を用いたPCR産物の増幅と、ダイレクトシークエンス法で得た塩基配列情報をもとに、データベース上で検索し、同定した。

全ての動物実験は杏林大学大学院医学研究科共同研究施設部門実験動物施設利用規定に基づいて、同施設内にて実施された。

結果および考察

*H. pylori*TK1402株1 x 10^7CFUを投与したスナネズミは、毎週1度6回実施した、糞便DNAを用いたnested PCR法による検出頻度より、高頻度検出群、中等度検出群、非検出群にわけることができた。糞便からの*H. pylori*の検出の結果は感染8週後の胃内*H. pylori*菌数と相関し、検出頻度の低い群には*H. pylori*が検出限界以下（陰性）となったスナネズミが含まれた。

さらに、上述の実験において保存したスナネズミ胃粘膜懸濁液を投与して、胃内細菌叢を修飾したのちに、*H. pylori*感染実験を実施した。その結果、*H. pylori*陰性のスナネズミ胃粘膜懸濁液を投与した群（A群）と、*H. pylori*高頻度検出の胃粘膜懸濁液を投与した群（B群）では、それぞれ、5匹中3匹、6匹中1匹が*H. pylori*の菌数が検出限界以下となった。この結果から、*H. pylori*陰性のスナネズミ胃粘膜懸濁液には*H. pylori*の持続感染に対して抑制的に働く細菌が多く含まれている可能性が示唆された。また、各群の*H. pylori*の平均胃内菌数はA群(log10;1.86±0.10以下同じ)に対して、B群(2.53±0.19)が高く、*Lactobacillus*属菌の平均胃内菌数はA群(11.08±0.64)がB群(10.46±0.91)と比べて高かった。すなわち、*Lactobacillus*属菌の菌数と*H. pylori*の菌数は逆相関する傾向が認められた。

さらに、両群の胃内細菌叢の解析の結果、通性嫌気性菌として、*E. coli*と*Kluyvera*属菌が両群から検出され、偏性嫌気性菌として、*L. reuteri*、*L. johnsonii*、*Lactobacillus*属菌が検出された。しかし、これらの細菌は*H. pylori*高頻度検出の胃粘膜懸濁液を投与したB群においても検出された。さらに、16SrDNA遺伝子増幅物の配列の違いにより、*L. reuteri*については2つのサブタイプが検出された。スナネズミA群は全例*L. reuteri*サブタイプ1が検出され、B群は6匹中3匹であった。この結果は、同じ胃粘膜懸濁液を投与したスナネズミの中にも*Lactobacillus*属菌の構成が異なることがわかり、これらの構成が*H. pylori*の持続感染に及ぼす作用についてさらに検討する必要性が示された。また、*H. pylori*陰性のスナネズミ1匹から*L. murinas*が検出されたため、本菌の*H. pylori*に対する増殖抑制効果を今後検討していく。

参考文献

1) Nakagawa S, Osaki T, Fujioka Y, et al. 12:347-53. 2005.
2) Osaki T, Matsuki T, Asahara T, et al. Microb Pathog. 53:12-8. 2012.
3) Osaki T, Taguchi H, Yamaguchi H, et al. J Clin Microbiol. 36:321-3. 1998.
4) Osaki T, Hanawa T, Manzoku T, et al. 55:1477-85. 2006.

