：発赤・発疹なし

入院時検査所見 (Table 1)：WBC 3,610/μl，Hb 14.2g/dl，Hct 42.2%，Plt $12.4\times10^4/\mu l$，AST 83IU/l，ALT 75IU/l，LDH 335IU/l，T-Bil 0.7mg/dl，Cr 0.85mg/dl，CRP 1.62mg/dl，尿蛋白(2+)，尿

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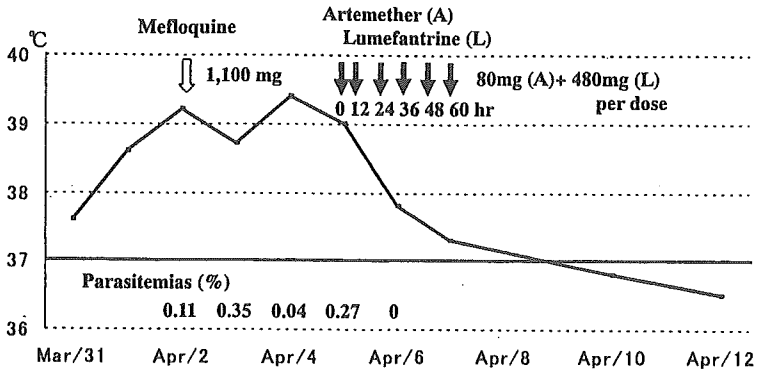
富山医科薬科大学感染症治療部
石崎有澄美

Table 1 Laboratory data on Admission

CBC		Blood chemistry		Urine Analysis	
WBC	3,610 /μl	Alb	4.7 g/dl	U-Prot	(2+)
Hb	14.2 g/dl	AST	83 IU/l	U-OB	(−)
Hct	42.2 %	ALT	75 IU/l	U-keton	(3+)
Plt	12.4×10^4 /μl	LDH	335 IU/l	U-Bil	(1+)
Coagulation		γGTP	62 IU/dl		
PT	13.1 sec	T-Bil	0.7 mg/dl		
APTT	40.3 sec	BUN	7 mg/dl		
Fib	429.7 mg/dl	Cr	0.85 mg/dl		
		CRP	1.62 mg/dl		

Fig. 1 Clinical Course



潜血（−），尿中ビリルビン（1+）．

心電図：異常なし．

胸部レントゲン写真：異常なし．

腹部超音波検査：肝脾腫あり，腹水なし，腎異常なし，胆道系異常なし．

入院後経過(Fig. 1)：症状，経過よりマラリアを疑った．末梢血薄層塗抹標本検査で熱帯熱マラリア原虫を認めた．マラリア原虫赤血球寄生率は0.11%であった．第1病日にメフロキン(メファキン「エスエス」錠275, SS製薬）を4錠（1錠につき塩酸メフロキン275mg含)を1回で内服した．内服約7時間後に食物残渣の嘔吐が見られた．第2病日朝の末梢血薄層塗抹標本検査ではマラリア原虫赤血球寄生率は0.35%と上昇していた．しかしながら頭痛・嘔気は若干軽快し，解熱傾向も認めた．第3病日朝の末梢血薄層塗抹標本検査ではマラリア原虫赤血球寄生率は0.04%と減少したが，夜半より再度39.4度の発熱が出現し，頭痛・嘔気・倦怠感も増強した．第4病日の末梢血塗抹検査ではマラリア原虫赤血球寄生率は0.27%と再上昇していた．メフロキン耐性熱帯熱マラリアを考え，第4病日昼よりCoartemの内服を開始した．投与にあたり患者本人に薬剤の効果と副作用を説明し，文書による同意を得た．投与量は多剤耐性熱帯熱マラリアの可能性を考え，1回4錠（1錠につきArtemether 20mg, Lumefantrine 120 mg含）とし，0, 12, 24, 36, 48, 60時間目に服用した．初回内服直後より著明に解熱し，第4病日夜の末梢血薄層塗抹標本検査では若干のマラリア原虫を認めたが，第5病日朝には完全に消失した．頭痛・嘔気・倦怠感などの症状も徐々に軽快し第11病日にはすべて消失した．その後再燃は認めず，熱帯熱マラリアは治癒した．また，入院時より熱帯熱マラリアに伴うと考えられる肝脾腫と肝機能障害を認めた．経過中肝機能は更に悪化し，第9病日にはAST 230IU/dl, ALT 381IU/dlと

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なった．経過からメフロキンによる薬剤性肝機能障害を疑い，経過観察したところ AST/ALT 値は正常化し肝腫大も改善した．その他重篤な合併症は認めなかった．

