C64T is extremely rare, which is why there has been no data describing the EFV plasma concentrations in homozygous C64T patients [15]. A limitation of this study is that we were not able to measure the EFV concentration. But the patient's CNS symptoms, such as insomnia and nightmare, strongly suggested a high EFV concentration in the plasma. Because the symptoms disappeared immediately after discontinuing EFV, even though the EFV concentration in C64T heterozygous is reported to be same as in wild type, homozygous C64T possibly has some differences in catalytic defects. Further studies are required concerning CYP2B6 genetic variants in Asians and the enzymatic activity of homozygous CYP2B6 C64T.

References

1 Marzolini C, Telenti A, Decosterd LA, Greub G, Biollaz J, Buclin T. Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. *Aids* 2001;**15**:71-5

2 Csajka C, Marzolini C, Fattinger K, *et al.* Population pharmacokinetics and effects of efavirenz in patients with human immunodeficiency virus infection. *Clin Pharmacol Ther* 2003;**73**:20-30

3 Burger D, van der Heiden I, la Porte C, *et al.* Interpatient variability in the pharmacokinetics of the HIV non-nucleoside reverse transcriptase inhibitor efavirenz: the effect of gender, race, and CYP2B6 polymorphism. *Br J Clin Pharmacol* 2006;**61**:148-54

4 Chen H, Chen W, Gan LS, Mutlib AE. Metabolism of (S)-5,6-difluoro-4-cyclopropylethynyl-4-trifluoromethyl-3, 4-dihydro-2(1H)-quinazolinone, a non-nucleoside reverse transcriptase inhibitor, in human liver microsomes. Metabolic activation and enzyme kinetics. *Drug Metab Dispos* 2003;**31**:122-32

5 Ward BA, Gorski JC, Jones DR, Hall SD, Flockhart DA, Desta Z. The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. *J Pharmacol Exp Ther* 2003;**306**:287-300

6 Rotger M, Colombo S, Furrer H, *et al.* Influence of CYP2B6 polymorphism on plasma and intracellular concentrations and toxicity of efavirenz and nevirapine in HIV-infected patients. *Pharmacogenet Genomics* 2005;**15**:1-5

7 Mathiesen S, Justesen US, Von Luttichau HR, Hansen AB. Genotyping of CYP2B6 and therapeutic drug monitoring in an HIV-infected patient with high efavirenz plasma concentrations and severe CNS side-effects. *Scand J Infect Dis* 2006;**38**:733-5

8 Ribaudo HJ, Haas DW, Tierney C, *et al.* Pharmacogenetics of plasma efavirenz exposure after treatment discontinuation: an Adult AIDS Clinical Trials Group Study. *Clin Infect Dis* 2006;**42**:401-7

9 Wang J, Sonnerborg A, Rane A, *et al.* Identification of a novel specific

CYP2B6 allele in Africans causing impaired metabolism of the HIV drug efavirenz. *Pharmacogenet Genomics* 2006;**16**:191-8

10 Klein K, Lang T, Saussele T, *et al.* Genetic variability of CYP2B6 in populations of African and Asian origin: allele frequencies, novel functional variants, and possible implications for anti-HIV therapy with efavirenz. *Pharmacogenet Genomics* 2005;**15**:861-73

11 Guan S, Huang M, Li X, Chen X, Chan E, Zhou SF. Intra- and Inter-ethnic Differences in the Allele Frequencies of Cytochrome P450 2B6 Gene in Chinese. *Pharm Res* 2006;**23**:1983-90

12 Guan S, Huang M, Chan E, Chen X, Duan W, Zhou SF. Genetic polymorphisms of cytochrome P450 2B6 gene in Han Chinese. *Eur J Pharm Sci* 2006;**29**:14-21

13 Hiratsuka M, Hinai Y, Konno Y, Nozawa H, Konno S, Mizugaki M. Three novel single nucleotide polymorphisms (SNPs) of the CYP2B6 gene in Japanese individuals. *Drug Metab Pharmacokinet* 2004;**19**:155-8

14 Hiratsuka M, Takekuma Y, Endo N, *et al.* Allele and genotype frequencies of

CYP2B6 and CYP3A5 in the Japanese population. *Eur J Clin Pharmacol* 2002;**58**:417-21

Tsuchiya K, Gatanaga H, Tachikawa N, *et al.* Homozygous CYP2B6 *6 (Q172H and K262R) correlates with high plasma efavirenz concentrations in HIV-1 patients treated with standard efavirenz-containing regimens. *Biochem Biophys Res Commun* 2004;**319**:1322-6

ウイルス感染とバイオディフェンス

－注目される補中益気湯の可能性－

監修・取材先
服部 俊夫 先生　東北大学大学院医学系研究科感染症・呼吸器病態学 教授
取材先
巽 浩一郎 先生　千葉大学大学院医学研究院加齢呼吸器病態制御学 助教授
資料協力先
岩垣 博巳 先生　岡山大学医学部・歯学部附属病院消化管外科 講師
取材・構成
佐久間光江（医学レポーター）

HIV、SARS、鳥インフルエンザ。医学が進歩してもなお、人類はウイルス感染の脅威にさらされ続けている。SARSについては一応の落ち着きをみたが、HIV、そしてインフルエンザのパンデミックに対して、果たして人類は勝利することができるのだろうか。

微生物との緊張感ある闘いのなかで、効果的なバイオディフェンス製剤を求めてさまざまなアプローチが試みられている。既存の薬剤や植物成分の見直しもその一つである。そのなかで、漢方薬も有効性が期待されるものとして研究対象になっている。

ここでは、かねてより免疫賦活作用が報告されている補中益気湯（TJ-41）を中心に、バイオディフェンスの視点で漢方薬の位置付けを考える。

今回、東北大学大学院感染症・呼吸器病態学の服部俊夫氏、千葉大学大学院加齢呼吸器病態制御学の巽浩一郎氏にお話を伺い、岡山大学医学部・歯学部附属病院消化管外科の岩垣博巳氏に資料協力をいただいた。

「Open Sesame」－開けゴマ－ ウイルスエントリーの呪文が破られるとき

今年（2006年）、Cell、JAMAに相次いでウイルス学の注目すべき論文が掲載された。Cell掲載の「Virus Entry: Open Sesame」というタイトルを冠した論文[1]は、ウイルス感染経路について最新の知見をレビューしたもので、標的細胞表面にあるウイルス受容体とウイルス膜との結合、ウイルスの刺激による細胞内シグナリング、エンドサイトーシスによるウイルス侵入経路などが明快に解説されている（図1）。ちなみに、インフルエンザウイルスはこの図が示すB、風邪の原因ウイ