Table.1　Detection of *H. pylor* and *Lactobacillus* spp. by real time PCR in both *H. pylori* (+) and *H. pylori* (-) groups

Gerbil group	A	B
number	5	6
pH(range)	2.5～3.0	3.0～4.5
胃重量(平均；g)	1.86±0.10	2.53±0.19
体重(平均；g)	55.30±2.95	61.83±2.30
H. pylori 菌数/g mucus (陽性動物数)	4.81±0.50 (2)	5.83±2.16 (4)
Lactobacillus 属菌数/g mucus	11.08±0.64	10.46±0.91

Helicobacter

Helicobacter ISSN 1523-5378
doi: 10.1111/j.1523-5378.2011.00930.x

Fragmented CagA Protein is Highly Immunoreactive in Japanese Patients

Kazuki Yamada,* Toshiro Sugiyama,* Hiroshi Mihara,* Shinya Kajiura,* Seiko Saito,* Yuko Itaya,* Hidemoto Yamawaki,* Takayuki Ando,* Takahiko Kudo,* Ayumu Hosokawa,* Masumi Okuda,† Kenichi Fukunaga,‡ Junko K Akada§ and Teruko Nakazawa§

*Department of Gastroenterology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan, †Department of Pediatrics, Sasayama Medical Center Hyogo College of Medicine, Sasayama, Japan, ‡Department of Scientific and Affairs, Kyowa Medex Co., Ltd., Tokyo, Japan, §Yamaguchi University Graduate School of Medicine, Ube, Japan

Keywords
antigenic epitope, ethnic difference, fragmented CagA, Helicobacter pylori, immunoassay.

Reprint requests to: Toshiro Sugiyama, Department of Gastroenterology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani 930-0194, Toyama, Japan.
E-mail: tsugi@med.u-toyama.ac.jp.

Abstract

Background: High-molecular-weight cell-associated proteins (HM-CAP) assay is the most popular serological immunoassay worldwide and has been developed from US isolates as the antigens. The accuracy is reduced when the sera are from adults and children in East Asia including Japan. To overcome the reduced accuracy, an enzyme immunoassay using Japanese strain–derived HM-CAP (JHM-CAP) was developed, in which the antigens were prepared by exactly the same procedure as HM-CAP. The performance of JHM-CAP was better than that of HM-CAP in Japanese adults as well as in children. The higher sensitivity was because of the presence of 100-kDa protein that was absent in the preparation of HM-CAP antigen.

Materials and Methods: Immunoblot analysis and peptide mass fingerprinting methods were used to identify the distinctive 100-kDa protein present in JHM-CAP antigens. The peptide sequence and identification were analyzed by Mascot Search on the database of *Helicobacter pylori*. The identified protein was confirmed by immunoblot with a specific antibody and inhibition assay by the sera.

Results: The distinctive 100-kDa protein was a fragment of CagA derived from Japanese clinical isolates, and the sera of Japanese patients had strongly reacted to the protein, probably to the exposed epitope on the fragmented CagA. The fragmentation of CagA had occurred in the process of antigen preparation in Japanese isolates, not in US isolates even under the same preparation.

Conclusion: The distinctive 100-kDa protein was a fragment of CagA protein of *H. pylori* derived from Japanese clinical isolates, and Japanese patients including children are likely to react strongly to the exposed epitopes on fragmented CagA.

Helicobacter pylori (*H. pylori*) infection is one of the most widespread infections in humans worldwide (about 50 million people in Japan are infected) and its cure prevents the recurrence of gastroduodenal ulceration. In addition, *H. pylori* infection has a causal relationship with histologic gastritis, atrophic gastritis, gastric cancer, and mucosa-associated lymphoid tissue (MALT) lymphoma in the stomach [1–4]. All patients with *H. pylori* infection have histologic gastritis, which is characterized by marked infiltration of neutrophils and other inflammatory cells. However, most of them are asymptomatic in life. Only one part of the infected individuals will develop gastric ulceration, duodenal ulceration, gastric cancer, or MALT lymphoma. It is unclear why only a minority of the infected peoples develop such diseases, probably due to the interactions between the strains and the host immune responses.