考　察

本症例は早期に熱帯熱マラリアの診断がつき，直ちにメフロキンによる治療を開始した．しかし症状が遷延したため，臨床的にメフロキン耐性熱帯熱マラリアと判断し Coartem の追加投与を行った．Coartem は合併症のない多剤耐性熱帯熱マラリア治療用に新しく開発され[2)5)]，チンハオスー（Artemisinin）誘導体である Artemether と Lumefantrine を含む合剤である．Artemisinin の薬効は完全には明らかにされていないが，その特徴は急速な原虫血症及び臨床症状の改善である．Lumefantrine の薬効も不明な点が多いがクロロキンと同様，マラリア原虫内でのヘム重合の阻害を起こすと考えられている．本薬剤は 1 錠に Artemether 20mg，Lumefantrine 120mg を含み，通常は 1 回の内服量を体重別に 1～4 錠とし，計 4 回（0, 8, 24, 48 時間目）内服する．多剤耐性マラリアでは計 6 回（0, 8, 24, 36, 48, 60 時間目）の内服を行う．妊婦での安全性は確立されていない．副作用はほとんど見られないが，めまいや倦怠感，消化器症状，動悸，関節痛，頭痛，不眠，発疹などの報告がある．すでに行われている海外での治験では，いずれも有効な成績を収めている[2)]．現在，クロロキンに対する耐性マラリアは世界各地で見られる[3)～5)]．メフロキン耐性熱帯熱マラリアは東南アジア，特にタイ・ミャンマーの国境付近で特に多いが，近年南米やアフリカ地域からの報告も散見される．その他の抗マラリア薬であるスルファドキシン/ピリメサミンや古くから使用されているキニーネへの耐性も報告されている[3)]．このような状況を鑑み，現在 WHO は多剤併用療法を推奨している[5)]．2 種類以上の抗マラリア薬の併用で相乗効果が期待できかつ，マラリア原虫の耐性獲得の確率低下も予測される．なかでもメフロキン耐性マラリアが大きな問題であるタイでの治療成績から[6)]，Artemisinin 誘導体を含んだ併用療法が有用と考えられている．今回，臨床的にメフロキン耐性が疑われたが，以上の知見に基づき Coartem を選択し，副作用もなく非常に良好な治療経過を得られた．尚，本症例における熱帯熱マラリア原虫の *in vitro* 薬剤感受性試験では，50%増殖阻害濃度（IC_{50}）は 10nM 未満でありメフロキン感受性株であったと判断された．メフロキンの効果が不十分であった原因として，十分に薬剤が吸収されていなかった可能性が考えられた．本症例は，我が国で初めて Coartem を使用して有効な治療をおさめることが出来た輸入熱帯熱マラリアの報告例である．

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An Imported Case of Falciparum Malaria Successfully Treated with Artemether-Lumefantrine in Japan

Azumi ISHIZAKI[1], Yoshiki KIKUCHI[1], Toshimitsu HATABU[2], Shigeyuki KANO[2], Akira YASUOKA[1] & Shinichi OKA[1]
[1]AIDS Clinical Center, International Medical Center of Japan
[2]Department of Appropriate Technology Development and Transfer, Research Institute, International Medical Center of Japan
*Present address : Department of Infectious Disease, Toyama Medical Pharmaceutical University

Spread of multi-drug resistant malaria in the endemic areas has made malaria control more difficult. Thus, WHO recommends combination therapy for the treatment of malaria. The aim of combination therapy is to improve efficacy and to reduce the incidence of resistance development to the each component of the combination. Particularly, the combination with artemisinin derivatives shows good outcome in Thailand where high resistance for mefloquine has already been found. We report the first case of falciparum malaria, successfully treated with Artemether-Lumefantrine in Japan. Artemether-Lumefantrine is a newly developed artemisinin-based combination agent for the treatment of uncomplicated multi-drug resistant malaria. This drug has proved highly effective and well tolerated by some clinical trials abroad. This Japanese female case showed a good clinical course without any side effect.

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Saquinavir (SQV) soft-gel capsule (SGC) + ritonavir (RTV) と SQV hard-gel capsule + RTV および SQV-SGC 単独投与時の薬物動態の比較

[1]国立国際医療センターエイズ治療・研究開発センター，[2]中外製薬株式会社，*現在，富山医科薬科大学

土屋 亮人[1] 平林 義弘[1] 今井 和成[2] 菊池 嘉[1]
立川 夏夫[1] 源河いくみ[1] 照屋 勝治[1] 安岡 彰[1]*
岡 慎一[1] 木村 哲[1]

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要 旨

抗 human immunodeficiency virus (HIV) 療法において，HIV プロテアーゼ阻害剤である saquinavir (SQV) は，同様のプロテアーゼ阻害剤である ritonavir (RTV) との併用により，単剤に比べより高い血漿中濃度を維持することができる．SQV は hard-gel capsule (SQV-HGC) と soft-gel capsule (SQV-SGC) とが知られているが，日本人 HIV 陽性患者において RTV 併用時の詳細な血漿中濃度の検討はなされていない．そこで筆者らは，RTV 併用時の SQV-HGC 400mg，1日2回 (BID，食後服用12時間毎) 群12例 (RTV + SQV-HGC 400mg BID 群) と SQV-SGC 400mg，1日2回 (BID，食後服用12時間毎) 群4例 (RTV + SQV-SGC 400mg BID 群) の薬物動態の比較，そして日本人健康成人における SQV-SGC 1,200 mg 食後単独投与群10例 (SQV-SGC 1,200mg 群) との薬物動態の比較を行った．その結果，RTV + SQV-HGC 400mg BID 群と RTV + SQV-SGC 400mg BID 群の薬物動態の比較では，RTV + SQV-SGC 400mg BID 群が最高血漿中濃度 (C_{max})，服用8時間後までの血漿中濃度時間曲線下面積 (AUC_{0-8h}) 共にそれぞれ 14.7%，25.5% 高値であった．また，SQV-SGC1,200mg 群との薬物動態の比較では，RTV + SQV-SGC 400mg BID 群が C_{max}，AUC_{0-8h} でそれぞれ 3.9 倍，8.5 倍高値であった．これらの結果より，RTV + SQV-SGC 400mg BID 群は，RTV + SQV-HGC 400mg BID 群と同等かそれ以上の，SQV-SGC 1,200mg 群よりも高い抗ウイルス効果を有すると期待される．