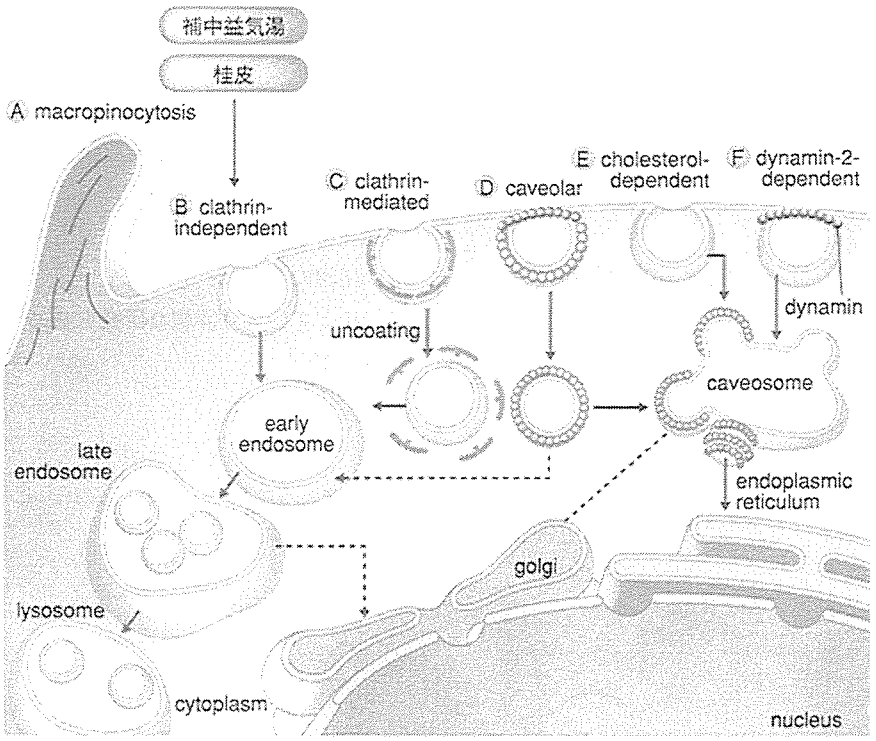


図1　ウイルス進入経路 文献1より引用、改変

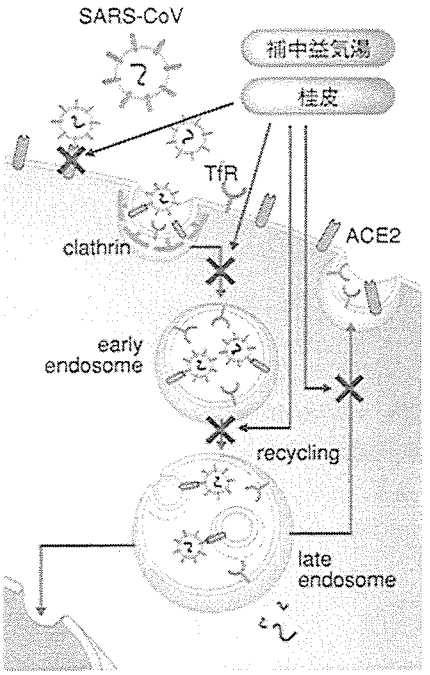


図2　生薬によるエンドサイトーシスの調節の可能性

ルスで最も多いとされるコロナウイルスは主にDの経路で細胞内に侵入すると考えられている。

こうしたウイルスエントリー、すなわちウイルスが細胞内に侵入する経路の解明は、エントリーインヒビターという新しいアプローチの抗ウイルス薬開発には欠かせない。例えば、HIV感染はウイルス表面のスパイク蛋白gp120と標的細胞表面の受容体CD4が結合し、gp120-CD4複合体がさらに細胞表面のケモカイン受容体と結合することでウイルス膜と標的細胞膜との融合が起こる。この融合を、ケモカイン受容体をブロックすることで阻害しようという設計で、いくつかの薬剤が開発段階にあり、そのうち一つはすでに上梓に至っている。ウイルスエントリー経路の詳細な分析は、ウイルスインヒビターのターゲットをより細かく絞り込み、特異的な阻害作用を得ることになる。

服部氏は、これまで一貫してウイルスエントリー阻害による抗ウイルス作用に焦点を当てて、さまざまな成分について精力的な研究を進めてきた。検討した成分には、感染防御作用が指摘される漢方薬の構成生薬や、HIV感染患者に対する臨床効果が報告されているアフリカ原産の植物エキスなども含まれている。また、技術面でもウイルスエントリーを高感度に測定する実験系も確立している。これはルシフェラーゼ遺伝子を組み込んだレポーター遺伝子にウイルスの外膜糖蛋白遺伝子を導入して外膜のスパイク蛋白を発現する疑似ウイルスを使うもので、この実験系ではルシフェラーゼ活性を測定することでウイルスエントリーを詳細に評価することができる。

こうした研究アプローチによって服部氏はこれまでに、漢方薬の補中益気湯や生薬の桂皮などがウイルス感染の抑制効果を示すこと、なかでも桂皮および丁子エキスがHIV/SARS疑似ウイ

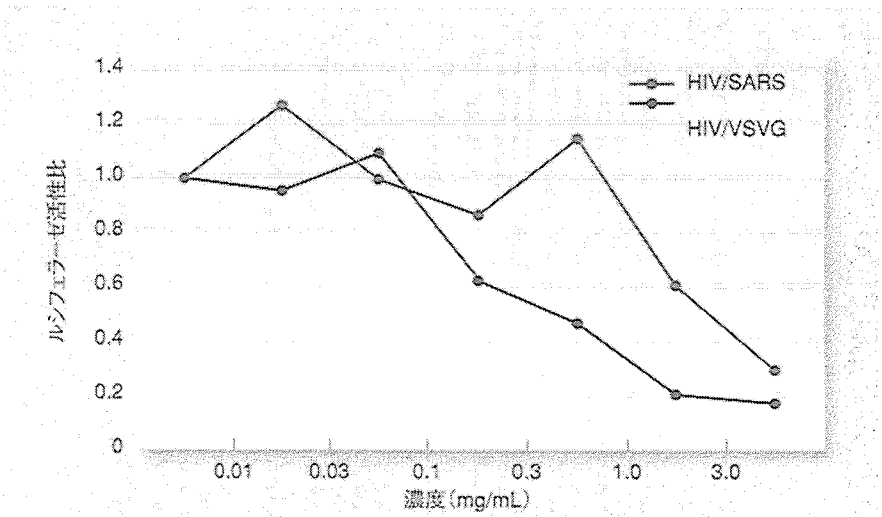


図3　HIV/SARS疑似ウイルスに対する補中益気湯の抑制効果

補中益気湯はHIV/SARSおよびHIV/VSVGを用量依存的に抑制した。(庄 敏・服部俊夫)文献2より引用

ルス感染に対し濃度依存性に抑制効果を示すことを明らかにしている[2)]。

特に桂皮エキスの抑制効果は大きく、多種類のウイルス感染に対し阻害作用を示すことから、桂皮エキスは「ウイルス感染の共通部分に作用している可能性」が考えられる。そこで服部氏は、桂皮成分がエンドサイトーシスの諸相に抑制的に作用しているとの仮説を立て、より詳細な研究を進めている(**図2**)。