The commercial available enzyme immunoassay (EIA) tests have a high diagnostic performance in the population study. However, the accuracy had been greatly reduced when sera from East Asia including Japan and younger subjects are evaluated by the most common EIA kit, high-molecular-weight cell-associated proteins (HM-CAP) (Scimedx Corp., Denville, NJ, USA)

[5–11]. In general, the use of *H. pylori* antigens derived from regions is speculated to achieve a higher diagnostic performance because of antigenic diversity among regions [1,5–7]. Okuda et al. [12] reported that the serodiagnostic accuracy of HM-CAP EIA was 51.4% sensitivity and 97.2% specificity with reference to the *H. pylori* stool antigen test HpSA (Meridian Bioscience Inc., Cincinnati, OH, USA) in asymptomatic Japanese children under 10 years of age. The low sensitivity may be due to antigen preparations or strain variations in the immunoassay. HM-CAP EIA was prepared from HM-CAP purified from US clinical isolates [13]. A serum-based EIA test using Japanese strain–derived high-molecular-weight proteins (JHM-CAP) prepared by exactly the completely same procedure as for HM-CAP has been developed [14]. The diagnostic performances of JHM-CAP EIA (Scimedx Corp.) and HM-CAP EIA in adult populations were reported using urea breath test (UBT) as the gold standard. For serum samples of Japanese populations, the performance of the JHM-CAP EIA was better than that of the HM-CAP EIA [14,15], although there was no difference between them on the accuracy in the serum samples from U.S. population. In addition, we have previously reported that in asymptomatic Japanese children under 10 years of age, the performance of JHM-CAP immunoassay was statistically better than that of HM-CAP EIA and found that the higher sensitivity of JHM-CAP was because of the presence of an immunoreactive 100-kDa protein, which was absent in HM-CAP[16]. The aim of this study was to identify this 100-kDa protein contained in the JHM-CAP EIA, which might contribute to a higher accuracy and a different immune response in ethnic.

Materials and Methods

Helicobacter pylori Clinical Isolates and Culture Conditions

Four clinical isolate strains from four Japanese patients with different diseases were used for culture. The strain numbers and the characteristics of the patients were described as follows: strain 159A from patients with gastric ulcer; strain 193C from patients with gastric cancer; strain 198C from patients with gastric ulcer; and strain 225C from patients with functional dyspepsia [14]. All strains were cultured on 10-cm-diameter blood agar plates (Trypticase Soy Agar II with 5% sheep blood: Becton Dickinson and Company, Tokyo, Japan) at 37 °C in a microaerobic atmosphere (5% O_2, 10% CO_2, and 85% N_2) and 100% humidity for 48–72 hours. Plate-grown bacteria were harvested and washed twice in phosphate-buffered saline and then resuspended in B-PER® bacterial protein extraction reagent (Pierce, Rockford, IL, USA). Bacterial proteins were then extracted according to the manufacturer's instructions.

HM-CAP EIA Antigens and JHM-CAP EIA Antigens

The HM-CAP used for HM-CAP EIA were purified from a U.S. strain (197SR-US) according to a published procedure [13]. In brief, crude extracts solubilized with 1% n-octyl alcohol containing phosphate-buffered saline were dialyzed against buffered saline and centrifuged to obtain soluble proteins. Then, high-molecular-weight proteins were separated by passing through an agarose A-5 m column equilibrated with 0.05 mol/L Tris–HCl buffer (pH 8.0), followed by ammonium sulfate precipitation and dialysis. The proteins used for JHM-CAP EIA were made from proteins mixed from four Japanese strains by the same purification method as that used for HM-CAP EIA. These purified proteins were obtained from Scimedx Corp.