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序 文

近年の抗 human immunodeficiency virus (HIV) 療法は，核酸系逆転写酵素阻害剤とプロテアーゼ阻害剤もしくは非核酸系逆転写酵素阻害剤を組み合わせる HAART (highly active anti-retroviral therapy) の導入により患者の予後が大幅に改善し，ある程度コントロール可能な疾患へと変化した[1]．しかしながら，薬剤服用スケジュールの煩雑さによる不十分なアドヒアランス，人種および個人間における薬物代謝酵素や薬物トランスポーターの活性や発現量の違いによる薬剤血漿中濃度の差等により，血漿中濃度が低値の場合には薬剤から逃れたウイルスが耐性化し，ウイルス学的失敗により治療変更を余儀なくされるケースもある[2]．

別刷請求先：(〒162-8655) 東京都新宿区戸山1—21—1
国立国際医療センターエイズ治療・研究開発センター 土屋 亮人

Saquinavir hard-gel capsule (SQV-HGC) は世界で最初に認可されたHIVプロテアーゼ阻害剤であったが，生体利用率が約4%であったため単剤での血漿中濃度の上昇が悪く，*in vitro* では優れた抗ウイルス効果が認められたにも関わらず，臨床での十分な治療効果が得られなかった[3]．その後同様のプロテアーゼ阻害剤であるritonavir (RTV) の登場以後は，RTVが肝臓の薬物代謝酵素であるチトクロムP450の酵素反応を阻害するため，RTVとSQV-HGCを組み合わせ，SQVの血漿中濃度を上昇させて高い抗ウイルス効果を得る，いわゆるRTV/SQV併用療法が多用されるようになった[4]．このRTV/SQV併用療法は優れた治療効果を収め，今日でも治療ガイドラインの第一選択薬の一つに挙げられている．その後SQVは高い生体利用率を示す薬剤として，フリー体のSQVをソフトカプセル製剤化したsaquinavir soft-gel capsule (SQV-SGC) が登場することとなった．

そこで本検討では，日本人HIV陽性患者におけるRTV/SQV併用療法でのSQV-HGCとSQV-SGCの薬物動態についての検討および日本人健康成人におけるSQV-SGC単独投与時の薬物動態との比較を行った．

対象患者および方法

対象患者は日本人HIV陽性患者で，逆転写酵素阻害剤2剤とRTV 300mgもしくは400mgを1日2回 (BID，12時間毎) 食後服用している16例について，SQV-HGC 400mgを1日2回(BID，12時間毎)食後服用している12例，SQV-SGC 400 mgを1日2回 (BID，12時間毎) 食後服用している4例の2群に分けて解析を行った．患者は服用開始2週間以上経過し，RTVおよびSQV血漿中濃度に影響を与える薬剤を服用していないことを確認した．SQV血漿中濃度測定は，薬剤服用前，服用1, 2, 3, 4, 6および8時間後にヘパリンナトリウム添加の採血管にて採血を行い，採血管を3,000 rpmで10分間遠心分離後血漿を分注し，測定開始時まで−80℃にて冷凍保存した．血漿中濃度測定は高速液体クロマトグラフィー (HPLC) 法を用い，株式会社ビー・エム・エル総合研究所にて実施した．SQV血漿中濃度測定の定量限界は0.005 μg/mlであった．血液生化学検査は測定日の薬剤服用前，測定日の血液生化学検査結果のない患者に関しては，大きな変化がない限り前後2週間以内の結果を用いた．また，日本人健康成人におけるSQV-SGC単独投与時の薬物動態については，「市販後臨床試験総括報告書フォートベイスカプセル（サキナビル）の健康成人男子における薬物動態試験」[5]を基にした．被験者はSQV-SGC 1,200 mgを食後服用した日本人健康成人男子10例で，SQV-SGC服用前，服用1, 2, 3, 4, 6および8時間後のSQV血漿中濃度測定結果を解析に用いた．また，血液生化学検査はSQV-SGC服用前の結果を用いた．SQV血漿中濃度測定結果の解析は，WinNonlin 3.1 (Pharsight，Mountain View，CA) を用いてノンコンパートメント解析法にて解析し，最高血漿中濃度 (C_{max})，最高血漿中濃度到達時間 (T_{max}) を算出した．また，服用前血漿中濃度をトラフ値とした．血漿中濃度半減期 ($T_{1/2}$) は ln (2) /λz (λzは消失半減期定数) を用い，T_{max} 以後の3採血点以上の血漿中濃度より算出した．また，血漿中濃度時間曲線下面積 (AUC) は直線台形法にて算出し，薬剤服用前から8時間後までを求めた (AUC_{0-8h})[6]．統計解析にはStatView 5.0 (SAS Institute，Cary，NC) を用い，Mann-WhitneyのU検定にて処理を行った．

成　績

対象患者の基礎データは，Table 1の通りである．RTV＋SQV-HGC 400mg BID群とRTV＋SQV-SGC 400mg BID群の2群間の年齢，体重および血液生化学検査結果において統計学的有意差は見られなかった．また，RTV＋SQV-HGC 400 mg BID群とRTV＋SQV-SGC 400mg BID群のSQV血漿中濃度は，平均トラフ値がそれぞれ1.088±1.415μg/ml，0.822±0.817μg/ml，平均C_{max}が2.939±2.128μg/ml，3.444±2.653μg/ml，平均T_{max}が3.7±1.6 h，4.5±1.7 h，平均$T_{1/2}$が4.6±2.7 h，5.4±0.6 h，平均AUC_{0-8h}が15.175±14.260 μg/ml，20.371±16.923μg/mlであった(Table 2)．この2群間のトラフ値，C_{max}，T_{max}，$T_{1/2}$およびAUC_{0-8h}において統計学的有意差は見られなかっ