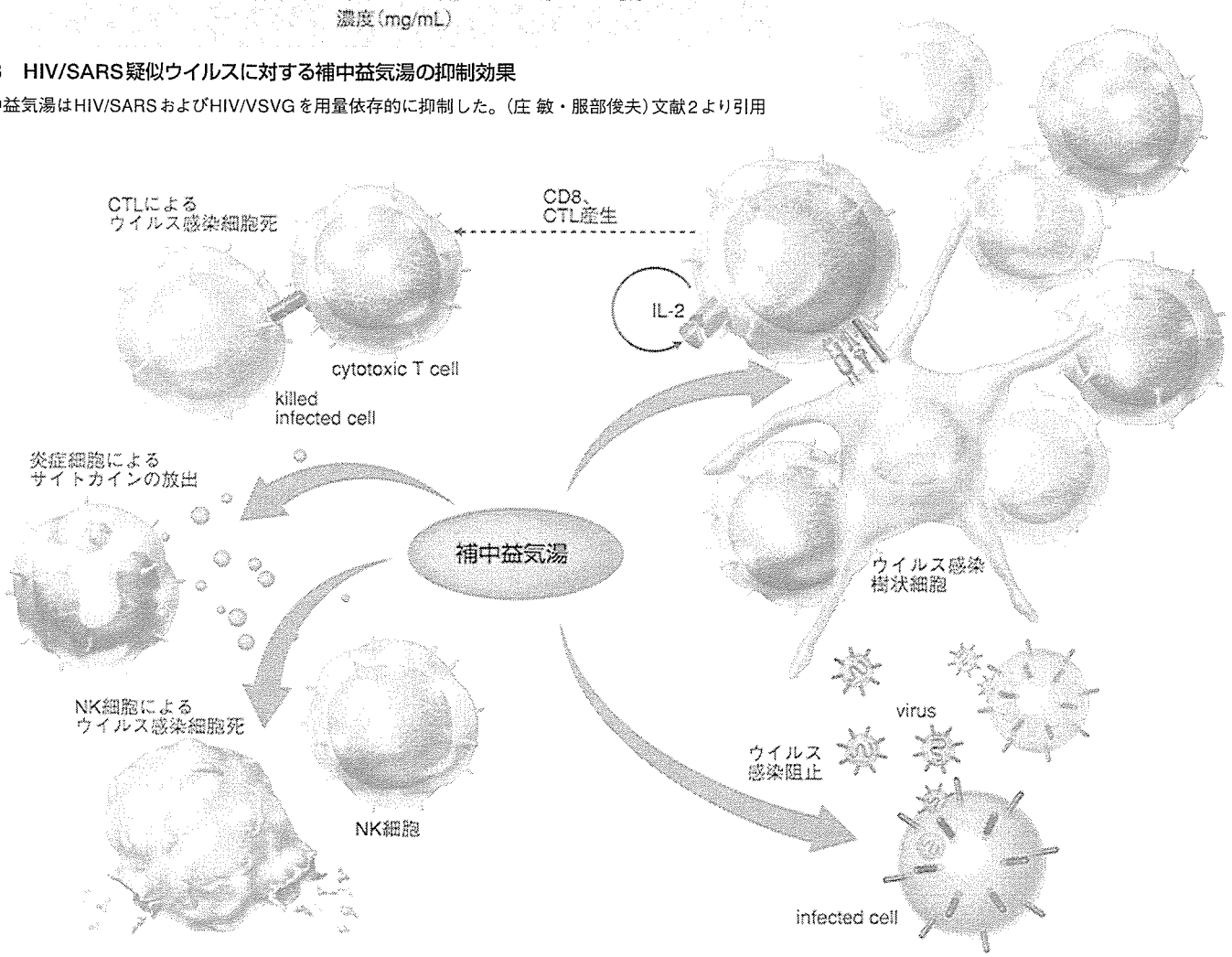


イラスト　補中益気湯の作用機序(監修：服部俊夫)

さらに同様の実験系で補中益気湯についても検討を行ない、桂皮と同様のウイルス感染抑制効果を補中益気湯に認めている（図3）。これについて服部氏は補中益気湯の構成成分である柴胡・黄蓍が特に抗ウイルス作用に関与している可能性を指摘している。

「ウイルス量はHIV感染予後因子にあらず」の衝撃

もう一つの論文は、今年のJAMA 9月27日号に載った「Predictive Value of Plasma HIV RNA Level on Rate of CD4 T-Cell Decline in Untreated HIV Infection」である[3]。これは、1984-2004年間に行なわれた約3000人対象のコホートスタディで明らかになった、HIV感染者の未治療段階での血清ウイルス量はその後のAIDS発症の予後予測因子にはならない、という解析結果である。この論文に対し、かつてHIV感染者のウイルス量がAIDS発症と生命予後を規定するとの論文を掲載したScienceが9月29日号でコメントを発表したのをみても、その論文の衝撃の大きさがうかがえる。JAMA掲載の論文の考察では、CD4T細胞の減少を規定するのはウイルス量ではなく他の因子-まだ確定はできないがおそらくは長期間の炎症持続状態の関与を示唆している。

服部氏はこの論文について「ウイルス量と予後は必ずしも一致しないということは、多くの臨床家が感じていたことで、その間を埋める因子として、全身性炎症の持続が指摘されたことになる」と評価。慢性ウイルス感染におけるバイオディフェンスを考えるうえで、炎症反応という新たなターゲットが提示されたのである。

全身性炎症抑制作用を補中益気湯で確認

全身性炎症の持続については、一見ウイルス感染とは関係のない領域でも興味深い報告がある。

その一つが、慢性閉塞性肺疾患（COPD）を全身性炎症としてとらえ、その対策として補中益気湯の有用性を検討した千葉大学の巽氏の研究である。

かつてのCOPDの疾患概念は、気道炎症と肺胞破壊という局所病変を背景にした、進行性で非可逆性の一秒量低下を病態の本質としてとらえるものだった。しかし、2004年にCelliにより、%FEV1・BMI・運動能力・運動時の息切れの4項目を指標としたBODE indexがCOPD患者の予後予測因子となることが報告[4]された頃から、COPDは全身性疾患として認識されるようになり、特に栄養状態と予後の関係が注目されるようになってきた。

巽氏は、COPD患者では気道炎症反応で生じた炎症性サイトカインが他組織におよび、全身性の炎症反応を惹

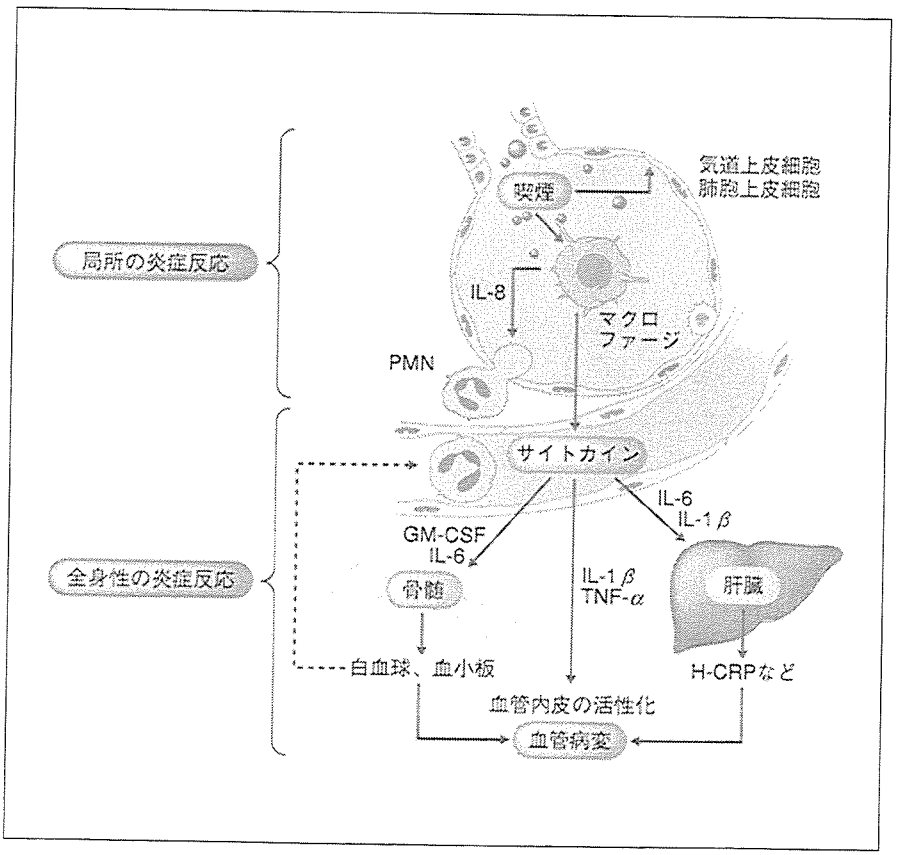


図4　COPDと全身性炎症

起しているとの考えに立ち(**図4**)、呼吸器疾患症状(SGRQのSymptom score)、漢方医学的な体調評価(気虚スコア)、感冒回数、急性増悪回数、炎症指標[5](高感度CRP、TNF-α、IL-6)、栄養指標(プレアルブミン、レプチン)、動脈硬化指標(高分子量アディポネクチン)を評価項目として、COPD患者に対する補中益気湯の効果を調べた。対象はCOPD症例35例で、うち17例に補中益気湯を投与し、18例を対照群とした。その結果、補中益気湯投与群ではSGRQのSymptom scoreおよび気虚スコアが改善し、感冒罹患頻度、急性増悪頻度が抑制され(**図5**)、高感度CRPおよびTNF-α値の低下が認められた。また、%一秒量と各種炎症指標とは負の相関がみられ、COPDが重症化するほど全身性炎症反応が強まっていることが示唆された。こうした結果は、COPDの病態は栄養状態および全身性炎症反応と深く関与し、補中益気湯はCOPDの栄養状態を改善し、全身性炎症反応を抑制している可能性を示唆するものといえる。

また、動脈硬化指標のアディポネクチン値も補中益気湯群で改善が認められた。これについて巽氏は、「近年、動脈硬化を血管壁の炎症反応としてとらえるようになり、また欧米ではCOPD患者に心血管イベントの発症頻度が高いという指摘がある。そういう意味でもCOPDを全身性炎症反応とする考え方は示唆に富んでいる」と、炎症反応に関与している可能性を指摘した。

過剰な炎症を抑え、免疫抑制を是正

このように、全身性炎症反応の抑制効果が指摘された補中益気湯だが、その一方で従来からNK細胞の活性化やCTL免疫誘導といった免疫賦活作用も知られている(**図6**)。