Immunoblot Analysis

Antigens were diluted and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli's method [17]. After electrophoresis, the proteins were transferred to a polyvinylidene difluoride membrane as described by Towbin et al. [18]. Immunoblot was carried out with 1000-fold-diluted serum (from adult patients with JHM-CAP-positive reaction). Horseradish peroxidase–labeled goat anti-human immunoglobulin G (American Qualex International Inc., San Clemente, CA, USA) diluted 10,000-fold in Tris-buffered saline with 0.5% Tween 20 was used to visualize the reactive bands detected by enhanced chemiluminescence (SuperSignal® West Pico Chemiluminescent Substrate; Pierce, Rockford, IL, USA). Immunoblot study was simultaneously carried out using rabbit anti-CagA antibody (Austral Biologicals Inc., San Ramon, CA, USA) diluted 2000-fold in Tris-buffered saline with 0.5% Tween 20. Anti-rabbit immunoglobulin G horseradish peroxidase–conjugated antibody (GE Healthcare Biosciences Corp., Piscataway, NJ, USA) was used to visualize the reactive antigens for detection by enhanced chemiluminescence.

Inhibition Study in Immunoblot Analysis

To confirm the antigenic characteristics in JHM-CAP EIA, an immunoblot analysis with 1000-fold-diluted

serum from patient showing strongly positive reaction in JHM-CAP EIA was carried out before the reaction with anti-CagA antibody (2000-fold dilution). Another immunoblot analysis was carried out with anti-CagA antibody reaction before the reaction with 1000-fold-diluted serum from patient showing positive reaction in JHM-CAP.

Mass analysis and Peptide Mass Fingerprinting

SDS-PAGE gel was stained with the Silver Staining Kit Protein (GE Healthcare Bio-science Corp.) using a modified protocol for mass spectrometry after electrophoresis, as described previously [19]. Protein bands were excised from the stained gel and digested with trypsin as described previously [20]. The peptide mixture was mixed (1 : 1) with a saturated solution of α-cyano-4-hydroxycinnamic acid in 33% acetonitrile–0.1% trifluoroacetate, and 1 μL was applied to the sample template of a mass analysis by matrix-assisted laser deposition–ionization time-of-flight mass spectrometer (Autoflex-T1; Bruker Daltonics Inc., Bellerica, MA, USA). Peptide mass fingerprinting (PMF) was searched using the program Mascot (Matrix Science, USA; http://www.martix-science.com/). Peptides that Mowse score was over 75 were investigated in the homology search. The search employed the database of the US National Center for Biotechnological Information (http://www.ncbi.nlm.-nih.gov) containing complete genomes of *H. pylori* strains 26695 and J99.

Results

Immunoblot of JHM-CAP and HM-CAP Antigens with Serum of Patient

The antigens used to develop JHM-CAP and HM-CAP EIA are purified by the same procedure, but the origins of bacterial strains are different, as described in the Materials and Methods. Figure 1A demonstrates immunoblot analysis to both purified antigens in SDS-PAGE followed by the same serum from patient with JHM-CAP-positive reaction. The JHM-CAP antigen had a distinctive 100-kDa band that was reactive with the serum, and the intensity of this band was the strongest among the bands detected in the JHM-CAP immunoblot. However, the HM-CAP antigen had a weak band of about 120 kDa, but no 100-kDa band as in the case of JHM-CAP. The 100-kDa protein was not a major protein in a silver-stained SDS-PAGE gel in the JHM-CAP antigen. The serum has reacted to a 60-kD protein that is a major antigen prepares in HM-CAP and JHM-CAP, both.

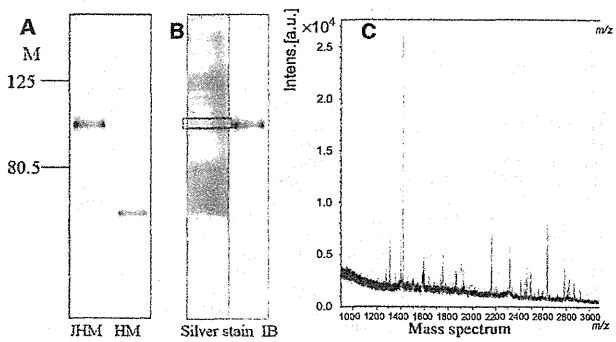


Figure 1 (*A*) Immunoblot of Japanese strain–derived high-molecular-weight proteins (JHM-CAP) antigen (JHM) and HM-CAP antigen (HM) with JHM-CAP-positive patient's serum. JHM (0.03 μg) and HM (0.03 μg) were separated by SDS-PAGE, followed by immunoblot. M: molecular size markers (kD), (*B*) Silver stain and immunoblot of SDS-PAGE gel of JHM-CAP protein. Boxed 100-kDa area was excised for the analysis. (*C*) Mass spectrometric analysis of the in situ tryptic-digested peptides of the excised protein.