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Table 1 Baseline characteristics[a]

	RTV+SQV-HGC 400mg BID	RTV+SQV-SGC 400mg BID	*p* value[b]	healthy subjects	*p* value[c]
n	12	4		10	
sex (M : F)	11 : 1	3 : 1		10 : 0	
age (year)	31.9 ± 7.4	32.5 ± 5.5	0.670	24.2 ± 1.7	0.022
weight (kg)	60.4 ± 10.1	59.5 ± 9.8	0.808	60.5 ± 5.8	0.671
GPT (U/l)	49.1 ± 28.0	42.0 ± 23.1	0.544	15.1 ± 4.7	0.005
GOT (U/l)	35.2 ± 14.7	28.8 ± 13.3	0.466	13.9 ± 1.4	0.004
ALP (U/l)	320.8 ± 88.4	280.0 ± 49.0	0.202	231.9 ± 68.0	0.203
Cr (mg/dl)	0.7 ± 0.19	0.6 ± 0.24	0.755	1.0 ± 0.11	0.004
T. Bil (mg/dl)	0.5 ± 0.25	0.4 ± 0.10	0.292	0.8 ± 0.36	0.019

[a]mean ± SD

[b]The baseline characteristics of RTV+SQV-HGC 400mg BID compared with the baseline characteristics of RTV+SQV-SGC 400mg BID.

[c]The baseline characteristics of RTV+SQV-SGC 400mg BID compared with the baseline characteristics of healthy subjects.

Table 2 Pharmacokinetic parameters of saquinavir in HIV-1-infected patients

Group	Subject No.	Plasma SQV concentration (μg/ml) Time after dose (h) 0	1	2	3	4	6	8	C_{max} (μg/ml)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{0\text{-}8h}$ (h*μg/ml)
RTV+SQV-HGC 400mg BID	1	1.403	0.321	0.950	1.009	1.227	1.122	0.719	1.403	0.0	5.2	7.785
	2	5.290	5.097	6.897	8.426	8.468	6.963	6.670	8.468	4.0	11.6	56.362
	3	1.129	1.051	0.998	1.934	3.509	2.161	2.206	3.509	4.0	6.0	16.338
	4	1.542	0.991	1.278	3.375	3.864	2.807	2.091	3.864	4.0	4.5	19.915
	5	0.516	0.528	1.311	1.933	2.393	1.456	1.114	2.393	4.0	3.6	11.646
	6	0.136	0.172	0.115	1.399	4.353	3.497	2.318	4.353	4.0	4.4	17.595
	7	0.145	0.353	0.304	0.299	0.595	0.485	0.219	0.595	4.0	2.8	3.109
	8	0.235	0.758	0.860	1.537	0.875	0.356	0.247	1.537	3.0	1.9	5.544
	9	0.355	0.275	0.235	0.241	0.313	0.960	0.506	0.960	6.0	n.d.	3.823
	10	0.669	0.690	0.777	1.182	2.299	2.428	1.371	2.428	6.0	n.d.	12.659
	11	0.403	0.712	1.946	1.649	0.917	0.791	0.467	1.946	2.0	3.0	7.933
	12	1.236	1.574	2.600	3.815	3.272	2.205	1.469	3.815	3.0	3.5	19.394
	Mean	1.088	1.043	1.523	2.233	2.674	2.103	1.616	2.939	3.7	4.6	15.175
	SD	1.415	1.337	1.835	2.219	2.284	1.815	1.761	2.128	1.6	2.7	14.260
RTV+SQV-SGC 400mg BID	13	0.624	1.025	2.917	3.202	2.940	n.t.	1.625	3.202	3.0	5.0	18.056
	14	2.024	4.757	5.844	7.080	6.139	6.246	3.641	7.080	3.0	5.9	44.035
	15	0.394	0.333	0.349	0.342	n.t.	0.726	0.574	0.726	6.0	n.d.	3.953
	16	0.245	1.037	1.570	1.742	2.143	2.768	2.219	2.768	6.0	n.d.	15.441
	Mean	0.822	1.788	2.670	3.092	3.741	3.247	2.015	3.444	4.5	5.4	20.371
	SD	0.817	2.007	2.362	2.904	2.115	2.791	1.280	2.653	1.7	0.6	16.923

n.t., not tested ; n.d., not determined ; C_{max}, maximum plasma concentration ; T_{max}, time to maximum plasma concentration ; $T_{1/2}$, plasma elimination half-life ; $AUC_{0\text{-}8h}$, area under the plasma concentration-time curve from 0 to 8 hour.

た．この2群のSQV血漿中濃度はFig. 1に示した．

日本人健康成人SQV-SGC 1,200mg単独投与群のSQV血漿中濃度は，平均C_{max}が0.875±0.295 μg/ml，平均T_{max}が2.2±0.4 h，平均$T_{1/2}$が1.6±0.5 h，平均$AUC_{0\text{-}8h}$が2.403±0.855μg/mlであった（Table 3）．この群のSQV血漿中濃度はFig. 2に示した．RTV+SQV-SGC 400mg BID群

Fig. 1 Time course of mean (+S.D.) saquinavir plasma concentration. ■ ; RTV + SQV-HGC 400 mg twice daily (BID) in HIV-1-infected patients (n = 12). ● ; RTV + SQV-SGC 400 mg twice daily (BID) in HIV-1-infected patients (n = 4).