ここで一つの疑問が生じる。一般に免疫応答は炎症反応をともなうものである。果たして補中益気湯の抗炎症作用と免疫賦活作用は両立するものなのだろうか。

これについては、岡山大学の岩垣氏の周術期患者に補中益気湯を投与したデータが一つの答えを示している。進行胃・大腸癌患者を対象とし、補中益気湯の術前投与が術後の全身性炎症反応症候群(SIRS)とそれに引き続き起こる代償性抗炎症反応症候群(CARS)への影響をみたもので、これによると、補中益気湯群では手術後の体温上昇が非投与群に対し抑えられ、術後感染症併発率(術後予防投与に用いた抗菌薬とは異なる抗菌薬を術後2週間以内に投与した症例を術後感染症併発と定義)も補中益気湯群で有意

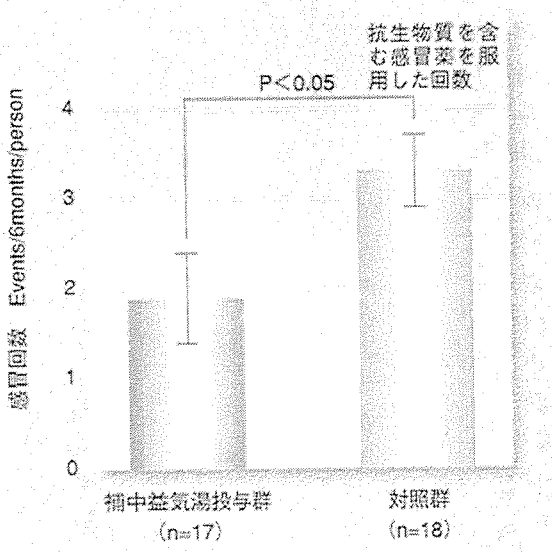


図5 COPD患者に対する補中益気湯投与群と対照群での感冒罹患頻度

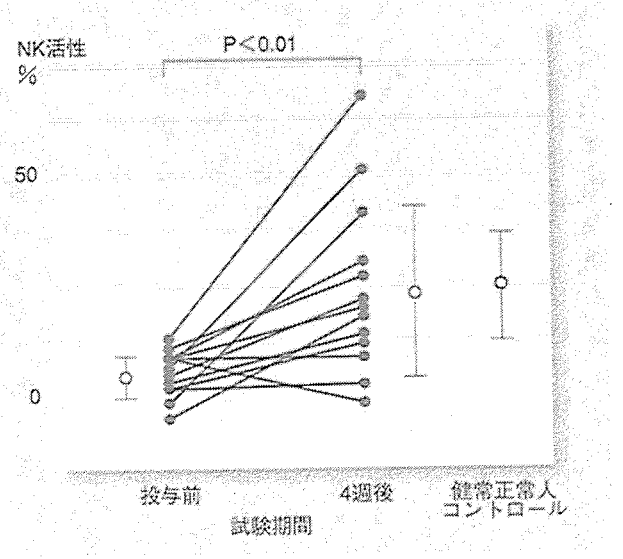


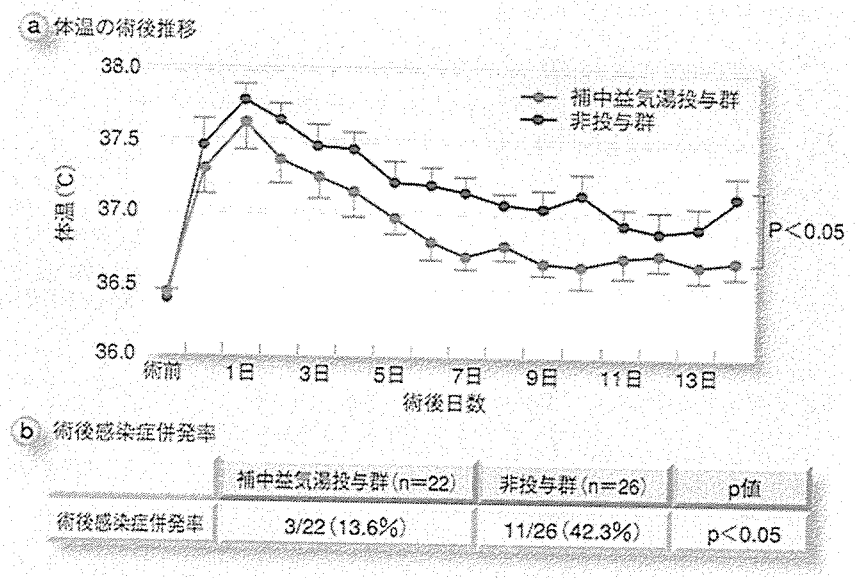
図6 NK活性低値例における補中益気湯の効果

に減少していた(図7)。このデータは、補中益気湯が術後の過度の炎症反応および免疫低下をどちらも改善していることを示すものといえる(図8)。

こうした補中益気湯がもつ作用の多面性について服部氏はこう述べる。「漢方は体内の環境バランスを整える方向で作用します。補中益気湯が免疫賦活作用・抗ウイルス作用・抗炎症作用を併せもつことを示すこれらのデータは、同剤が生体防御に有利な状態に体内環境を整える方剤であることを示しているといえます」。

ここで紹介したデータの他にも、補中益気湯についてはインフルエンザ感染マウスの生存率向上、感冒(ライノウイルス)感染抑制、ヘルペスウイルス感染抑制、タバコ刺激に対する抗炎症効果、などの報告もある。こうした作用は同剤の多面的作用によるバイオディフェンス増強作用によるものといえるだろう。

ひたひたと着実に広がりを見せる鳥インフルエンザの人への感染例、そしてHIV感染は世界的に増加の一途をたどっている。バイオディフェンスを増強する手段は、選択肢は多いほど人類にとっては好ましい。服部氏らが取り組む、こうした漢方薬の薬理効果の新たな解明に、多くの期待が集まっている。



	補中益気湯投与群(n=22)	非投与群(n=26)	p値
術後感染症併発率	3/22(13.6%)	11/26(42.3%)	p<0.05

図7 体温の術後推移と術後感染症併発率に対する補中益気湯の効果

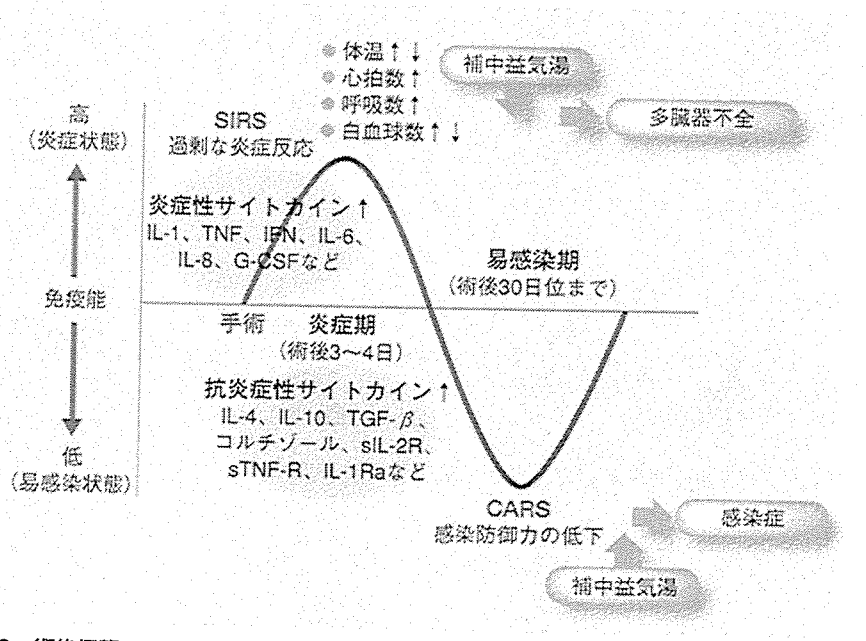


図8 術後侵襲による炎症反応・感染防御力低下に対する補中益気湯の可能性

文献

1) Marsh M, Helenius A. Review Virus Entry: Open Sesame Cell. 2006; 124: 729-40.
2) インタビュー 感染症と漢方薬の抗ウイルス作用.漢方医学 2006;30,1:11-3
3) Rodriguez B. et al. Predictive value of plasma HIV RNA level on rate of CD4 T-cell decline in untreated HIV infection. JAMA. 2006; 296: 1498-506.
4) Celli BR, et al. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. N Engl J Med. 2004; 350: 1005-12.
5) Shinozuka N, et al. The traditional herbal medicine hochuekkito improves systemic inflammation in patients with chronic obstructive pulmonary disease. Journal of the American Geriatrics Society. in press