Peptide Mass Fingerprinting of the 100-kDa Protein

To identify the highly immunoreactive 100-kDa protein, we tried to visualize the protein band in order to excise it from the gel. However, usual silver staining did not visualize this 100-kDa protein band when the amount of protein increased more than 10 times, as shown in Fig. 1B. Figure 1B shows the results of simultaneous silver staining and immunoblot to determine the 100-kDa protein in the silver-stained lane. We excised a piece of gel containing the 100-kDa protein band from the SDS-PAGE gel and digested the protein with trypsin, using an in-gel digestion method. The digested peptides were analyzed by mass spectrometry. Figure 1C shows the mass spectrum of the observed peptides. Nine peaks of peptides were analyzed. PMF was performed by using these lists and was analyzed by Mascot Search. Table 1 demonstrates the top five hit

Table 1 All of five peptides that Mowse score was over 75 were identical to the peptide sequence of CagA, which was determined by Mascot PMF search on database

Mowse score	NCBI-gi	Protein	Peptides matched
94	52693780	CagA	13
82	46091352	CagA	12
82	52693804	CagA	12
82	12225014	CagA	12
81	22335393	CagA	12

PMF, peptide mass fingerprinting.

1 MANETIDQTI TPDQTPNRTD FVPQRFINNL QVAFLKVDNA VASFDPDQKP
51 IVDKNDRDNR QAFEKISQLR EEYANKAIKN PTKKNQYFSD FINKSNDLIN
101 KDNLIAVDSS VESFRKFGDQ RYQIFTSWVS LQKDPSKINT QQIRNFMENI
151 IQPPISDDKE KAEFLRSAKQ SFAGIIIGNQ RSDEKFMGV FDESLKERQE
201 AEKNAEPAGG DWLDIFLSFV FNKKQSSDLK ETLNQEPRPD FEQNLATTTT
251 DIQGLPPESR DLLDERGNFF KFTLGDVEML DVEGVADKDP NYKFNQLLIH
301 NNALSSVLMG GHSNIEPEKV SLLYGDNGGP EARHDWNATV GYKNQQGNNV
351 ATLINAHLNN GSGLVIAGNE NGIKNPSFYL YKEDQLTGLK QAMSQEEIQN
401 KVDFMEELAQ NNAKLDNLSE KEKEKFQTEI ENFQKDRKAY LDALGNDHIA
451 FVSKKDPKHL ALVTEFGNGE VSYTLKDYGK KQDKALDGET KTTLQGSLKV
501 DGVMFVNYSN FKYTNASKSP DKGVGATNGV SHLEANFSKV AVFNLPNLNN
551 LAITNYIRRD LEDKLWAKGL SSQEANKLIK DFLNSNKELL GKVSNFNKAV
601 AGAKNTGNYD EVKKAQKDLE KSLRKREHLE KEVAKKLESR NDNKNRMEVK
651 AQAQSQKDKI FALINQEASK EARAAAFDPS LKGVRSELSD KLENINKNLK
701 DFGKSFDELK NGKNNDFSKA EETLKALKDS VKDLGINPEW ISKIENLNAA
751 LNDFKNGKNK DFSKVTQAKS DLENSIKDVI INQKITDKVD NLNQAVSETK
801 LTGDFSKVEQ ALAELKSLSL DLGKNSDLQK SVKNGVNGTL VGNGLSKTEA
851 TTLTKSFSDI RKELNEKLFG NSNNNNNGLK NNTEPIYAKV NKKKTGQVAS
901 PEEPIYAQVA KKVSAKIDQL NEATSAINRK IDRINKIASA GKGVGGFSGA
951 GQSASPEPIY ATIDFDETNQ AGFPLRRSAA VNDLSKVGLS REQELTRRIG
1001 DLNQAVSEAK TGHFGNLEQK IDELKDSTKK NALKLWVESA KQVPTGLQAK
1051 LDNYATNSHT RINSNVQSGT INEKATGMLT QKNPEWLKLV NDKIVAHNVG
1101 SAHLSEYDKI GFNQKNMKDY SDSFKFSTKL NNAVKDIKSS FVQFLTNTFS
1151 TGSYSLMKAN AEHGVKNTNT KGGFQKS