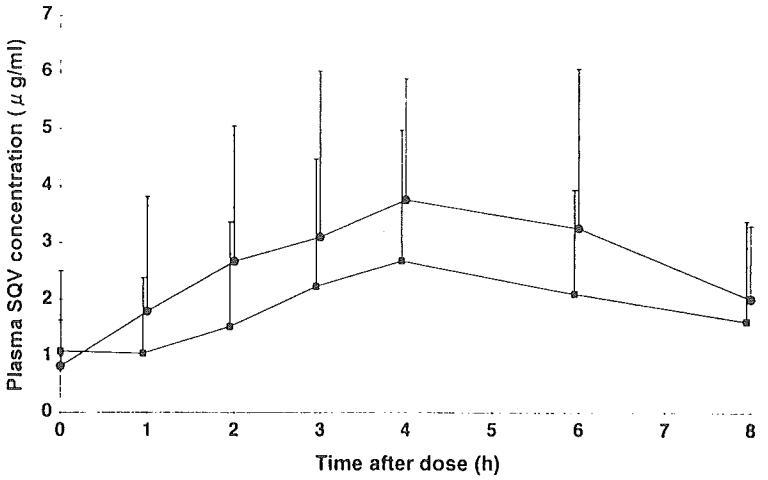


Table 3 Pharmacokinetic parameters of saquinavir soft-gel capsule in healthy subjects

Subject No.	Plasma SQV concentration (μg/ml) Time after dose (h)							C_{max} (μg/ml)	T_{max} (h)	$T_{1/2}$ (h)	AUC_{0-8h} (h*μg/ml)
	0	1	2	3	4	6	8				
1	0.000	0.288	0.843	0.856	0.362	0.066	0.049	0.856	3.0	1.2	2.710
2	0.000	0.064	0.812	1.143	0.707	0.138	0.073	1.143	3.0	1.2	3.428
3	0.000	0.460	0.539	0.090	0.053	0.028	0.022	0.539	2.0	2.5	1.247
4	0.000	0.320	0.870	0.774	0.671	0.130	0.065	0.870	2.0	1.3	3.295
5	0.000	0.822	1.500	0.555	0.186	0.088	0.058	1.500	2.0	2.4	3.389
6	0.000	0.355	0.793	0.368	0.144	0.030	0.021	0.793	2.0	1.1	1.813
7	0.000	0.394	1.012	0.748	0.315	0.063	0.049	1.012	2.0	1.2	2.801
8	0.000	0.438	0.683	0.172	0.065	0.028	0.024	0.683	2.0	1.8	1.471
9	0.000	0.113	0.497	0.445	0.169	0.047	0.022	0.497	2.0	1.2	1.424
10	0.000	0.661	0.857	0.370	0.221	0.088	0.057	0.857	2.0	1.8	2.454
Mean	0.000	0.392	0.841	0.552	0.289	0.071	0.044	0.875	2.2	1.6	2.403
SD	0.000	0.228	0.279	0.328	0.232	0.040	0.020	0.295	0.4	0.5	0.855

C_{max}, maximum plasma concentration ; T_{max}, time to maximum plasma concentration ; $T_{1/2}$, plasma elimination half-life ; AUC_{0-8h}, area under the plasma concentration-time curve from 0 to 8 hour.

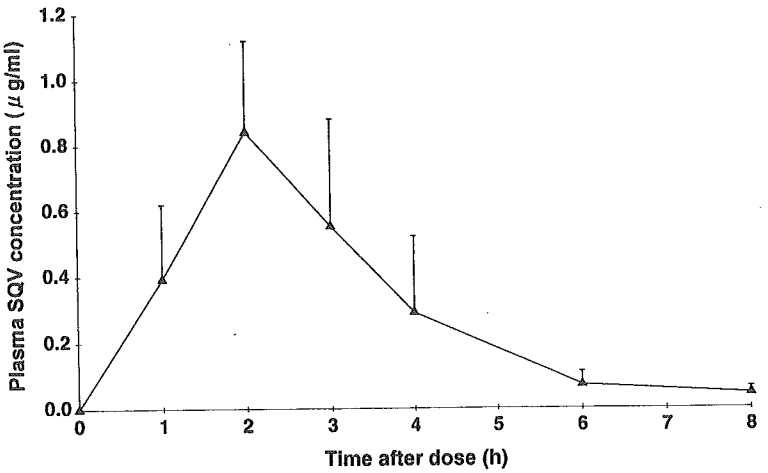
との統計学的解析を行ったところ，年齢 ($p<0.05$)，GPT 値 ($p<0.01$)，GOT 値 ($p<0.01$)，Cr 値 ($p<0.01$) および T. bil 値 ($p<0.05$) において有意差が見られた．また T_{max} ($p<0.01$)，$T_{1/2}$ ($p<0.05$) および AUC_{0-8h} ($p<0.01$) においても有意差が見られた．

考　察

HIV プロテアーゼ阻害剤の血漿中濃度は，肝臓の薬物代謝酵素であるチトクロム P450 による代謝や小腸の薬物トランスポーターである P-糖蛋白質の排出等の影響を受けることが知られているが[7]，それらの活性や発現には人種差があることが知られている[8][9]．したがって，欧米人と日本人

平成15年6月20日

Fig. 2 Time course of mean (+S.D.) saquinavir plasma concentration. SQV-SGC 1,200 mg single dose in healthy subjects (n=10).