※図7は別刷制作時に追記したものです。

Images in *Thorax*

Secondary bronchiolitis obliterans organising pneumonia in a patient with carbamazepine-induced hypogammaglobulinemia

A 49-year-old woman had been treated with carbamazepine for 2 years because of epilepsy. She was referred to us for progressive exertional dyspnea and prolonged productive cough. Chest computed tomography (CT) scan showed bilateral infiltrates including ground glass opacities and consolidations predominantly in the lower lung fields. Her laboratory findings showed severe hypogammaglobulinemia, that is, immunoglobulin (Ig) G 418 mg/dl (normal, 748–1694 mg/dl), Ig A 20 mg/dl (91–391 mg/dl) and Ig M 51 mg/dl (33–254 mg/dl). Carbamazepine and other suspected antibiotics were all negative for drug-induced lymphocyte stimulation tests. Histological examination by trans-bronchial lung biopsy showed intraluminal fibrosis of distal airspaces with foamy alveolar macrophages, suggesting bronchiolitis obliterans organising pneumonia (BOOP). After the cessation of carbamazepine, all abnormalities in gammaglobulins and roentgenogram findings gradually improved without any medication. This good clinical course also considerably supports the diagnosis of BOOP.

BOOP may result from diverse causes such as drugs, acute respiratory infections and radiation treatment, or appear idiopathically.[1 2] Here, we show a case of secondary BOOP, which was associated with repeated respiratory infections caused by carbamazepine-induced hypogammaglobulinemia. Although the exact mechanisms of carbamazepine-induced hypogammaglobulinemia are unknown, they can be classified into three groups, that is, an absence of B cells,[3] an extensive impairment of the synthesis of Igs in B cells[3] and a disorder of the class-switch of Igs in B cells.[4] Our case described above would belong to the second group. Generally, drug-induced BOOP often develops within several weeks or less. However, our report indicates that even in the case of several years after use, anticonvulsants such as carbamazepine may have some adverse effects on the immune system and cause frequent airway infections, resulting in the development of secondary BOOP.

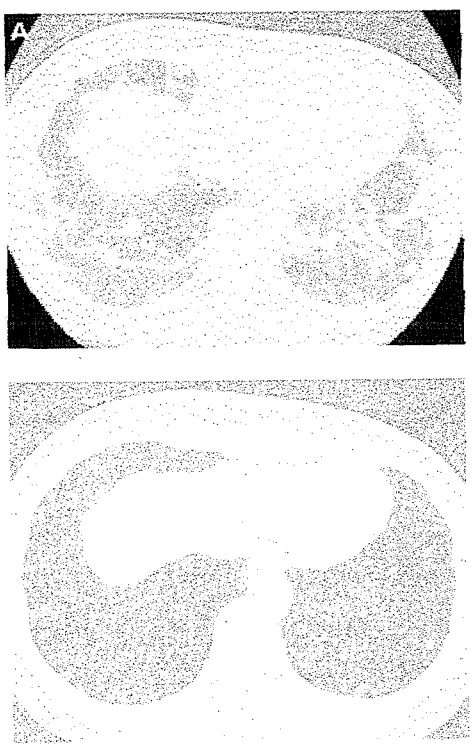


Figure 1 (A) Chest CT scans on admission. Bilateral infiltrates including ground glass opacities and consolidations are seen predominantly in lower lung fields. (B) Chest CT scans seven months after the cessation of carbamazepine showing marked improvement. The serum levels of Ig G, Ig A and Ig M are also increased to 1328, 69 and 355 mg/dl, respectively.

Learning points

- A drug-induced hypogammaglobulinemia after long term use of carbamazepine is very rare.
- A hypogammaglobulinemia should be considered as one of the causes of secondary bronchiolitis obliterans organising pneumonia with repeated airway infections.

ACKNOWLEDGEMENTS

The authors acknowledge Mr Brent K. Bell for reading the manuscript.

T Tamada, M Nara, M Tomaki, Y Ashino, T Hattori,
Division of Infectious and Respiratory Diseases, Department of Internal Medicine, Tohoku University Hospital, Sendai, Japan

Correspondence to: Tsutomu Tamada, Division of Infectious and Respiratory Diseases, Department of Internal Medicine, Tohoku University Hospital, Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan; tamada@rid.med.tohoku.ac.jp

doi: 10.1136/thx.2006.063842

Competing interests: None declared.

REFERENCES

1 **Cordier J-F.** Organising pneumonia. *Thorax* 2000;55:318–28.
2 **Cazzato S,** Zompatori M, Baruzzi G, *et al.* Bronchiolitis obliterans-organizing pneumonia: an Italian experience. *Respir Med* 2000;94:702–8.
3 **Castro AP,** Redmershi MG, Pastorino AC, *et al.* Secondary hypogammaglobulinemia after use of carbamazepine: case report and review. *Rev Hosp Clin Fac Med Sao Paulo* 2001;56(6):189–92.
4 **Go T.** Carbamazepine-induced IgG1 and IgG2 deficiency associated with B cell maturation defect. *Seizure* 2004;13:187–90.

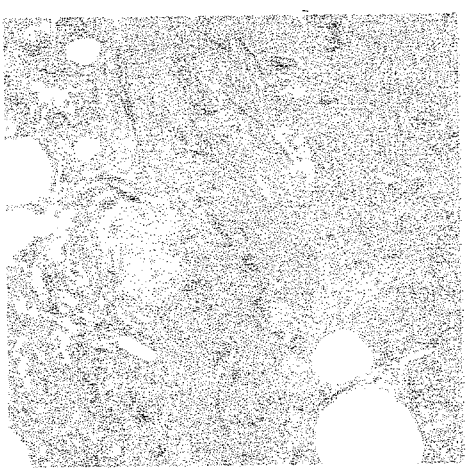


Figure 2 Elastica-Masson staining of specimens from TBLB. Immature fibroblastic foci and foamy alveolar macrophages are obstructing the alveolar ducts and adjacent alveoli. These features are consistent with BOOP.

Tohoku J. Exp. Med., 2004, **202**, 275-282

Long-Term Use of Corticosteroid Eye Drops Delays the Spontaneous Remission of Pulmonary Sarcoidosis

MASAYUKI NARA, KAN SASAMORI, SANAE SHIMURA, HIROMASA OGAWA, SATSUKI ISHIGAKI-SUZUKI[1], MIYUKI NAGAOKA, TSUTOMU TAMADA, MASAKAZU ICHINOSE[2], GEN TAMURA and TOSHIO HATTORI

Department of Respiratory and Infectious Diseases, [1]Department of Cellular Pharmacology, Postgraduate Division, Tohoku University School of Medicine, Sendai 980-8574, and
[2]The Third Department of Internal Medicine, Wakayama Medical University, Wakayama 641-8509