Figure 2 Protein sequences of NCBI-gi52693780 (CagA), which was the top-hit protein by Mascot Search in Table 1. Peptide mass fingerprinting-matched peptides were shown as underlined-bold.

proteins according to the results of peptide sequencing, all of which were CagA peptides. Figure 2 shows the protein sequences of the top-hit protein NCBI-gi52693780 (CagA), and the underlined peptides were hit to that of CagA protein.

Immunoblot of JHM-CAP and HM-CAP Antigens with Anti-CagA Antibody

From these results, we presumed that this 100-kDa protein was a part of the CagA protein. Therefore, we performed immunoblot analysis using anti-CagA antibody (Fig. 3A). The JHM-CAP antigen was a 100-kDa band reacted with anti-CagA antibody, whereas the HM-CAP had an about 120-kDa band, but not a 100-kDa band, reacted with anti-CagA antibody. These results suggest that the molecular weight of CagA is different in JHM-CAP antigen and HM-CAP antigen, although both were reacted with anti-CagA antibody.

Inhibition Study in Immunoblot Analysis

To confirm whether the 100-kDa protein containing JHM-CAP antigen is a fragment of the CagA protein, an inhibition study in immunoblot analysis was performed. Before immunoblot with anti-CagA antibody (2000-fold dilution), an immunoblot was carried out with diluted serum (1000-fold dilution was performed). The diluted serum almost completely inhibited anti-CagA antibody binding (Fig. 3B,C). However, the band in immunoblot by the patient's serum was slightly inhibited by pretreatment with anti-CagA antibody (Fig. 3D,E).

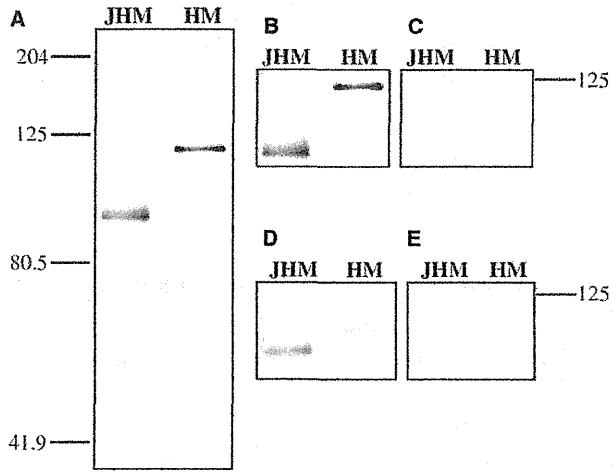


Figure 3 (*A*) Immunoblot of Japanese strain–derived high-molecular-weight proteins (JHM-CAP) antigen (JHM) and high-molecular-weight cell-associated proteins (HM-CAP) antigen (HM) with anti-CagA specific antibody. (*B–D*) Inhibition assays of JHM-CAP and HM-CAP antigens. (*B*) Anti-CagA antibody was used as primary antibody (1 : 3000). (*C*) Before primary antibody (anti-CagA antibody) reaction, diluted JHM-CAP-positive patient's serum (1 : 2000) was added. The binding of primary antibody, anti-CagA antibody, was completely inhibited. (*D,E*) Before primary antibody (JHM-CAP-positive patient's serum) reaction, anti-CagA antibody (1 : 1000) was added, and the amount of primary antibody binding was slightly decreased.