における血漿中濃度にも差異があることが予想され，日本人におけるプロテアーゼ阻害剤の血漿中濃度を解析することは極めて意味深い．

今回検討したRTV+SQV-HGC 400mg BID群とRTV+SQV-SGC 400mg BID群の薬物動態は，C_{max}，AUC_{0-8h}の比較から，統計学的有意差はなかったもののRTV+SQV-SGC 400mg BID群がそれぞれ14.7%，25.5%高値であった．またT_{max}，$T_{1/2}$の延長傾向も認められた．これらの結果より，SQV-SGCはRTVとの併用においても生体利用率が高く，RTV+SQV-HGC 400mg BID群と同等かもしくはそれ以上の抗ウイルス効果が得られる血漿中濃度を維持されることが確認された．また，SQV-SGC 1,200mg群との比較では，RTV+SQV-SGC 400mg BID群と，GPT値，GOT値，Cr値およびT. bil値において統計学的有意差が見られたが，いずれも正常値に近いものであり，薬剤血漿中濃度に影響したとは考えにくい．また，年齢もSQV-SGC 1,200mg群が統計学的には有意に低かったが，実際にはやはりこの程度の差が薬剤血漿中濃度に影響したとは考えにくい．しかし今後は，健康成人コントロールの年齢もHIV陽性患者に揃えるべきであろう．薬物動態での比較では，反復投与と単回投与とでは単純に比較はできないが，C_{max}，AUC_{0-8h}でそれぞれ3.9倍，8.5倍RTV+SQV-SGC 400mg BID群が高値であった．また，T_{max}，$T_{1/2}$でも大幅な延長傾向が認められ，統計学的有意差(それぞれ$p<0.05$，$p<0.01$)が得られた．AUC_{0-8h}の比較でも統計学的有意差($p<0.01$)が得られた結果から，SQV-SGCはRTVとの併用により高い血漿中濃度が維持され，単剤よりも優れた抗ウイルス効果を得られることが期待される．

欧米におけるRTV400mg+SQV-SGC 400mg BID群の薬物動態は，平均トラフ値が0.84μg/ml，平均C_{max}が2.92μg/ml，平均AUC_{0-24h}が30.92μg/mlとの報告[10]があるが，今回の結果と比較すると平均トラフ値はほぼ同等であったのに対し，平均C_{max}は1.2倍高値であった．また，AUCも同時間値のデータではないが，今回の結果の方が高値であることが予想される．おそらくこの差は日本人と欧米人との人種差および体重差等により生じたものと考えられる．今後日本人における最適な服薬量を考えていく上でも，多くの血漿中濃度測定データの蓄積および解析が望まれる．また，抗ウイルス効果の面より平均トラフ値とSQVの*in vitro*での50%増殖阻害濃度(IC50) 0.023μg/ml[11]を比較したところ，RTV+SQV-SGC 400mg BID群は35.7倍高値であった．つまりRTV+SQV-SGC 400mg BID群は，平均トラフ値からも十分な抗ウイルス効果が得られるSQV血漿中濃度を維持し

ていることが示された.

RTV/SQV 併用療法は,今後も抗 HIV 療法においてより強い治療効果を得たい場合,例えば前治療の失敗におけるサルベージ療法などに多用されていくと考えられるが,今回の結果より SQV-SGC は SQV-HGC よりも,また単剤よりも高い抗ウイルス効果が期待でき,より良い治療効果が得られるものと考えられる.

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平成15年6月20日

Comparison of Pharmacokinetics of Saquinavir Soft-gel Capsule (SQV-SGC) Combined with Ritonavir (RTV), SQV Hard-gel Capsule with RTV, and SQV-SGC Alone

Kiyoto TSUCHIYA[1], Yoshihiro HIRABAYASHI[1], Kazunari IMAI[2], Yoshimi KIKUCHI[1], Natsuo TACHIKAWA[1], Ikumi GENKA[1], Katsuji TERUYA[1], Akira YASUOKA[1]*, Shinichi OKA[1] & Satoshi KIMURA[1]

[1]AIDS Clinical Center, International Medical Center of Japan, [2]Chugai Pharmaceutical Co., LTD

*Current Address : Faculty of Medicine, Toyama Medical and Pharmaceutical University

Saquinavir (SQV) is a human immunodeficiency virus (HIV) specific protease inhibitor. When combined with ritonavir (RTV), plasma concentration of SQV is increased. In this study, we examined pharmacokinetics of SQV soft-gel capsule (SQV-SGC) 400 mg twice daily (BID) combined with RTV in HIV-1-infected patients (n = 4) and compared with those of SQV hard-gel capsule (SQV-HGC) 400 mg BID combined with RTV (n = 12). Pharmacokinetics of SQV-SGC 1,200mg single dose in healthy subjects (n = 10) were also studied. Peak SQV concentration in plasma (C_{max}) and area under the plasma concentration-time curve from 0 to 8 hour (AUC_{0-8h}) of SQV-SGC 400 mg BID combined with RTV group were higher than those of SQV-HGC 400 mg BID combined with RTV group ; increase of 14.7% and 25.5%, respectively. C_{max} and AUC_{0-8h} of SQV-SGC were higher than SQV-SGC 1,200 mg single dose group ; increase of 3.9 fold and 8.5 fold, respectively.

These results indicated that SQV-SGC combined with RTV therapy is the most potent antiviral effect among SQV-SGC with RTV, SQV-HGC with RTV, and SQV-SGC alone.