NARA, M., SASAMORI, K., SHIMURA, S., OGAWA, H., ISHIGAKI-SUZUKI, S., NAGAOKA, M., TAMADA, T., ICHINOSE, M., TAMURA, G. and HATTORI, T. *Long-Term Use of Corticosteroid Eye Drops Delays the Spontaneous Remission of Pulmonary Sarcoidosis.* Tohoku J. Exp. Med., 2004, **202** (4), 275-282 —— Topical corticosteroid eye drops are commonly used for ocular sarcoidosis. That systemic absorption of corticosteroids by eye drops may influence the clinical course of sarcoidosis may be speculated because it has been reported that the serum concentration of corticosteroids after drop administration was dose-related. To evaluate the effects of corticosteroid eye drops on the clinical course of patients with stage I pulmonary sarcoidosis, we compared the serum levels of angiotensin converting enzyme (ACE) and bilateral hilar lymphadenopathy (BHL) on chest radiographs of group CS, which is consisted of patients who received topical therapy of betamethasone in the form of eye drops for anterior uveitis, and group CN, which is consisted of patients who did not receive any medications throughout the entire course of the disease. Although the serum ACE level was not significantly different between groups CS and CN at the time of the diagnosis of pulmonary sarcoidosis, the level of serum ACE in group CS was significantly higher than that in group CN 20 months after the topical corticosteroid treatment (24 IU/ml and 16 IU/ml, respectively). Further, the size of BHL on chest radiography in group CS was significantly larger than that in group CN 20 months after the topical treatment (82% and 37% of before control, respectively). These findings suggest the possibility that the topical corticosteroid therapy influenced the clinical course of pulmonary sarcoidosis, inducing some delay in the spontaneous remission in the long-term course. ——— angiotesin converting enzyme; bilateral hilar lymphadenopathy; betamethasone eye drops; spontaneous remission; pulmonary sarcoidosis

Received November 26, 2003; revision accepted for publication February 16, 2004.
Address for reprints: Masayuki Nara, M.D., Ph.D., Department of Respiratory and Infectious Diseases, Postgraduate Division, Tohoku University School of Medicine, Seiryomachi, Aoba-ku, Sendai 980-8574, Japan.
e-mail: nara@intl.med.tohoku.ac.jp

Sarcoidosis is a chronic and systemic disease of unknown etiology that is characterized by non-caseating epithelioid cell granuloma (so called sarcoidosis granuloma). Lungs and thoracic lymph nodes are almost always involved, and extrathoracic involvements, such as erythema nodosum or uveitis, are also seen although their incidences differ among races (Newman et al. 1997). Both bilateral hilar lymphadenopathy (BHL) and anterior uveitis are reported to be more common in Japanese as pulmonary and intraocular manifestations of sarcoidosis, respectively (Ohara et al. 1992).

Generally, systemic or oral corticosteroids are indicated for severe ocular, neurologic, or cardiac sarcoidosis, malignant hypercalcemia and progressive stage II (hilar lymphadenopathy with parenchymal infiltrates), III (parenchymal infiltrates without hilar lymphadenopathy) and IV (fibrosis) pulmonary sarcoidosis (Newman et al. 1997). Other sarcoidosis patients should be observed without therapy because of the potential for spontaneous remission (Silver and Messner 1994; Newman et al. 1997). There have been many reports showing evidence of adverse outcomes in sarcoidosis patients systemically treated with corticosteroids (Young et al. 1970; Israel et al. 1973; Selroos et al. 1974; Harkleroad et al. 1982; Eule et al. 1986; Izumi 1994; Gottlieb et al. 1997; Reich 2002). Meanwhile, topical corticosteroid eye drops are efficient and commonly used for ocular sarcoidosis (Silver and Messner 1994; Newman et al. 1997). It has been reported that the serum concentrations of corticosteroids after single-drop administration to the eyes were dose-related and that high serum concentrations were retained in the sera, resulting in adrenal suppression (Krupin et al. 1976; Baba et al. 1983). Taken together with these findings, we can speculate that systemic absorption of corticosteroids by topical therapy may influence the clinical course of sarcoidosis. To our knowledge, however, there are no studies that show the effect of glucocorticosteroid eye drops on the clinical course of pulmonary sarcoidosis.

In this preliminary report, we examined the effects of such eye drops on serum angiotensin converting enzyme (ACE) and BHL on chest radiograph in patients with stage I pulmonary sarcoidosis and found that the topical corticosteroid therapy appeared to influence the clinical course of pulmonary sarcoidosis, inducing some delay in the spontaneous remission in the long-term usage, as shown in the case of systemic corticosteroid treatment of pulmonary sarcoidosis (Young et al. 1970; Israel et al. 1973; Selroos et al. 1974; Harkleroad et al. 1982; Eule et al. 1986; Izumi 1994; Gottlieb et al. 1997; Reich 2002).

Methods

Subjects

During 1990 to 1997, we experienced 128 sarcoidosis patients in Tohoku University Clinic and Hospital. From the complete medical records including cardiac and ophthalmologic examinations and serial examinations of serum ACE and chest radiograph for 2 years or more, 24 patients with stage I pulmonary sarcoidosis were selected for the present study. The diagnosis of pulmonary sarcoidosis was made by the combination of the histology of transbronchial lung biopsy (TBLB) (the presence of non-caseating epithelioid cell granuloma etc.), serum ACE, tuberculin skin test, gallium citrate Ga^{67} scintigraphy, serum lysozyme, computed tomographic (CT) scan, bronchoalveolar lavage (BAL), and clinical features for 4 years or more, excluding pulmonary tuberculosis, hypersensitive pneumonitis, and fungus diseases.

Group CN consisted of patients with stage I pulmonary sarcoidosis who were free of ocular lesions by ophthalmologic examinations and did not receive any medications throughout the entire course of disease (Table 1A). Group CS consisted of patients with stage I pulmonary sarcoidosis who received betamethasone sodium phosphate (BM) solution eye drops for anterior uveitis (Table 1A). There were no significant differences between the two groups in the findings of Ga^{67} scintigram, serum lysozome, CT, or BAL. BM solution (0.1%) was used in 10 patients of group

CS; 3,5, and 2 patients received single drops of the steroid solution in both eyes 6, 4, and 3 times a day, respectively. A single drop of 0.1% BM solution contained about 0.05 mg of betamethasone sodium (Baba et al. 1983). All patients in group CS received the topical therapy for 1 year or more and showed some improvement in both their symptoms and ophthalmologic findings (except for one patient).

To determine the early effect of BM solution eye drops on lung histology, we selected 10 patients with stage I and II pulmonary sarcoidosis (group CS-2) who had received eye drops for 4 to 6 weeks until TBLB (Table 1B). BM solution (0.1%) was used in 10 patients of the CS-2 group; 2 and 8 patients received single drops of the steroid solution in both eyes 6 and 4 times a day, respectively. As the controls, there were 30 patients with I and II pulmonary sarcoidosis (group CN-2) who had received no treatment for at least 4 to 6 weeks until TBLB. The histological findings of biopsy samples from group CS-2 and CN-2 were compared.

Serum angiotensin-converting enzyme

Serum angiotensin-converting enzyme (ACE) activity from each patient was measured an assay using the colorimetric method as described previously (Kasahara and Ashihara 1981) at intervals of 1 to 2 months for 2 years or more. A value of 21.4 U/ml or greater was ascertained to be elevated.

Chest radiograph

Chest radiograph was obtained from each patient with the same machine (FCR5000, Fuji film medical, Tokyo) under the same exposure conditions and printed with the same type of film