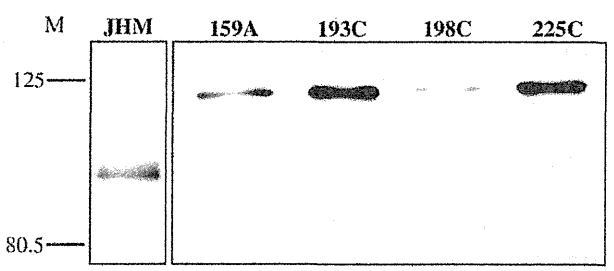


Figure 4 Immuoblot of the Japanese strain–derived high-molecular-weight proteins (JHM-CAP) antigen and the ultrasonicated antigens extracted from four Japanese *Helicobacter pylori* strains with anti-CagA antibody.

Immunoblot of JHM-CAP Antigen and Isolated *Helicobacter pylori* Strains

Immunoblot analysis using the JHM-CAP antigens as well as the four clinical isolates was carried out with anti-CagA antibody. Anti-CagA antibody strongly reacted with a 100-kDa band of the JHM-CAP antigens; however, it reacted with a 120-kDa band of all four clinical strains, where the antigens were extracted by a ultrasonic antigen preparation [21], not a high-molecular-weight cell-associated protein preparation (Fig. 4).

Discussion

There are many methods for the diagnosis of *H. pylori* infection. HM-CAP is one of the most commonly used EIA kits worldwide, and this assay is based on the HM-CAP purified from one U.S. strain (197SR-US) [13]. The sensitivity and specificity of the HM-CAP EIA kit have been reported to be 95–100% in Western countries [3,12,13,15]. However, other studies reported that the accuracy of Western antigen-based EIA kits had been significantly decreased when used in East Asia, high frequency area of gastric cancer. For example, Leung et al. [8] reported that the sensitivity and specificity of HM-CAP were 72.7% and 68.4%, respectively, in Chinese patients. Matsuo et al. [9] reported that the sensitivity and specificity of HM-CAP were 85.7 and 77.1%, respectively, in 251 Japanese patients who showed positive results for *H. pylori* infection. There may be several reasons for the different diagnostic accuracies, for example, the strain heterogeneity of *H. pylori* in different geographic regions [3,9], geographic variation in cross-reactivity to other intestinal pathogens [22], and different immunologic responses to *H. pylori* antigens in different patient populations [6].

However, the reason for lower diagnostic accuracy in an imported serologic EIA kits is not well understood. Several studies have reported the advantages of regional strain-derived antigens in EIA systems [1,5–7,23]. A serum-based EIA test of JHM-CAP prepared by exactly the same procedure as that used to prepare HM-CAP EIA has been developed. When this EIA kit was used in Japanese patients, the sensitivity and specificity were 97.4 and 96.4%, respectively, and the number of intermediate cases decreased dramatically [14]. We have also reported the accuracy of JHM-CAP EIA and HM-CAP EIA in Japanese children [16]. The sensitivity and specificity of these kits were 94.4 and 95.4%, respectively, for JHM-CAP EIA and 67.4 and 93.2% for the HM-CAP EIA. When immunoblot analysis was used, JHM-CAP EIA was found to contain a 100-kDa antigen recognized by JHM-CAP-positive sera, but not HM-CAP EIA. We concluded that the presence of this antigen in the Japanese strains used to prepare JHM-CAP EIA was the reason the high sensitivity of this kit, and hypothesized that this antigen was strongly recognized by the immune system in Japanese patients, even by that in Japanese childhood.