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Research Article

Environmental isolation of *Cryptococcus neoformans* from an endemic region of HIV-associated cryptococcosis in Thailand

Misuzu Kuroki[1], Cheseda Phichaichumpon[2], Akira Yasuoka[3], Piphat Chiranairadul[4], Toru Chosa[2], Petchsri Sirinirund[5], Taiga Miyazaki[1], Hiroshi Kakeya[1], Yasuhito Higashiyama[1], Yoshitsugu Miyazaki[1], Yutaka Ishida[6] and Shigeru Kohno[1]*

[1] *The Second Department of Internal Medicine Nagasaki University School of Medicine, Nagasaki, Japan*
[2] *Chiang Muan Hospital, Chiang Muan, Thailand*
[3] *International Medical Center of Japan, Tokyo, Japan*
[4] *Medical Department of Phayao Hospital, Phayao, Thailand*
[5] *Provincial Health Office, Phayao, Thailand*
[6] *The Japan International Cooperation Agency, Tokyo, Japan*

*Correspondence to:
Shigeru Kohno, The Second Department of Internal Medicine, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan.
E-mail:
s-kohno@net.nagasaki-u.ac.jp

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Abstract

We successfully isolated *Cryptococcus neoformans* from chicken faeces in suburban areas of Thailand. *C. neoformans* was isolated from 36/150 houses (24.0%) in the dry season and 6/150 (4.0%) in the rainy season. All environmental isolates were of serotype A. The high isolation rate of 24% from chicken faeces has never been reported previously. Our environmental study could probably explain the high incidence of cryptococcal meningitis in HIV patients in Thailand. Copyright © 2004 John Wiley & Sons, Ltd.

Keywords: ***Cryptococcus neoformans*; chicken faeces; Phayao; Thailand; HIV; AIDS; cryptococcal meningitis; isolation**

Introduction

Cryptococcal meningitis caused by *Cryptococcus neoformans* is one of the most serious opportunistic infections in patients with acquired immunodeficiency syndrome (AIDS), and also the gravest complication with respect to prognosis, especially in developing countries. A higher incidence of cryptococcal meningitis (18.5%) in patients with human immunodeficiency virus (HIV) infection has been described in Thailand compared to other developed countries. Especially, the percentage of AIDS and cryptococcosis in HIV-infected patients reported from the northern area of Thailand is larger than that from other areas (Suwat *et al.*, 2001).

C. neoformans is known to inhabit natural environments such as soil and grows in bird excreta, especially that of pigeons (Ajello, 1958; Denton and DiSalvo, 1968; Yamamoto *et al.*, 1995a, 1995b). *C. neoformans* has rarely been isolated from fruits, eucalyptus trees or other natural sources. The fungus spreads in the air and infects humans through inhalation. Although environmental studies have been performed, the source of *C. neoformans* in endemic areas of northern Thailand has never been elucidated.

As there is an office of the Japan International Cooperation Agency (JICA) in Phayao province, in northern Thailand, we were able to benefit from their cooperation. In Phayao province people breed many chickens under stilt houses and are exposed to potent aerosols of chicken faeces. To identify the source of *C. neoformans* in patients with cryptococcal meningitis, we focused on the layers of chicken faeces which are piled up under most houses or in the backyards of residential areas in Phayao province in which we could not find any pigeons, and determined whether they contained *C.*

neoformans. Chiang Muan, Pon and Chiang Kham districts in Phayao were chosen for collection of faeces because the JICA office in Phayao were able to negotiate with medical staff there. We expected a higher isolation rate of *C. neoformans* from the dry faeces that can be collected in sunny periods. To our knowledge, there have been no studies comparing *C. neoformans* isolation rates in the dry and rainy seasons. In the present study, we compared the number of fungi that could be isolated from the chicken excreta in the dry month of March and the rainy month of December. Figure 1 shows climatic data for Chiang Rai in northern Thailand.

Materials and methods

Approximately 3.0–6.0 g weathered chicken excreta was added to 20 ml sterilized saline. The samples were allowed to soak for more than 1 h with frequent vortexing. The solution was filtered using sterilized nylon mesh with a pore size of 292 ± 10 μm (mean ± SEM) and was centrifuged at 3000 rpm for 10 min. The precipitates were inoculated onto birdseed agar plate, pH 6.8 ± 0.2 (BBL, MD). All plates were incubated at 30 °C for 2–6 days and a brown colony was streaked onto a Sabouraud dextrose agar, pH 5.6 ± 0.2 (Yamamoto *et al.*, 1995b). All isolates were identified as *C. neoformans* by the Auxacolor system (Sanofi Diagnostics, Pasteur). Serotypes were confirmed by the Crypto check system (Iatron, Tokyo, Japan) (Ikeda *et al.*, 1982; Kabasawa *et al.*, 1991).