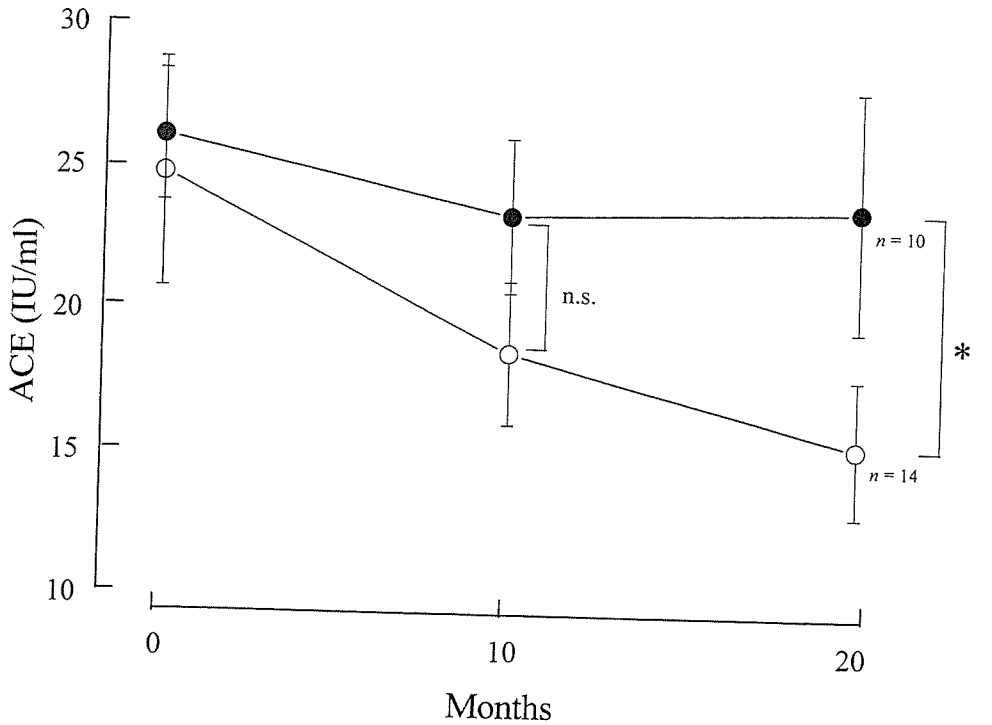


Fig. 1. The effect of topical treatment of betamethasone on the serum ACE in the pulmonary sarcoidosis patients. The serum ACE level of the non-therapeutic (CN) group (15.5±2.4 IU/ml, n=14) was significantly lower than that of the topical therapeutic (CS) group (23.8±4.2 IU/ml, n=10) 20 months after diagnosis, at which time these levels were not different (24.9±4.0 IU/ml and 26.2±2.3 IU/ml, respectively, p=0.037) at the time of diagnosis. $^{*}p<0.05$.
●, Topical therapy (CS) group; ○, Non-therapy (CN) group.

at intervals of 1 to 2 months for 2 years or more. The size of BHL was estimated by weighing cutouts of the drawings of the chest radiographs. The measurement of BHL on chest radiographs was performed without knowledge of the case number by 2 chest physicians and the mean value was used for data analysis.

Transbronchial lung biopsy

Under topical anesthesia of lidocaine hydrochloride, transbronchial lung biopsy (TBLB) was performed. Three to 5 biopsy samples were obtained from the right lung of each patient.

Statistics

Statistical analysis was performed using the unpaired *t*-test (histologically positive rate of non-caseating granuloma), Mann-Whitney's U-test (age, serum ACE levels, and %BHL) and Fischer's exact probability test (sex). A p-value <0.05 was considered significant. Data were expressed as mean±S.E.

Results

As shown in Table 1A, the patients in the CN group were older than those in the CS group although not significantly (p=0.11), and both the sex and race distribution were similar in the two groups.

Serum ACE levels

At the time of diagnosis of pulmonary sarcoidosis, there were no significant differences in the serum ACE levels between the CN and CS groups (24.9±4.0 IU/ml and 26.2±2.3 IU/ml, respectively). The serum ACE level in each patient showed a gradual decrease for 2 years or more. However, the serum ACE levels from the CN group showed greater decreases than those from the CS group, and the ACE levels from the CN group were significantly lower than those from the CS group 20 months after the diagnosis or topical treatment (15.5±2.4 and 23.8±4.2 IU/ml, respectively, p=0.037) (Fig. 1).

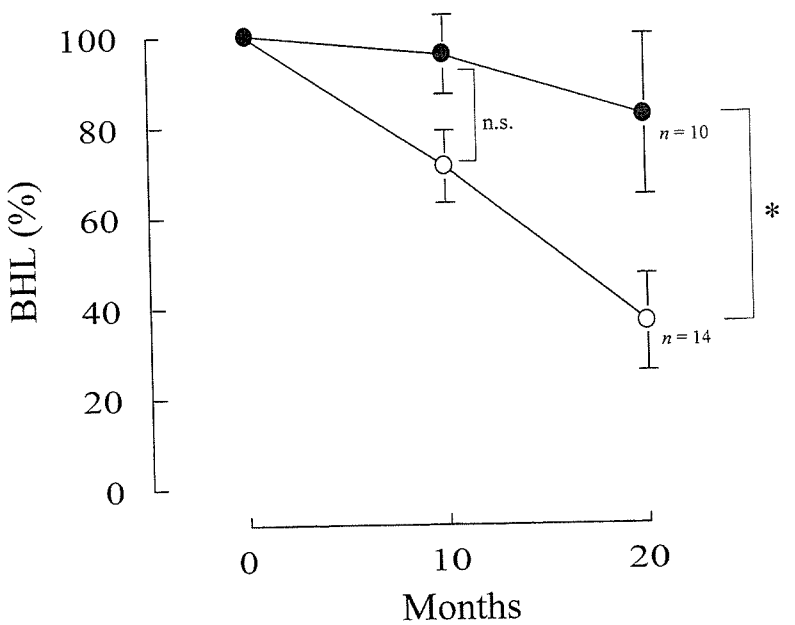


Fig. 2. The effect of topical treatment of betamethasone on the size of BHL on chest radiograph in the pulmonary sarcoidosis patients. The size of BHL was expressed as a percentage of that at the time of diagnosis. The BHL on chest radiography of the non-therapeutic (CN) group was significantly diminished in size 20 months after diagnosis compared to that of the topical therapeutic (CS) group (36.9±11.9% and 82.2±17.7% of before control, respectively, mean±S.E., p=0.029). *$p<0.05$. ●, Topical therapy (CS) group; ○, Non-therapy (CN) group.

BHL size on chest radiograph

Coincident with the changes in the serum ACE level, the BHL sizes showed a gradual decrease in each patient, and those from the CN group showed a greater decrease than those from the CS group. There were significant differences in the BHL sizes between the CN and CS groups 20 months after the diagnosis or topical treatment (36.9±11.9 vs. 82.2±17.7% of before control, respectively, p=0.029) (Fig. 2).

Non-caseating granuloma by TBLB

When one or more biopsy samples from each patient contained a non-caseating granuloma, it was determined to be histologically positive. Although it did not reach statistical significance, the positive rate of the CS-2 group had a tendency to be lower than that of the CN-2 group (50.0 and 73.3%, respectively, p=0.31).

Discussion

The results of the present study of the serial serum ACE and chest radiograph BHL-size measurements suggest that long-term BM solution eye drops influence the clinical course of pulmonary sarcoidosis, including some delay in the spontaneous remission.

It may be difficult to assess the effects of the corticosteroids on pulmonary sarcoidosis since sarcoidosis tends to follow a benign clinical course in the majority of patients (Newman et al. 1997). The serum ACE activity is a sensitive index for evaluating the clinical course of sarcoidosis including spontaneous remission (DeRemee and Rohrbach 1980; Rohatgi et al. 1981), and the size of BHL on chest radiograph has been shown to change in parallel with the activity of stage I pulmonary sarcoidosis that shows spontaneous remission (Newman et al. 1997; The American Thoracic Society et al. 1999). Chest CT examination, which would enable to accurate measurement, was not used for the present study because of the substantial radiation exposure.

The severity of the two groups at the time of diagnosis in the present study was almost the same since both ACE levels and BHL sizes were not significantly different between the two groups. The corticosteroid eye drops delayed the improvement or decrease of both the ACE level and the size of BHL 20 months after the treatment. It has been reported that the serum concentrations of corticosteroids after single-drop administration to the eyes were dose-related and that high serum concentrations were retained in the sera (Krupin et al. 1976; Baba et al. 1983), although our report lacks data of the serum concentrations because it was a retrospective study. The doses of corticosteroids by eye drops in the CS group of the present study were similar to those reported to effect adrenal function through systemic absorption with long-term usage (Baba et al. 1983). Taken together with these reports, we can speculate that systemic absorption of topical corticosteroid therapy adversely affects the course of pulmonary sarcoidosis.

We have assumed that the course of pulmonary sarcoidosis is uninfluenced by the presence of uveitis since we could not find any reports concerning the adverse effect of uveitis on the clinical course. Additionally, advanced age is known to be one of the adverse prognostic factors of sarcoidosis (The American Thoracic Society et al. 1999). As shown in Table 1A, the patients in the CN group were older than those in the CS group although not significantly. Therefore, the difference between the two groups in both serum ACE and the size of BHL 20 months after the topical treatment would have been more prominent if the ages between the two groups had been matched.

The adverse effects of corticosteroids on the course of sarcoidosis have been reported by several investigators (Young et al. 1970; Israel et al. 1973; Selroos et al. 1974; Harkleroad et al. 1982; Eule et al. 1986; Izumi 1994; Gottlieb et al. 1997; Reich 2002). For example, Eule et al. (1986) reported an evaluation of the long-term influence of oral corticosteroid therapy on the natural course of asymptomatic pulmonary sarcoidosis. They reported that relapse occurred in 22% of the patients in the treated group and concluded

TABLE 1. *Patients' demographics*

A

	CN Group[1]	CS Group[2]	*p*-value
No. of patients	14	10	
Sex			p=0.68
Male	9 (64%)	5 (50%)	
Female	5 (36%)	5 (50%)	
Race			
Japanese	14 (100%)	10 (100%)	
Age (years±S.E.)	50.3±18.6	38.3±18.5	p=0.11
Range	23-78	20-68	

[1]The CN group consisted of patients with stage I pulmonary sarcoidosis who did not receive any medications throughout the entire course of the disease.

[2]The CS group consisted of patients with stage I pulmonary sarcoidosis who received topical therapy of betamethasone sodium phosphate solution eye drops for anterior uveitis.

B

	CN-2 Group[1]	CS-2 Group[2]	*p*-value
No. of Patients	30	10	
Stage			p=0.714
Stage I	18 (60%)	7 (70%)	
Stage II	12 (40%)	3 (30%)	
Sex			
Male	18 (60%)	6 (60%)	
Female	12 (40%)	4 (40%)	
Race			
Japanese	30 (100%)	10 (100%)	
Age (years)±S.E.	41.0±2.7	32.9±6.5	p=0.096
Range	22-78	20-75	

[1]The CN-2 group consisted of 30 patients with stage I and II pulmonary sarcoidosis who had received no treatment for at least 4 to 6 weeks until TBLB.

[2]The CS-2 group consisted of 10 patients with stage I and II pulmonary sarcoidosis who had received BM solution eye drops for 4 to 6 weeks until TBLB.

that corticosteroid therapy did not have a real influence on the long-term course of sarcoidosis regardless of the radiographic stage (Euel et al. 1986). Further, Izumi (1994) reported that, at 10 years, the outcome favored the untreated group: 24% in the group treated orally with corticosteroids vs. 8% in the untreated group had persistent radiographic abnormalities. Also, clinical and radiographic deterioration was observed in 5% in the treated group – in one instance severe enough to require continuous oxygen therapy – but in no patients of the untreated group. Our results are

mostly compatible with these results in spite of the fact that the treatment of corticosteroids was only with topical eye drops.

Serial measurement of serum ACE or chest radiograph failed to show an early effect of decreased sarcoidal inflammation by the steroid eye drops. Meanwhile, after 4 to 6 weeks of topical treatment, the histologically positive rates of non-caseating granuloma in the TBLB biopsy samples from the CS group had a tendency to be lower (50.0%) compared with those from the CN group (73.3%), although the difference was not statistically significant. Treatment with oral corticosteroids is known to prevent short-term deterioration of chest roentgenographic findings and lung functions (du Bois 1994; Hunninghake et al. 1994; Gibson et al. 1996; The American Thoracic Society et al. 1999). Therefore, the finding that the topical administration (eye drops) of corticosteroids had a tendency to reduce the non-caseating granulomas (sarcoidosis granuloma) in lung tissue is consistent with the known effects of oral corticosteroids in suppressing the acute consequences of widespread pulmonary granuloma (Gibson et al. 1996).

In conclusion, topical administration of corticosteroids by eye drops influences the clinical course of pulmonary sarcoidosis through the systemic absorption, producing some delay in the spontaneous remission. These findings should be confirmed by a prospective randomized double-blind study with a larger number of patients in the future.

Acknowledgments

We thank Mr. B. Bell for reading the manuscript.

References

Baba, S., Mishima, H., Okimoto, M. & Miyachi, Y. (1983) Plasma steroid levels and clinical effects after topical application of bethamethasone. *Graefe's Arch. Clin. Exp. Ophthalmol.*, **220**, 209-214.

DeRemee, R.A. & Rohrbach, M.S. (1980) Serum angiotensin-converting enzyme activity in evaluating the clinical course of sarcoidosis. *Ann. Intern. Med.*, **92**, 361-365.

du Bois, R.M. (1994) Corticosteroids in sarcoidosis: friend or foe? *Eur. Respir. J.*, **7**, 1203-1209.

Eule, H., Weinecke, A. & Roth, I. (1986) The possible influence of corticosteroid therapy on the natural course of pulmonary sarcoidosis. *Ann. NY Acad. Sci.*, **465**, 695-701.

Gibson, G.J., Prescott, R.J., Muers, M.F., Middleton, W.G., Mitchell, D.N., Connolly, C.K. & Harrison, B.D.W. (1996) British thoracic society sarcoidosis study: effects of lomg term corticosteroid treatment. *Thorax*, **51**, 238-247.

Gottlieb, J.E., Israel, H.L., Steiner, R.M., Triolo, J. & Patrick, H. (1997) Outcome in Sarcoidosis. *Chest*, **111**, 623-631.

Harkleroad, L.E., Young, R.L., Savage, P.J., Jekins, D.W. & Lordon, R.E. (1982) Pulmonary sarcoidosis: Long-term follow-up of the effects of steroid therapy. *Chest*, **82**, 84-87.

Hunninghake, G.W., Gilbert, S., Pueringer, R., Dayton, C., Floerchinger, C., Helmers, R., Merchant, R., Wilson, J., Galvin, J. & Schwartz, D. (1994) Outcome of he treatment for sarcoidosis. *Am. J. Respir. Crit. Care Med.*, **149**, 893-898.

Israel, H.L., Fouts, D.W. & Beggs, R.A. (1973) A controlled trial of prednisone treatment of sarcoidosis. *Am. Rev. Respir. Dis.*, **107**, 609-614.

Izumi, T. (1994) Are corticosteroids harmful to sarcoidosis: a conclusion drawn from a retrospective study on the chest radiographic prognosis of 185 asymptomatic patients with pulmonary sarcoidosis followed up for more than 10 years. *Sarcoidosis*, **11**, 119-122.

Kasahara, Y. & Ashihara, Y. (1981) Colorimetry of angiotensin-I converting enzyme activity in serum. *Clin. Chem.*, **27**, 1922-1925.

Krupin, T., Mandell, A.I., Podos, S.M. & Becker, B. (1976) Topical corticosteroid therapy and pituitary-adrenal function. *Arch. Ophtalmol.*, **94**, 919-920.

Newman, L.S., Rose, C.S. & Maier, L.A. (1997) Sarcoidosis. *N. Engl. J. Med.*, **336**, 1224-1234.

Ohara, K., Okubo, A., Sasaki, H. & Kamata, K. (1992) Intraocular manifestation of systemic sarcoidosis. *Jpn. J. Ophtalmol.*, **36**, 452-457.

Reich, J.M. (2002) Mortality of intrathoracic sarcoidosis in referral vs. population-based settings: influence of stage, ethnicity, and corticosteroid therapy. *Chest*, **121**, 32-39.

Rohatgi, P.K., Ryan, J.W. & Linderman, P. (1981) Value of serial measurement of serum angioten-

sin converting enzyme in the management of sarcoidosis. *Am. J. Med.*, **70**, 44-50.

Selroos, O., Niemistö, M. & Riska, N. (1974) A follow-up study of treated and untreated early sarcoidosis. In: *Proceedings of the VI International Conference on Sarcoidosis. Tokyo 1972*, edited by K. Iwai & Y. Hosoda, University of Tokyo Press, Tokyo, pp. 525-528.

Silver, M.R. & Messner, L.V. (1994) Sarcoidosis and its ocular manifestation. *J. Am. Optom. Assoc.*, **65**, 321-327.

The American Thoracic Society, the European Respiratory Society & the World Association of Sarcoidosis and Other Granulomatous Disorders (1999) Statement on sarcoidosis. *Am. J. Respir. Crit. Care Med.*, **160**, 736-755.

Young, R.L., Harkleroad, L.E., Lordon, R.E. & Weg, J.G. (1970) Pulmonary sarcoidosis: a prospective evaluation of glucocorticoid therapy. *Ann. Intern. Med.*, **73**, 207-212.