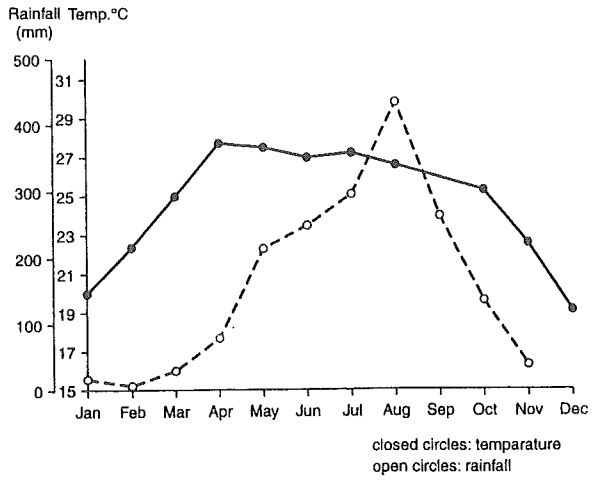

Figure 1. Climatic data for Chiang Rai in northern Thailand

Results

We collected chicken faeces from 90 dwellings in the Chiang Muan district, 30 in the Pon district and 30 in the Chiang Kham district, from under or around the private houses. In addition, 16 *C. neoformans* isolates from the cerebrospinal fluid of HIV patients were provided by Chiang Kham Hospital. Differences between groups were examined for statistical significance using the Fisher exact test. A p-value of <0.05 denoted the presence of a statistically significant difference.

C. neoformans was isolated from 36/150 (24.0%) houses in the dry season and 6/150 (4.0%) in the rainy season. All environmental isolates were serotype A (Table 1). Similarly, all strains from patients were of type A.

Discussion

Cryptococcosis is a deep-seated mycosis that can be classified roughly into three types of diseases; pulmonary cryptococcosis, cryptococcal meningitis and disseminated cryptococcosis. Cryptococcal meningitis is a fatal infection in patients with AIDS. As mentioned above, *C. neoformans* is prevalent in the natural environment, especially in the weathered excreta of birds. A high prevalence of *C. neoformans* in pigeon excreta has been well documented by numerous investigators in several parts of the world (Ajello, 1958; Denton *et al.*, 1968; Yamamoto *et al.*, 1995a, 1995b). Ajello (1958) isolated *C. neoformans* from both pigeon and chicken habitats. However, there are only a few reports of isolation of this organism from chicken habitats, and the isolation rate of *C. neoformans* from such environments is quite low. Walter and Yee (1968) attributed the growth inhibitory effect of chicken droppings on *C. neoformans* to the presence of a high molecular growth

Table 1. Incidence of *C. neoformans* isolated from chicken excreta

	Houses	Villages	Serotype
Dry season	36/150 (24%)*	24/50 (48%)*	All type A
Rainy season	6/150 (4%)	6/50 (12%)	All type A

Data are number of isolates/total number examined (percentage).
* $p < 0.05$, compared with the rainy season.

inhibitory substance and the high alkalinity of the droppings, which may explain the failure of isolation of the organism from chicken excreta. Also, Kielstein (1996) showed that the increase of pH is not regarded as responsible for the survival of *C. neoformans* in the birds' droppings.

In the Phayao province of northern Thailand, most people breed chickens in their yards. In the present study, we cultured aged and well-weathered faeces because birds faeces are not a primary source of *C. neoformans* (Monga *et al.*, 1971; Littman and Walter, 1968). We succeeded in isolating *C. neoformans* from Phayao chicken excreta with a high isolation rate of 24% (36/150 houses) and showed an even higher isolation rate of 48% in 24/50 villages. Once *C. neoformans* is detected from one house, it is likely that there is *C. neoformans* in faeces from other houses in the same village. The isolation of *C. neoformans* is difficult because its growth on media may be affected by a variety of biotic factors, such as soil bacteria, amoebae, mites or sow bugs that are capable of inhibiting or killing the fungi. The weather in Thailand is considered appropriate for the growth of *C. neoformans*, with a mean temperature of 26–30 °C, and differs in rainy and dry seasons (Figure 1). Several studies have reported seasonal variation in isolation of *C. neoformans* from patients with AIDS (Bell *et al.*, 2001; Bogaerts *et al.*, 1999; Sorvillo *et al.*, 1997). According to these reports, cryptococcosis in patients with AIDS is predominant in the rainy season, although our data showed the counterview. In cases where a patient inhales a lot of the pathogen in the dry season, the infection may become manifest after about a 6 month latency period. Thus, it is conceivable that the seasonal predominance of *C. neoformans* isolation is an important factor in cryptococcal infection. On the other hand, seasonal differences in the isolation rates do not necessarily account for the latency period of cryptococcosis. Considering a report of years-long latency, however, the most important factor that affects cryptococcal sideration is the host immune status. Endogenous reactivation of cryptococcosis, like tuberculosis, is also possible (Dromer *et al.*, 1992; Blasi *et al.*, 2001).

Generally, there is a high incidence of *C. neoformans* serotype B infection among the non-AIDS population in Thailand (Kwon-Chung and Bennett, 1984). However, our results showed that CSF isolates from AIDS patients were all of serotype A. These findings suggest that *C. neoformans* in the natural environment could cause cryptococcal meningitis. Yamamoto *et al.* (1995a) used strain-typing analysis by random amplified polymorphic DNA (RAPD) to confirm the genetic correlation between environmental isolates of *C. neoformans* and clinical isolates from patients with pulmonary cryptococcosis. In this regard, strain-typing studies are essential to determine the relationship between clinical and environmental isolates. Once the origin of infection is confirmed, this information could be useful for prevention of cryptococcal meningitis in such endemic areas as Phayao. In this study, although we tried to examine the relationship between environmental and clinical isolates of *C. neoformans* in Phayao province using RAPD analysis, RAPD did not reveal any clear relationships due to its poor resolution.

Our ultimate goal is to reduce the incidence of fatal cryptococcal infections. Further studies are needed to confirm our results in order to establish the importance of simple methods to prevent infection, such as effective removal or elimination of dry piled faeces.

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