We investigated the 100-kDa protein in this study by mass spectrometry and PMF method and showed that this protein was CagA, and we confirmed that this protein was a fragment of the CagA protein by immunoblot analysis using anti-CagA antibody and an inhibition assay with the serum from JHM-CAP-positive patient before anti-CagA antibody reaction. In this assay, the inhibitory effect to the reaction of anti-CagA antibody by diluted serum was perfect (Fig. 3B,C); however, the inhibitory effect to the reaction of the diluted serum by anti-CagA antibody was incomplete (Fig. 3D,E). The commercially available anti-CagA antibody was a rabbit antibody produced by immunization of a purified CagA protein and was a chemically purified monospecific antibody. On the other hand, the anti-CagA antibody in patient's serum was polyspecific and it might recognize several broad antigenic epitopes on the CagA protein. Therefore, we speculate that the commercially available anti-CagA antibody may incompletely inhibit all antigenic epitopes of the CagA protein reacted by the patient's serum antibody and result in the partial inhibitory effect to the reaction of the serum from a JHM-CAP-positive patient.

There are several reports about the differences in CagA protein between East Asian and Western strains. Analysis of the cagA gene in *H. pylori* strains isolated from patients in East Asia has shown differences in the cagA variable region between East Asian and Western isolates [24,25] and an association between the number of tyrosine phosphorylation motifs (EPIYA motif) and the diseases in Japan [26]. The four clinical isolates that were the origins of the JHM-CAP antigen had a 120-kDa CagA protein (Fig. 4). Therefore, we presumed that the 100-kDa protein is a fragment of the CagA protein and it might be cut during a high-molecular-weight cell-associated protein preparation. Intriguingly, HM-CAP antigen of US clinical isolate had also contained CagA antigen, but its molecular weight was a 120 kDa, not a 100 kDa. This indicates that the CagA protein from Western strain using HM-CAP is not cut during the same process of HM-CAP antigen. However, the comparative structural stability of CagA protein in East Asian and Western strains had not been studied in detail, and little is known regarding the mechanisms of the host immune response to the epitopes on CagA protein or fragmented, small CagA protein in terms of ethnic different responses. In the estimation of the molecular weight of the fragmented protein, EPIYA motif on CagA is not exposed and will not be the antigenic epitopes after fragmentation, which is the remarked different structure between East Asia strains and Western strains. However, the possibility will not be excluded that the different structure around EPIYA motif on CagA has changed the conformational structures linked to the antigenic epitopes after fragmentation.

We hypothesize that there are some structural differences in the CagA protein between the Western and Japanese strains and that the cleavage of CagA protein may have occurred during the antigen purification of protein used in EIA. In addition, Japanese patients

might have highly reacted to the exposed immunoreactive epitopes of the fragmented CagA after antigen purification of JHM-CAP. These observations suggest that the different structure of CagA protein as well as the unique immune responses to the exposed antigenic epitope on CagA protein in Japanese patients including children may be linked to the different disease outcome from Eastern patients.

Acknowledgements and Disclosures

Competing interests: The authors have no competing interests.

References

1 Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, Orentreich N, Vogelman JH, Friedman GD. *Helicobacter pylori* infection and gastric lymphoma. *N Engl J Med* 1994;330:1267–71.
2 Suerbaum S, Michetti P. *Helicobacter pylori* infection. *N Engl J Med* 2002;347:1175–86.
3 Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 2001;345:784–9.
4 Yuan J-M, Yu MC, Xu W-W, Cockburn M, Gao Y-T, Ross RK. *Helicobacter pylori* infection and risk of gastric cancer in Shanghai, China: updated results based upon a locally developed and validated assay and further follow-up of the cohort. *Cancer Epidemiol Biomarkers Prev* 1999;8:621–4.
5 Hoang TTH, Wheeldon TU, Bengtsson C, Phung DC, Sorberg M, Granstroem M. Enzyme-linked immunosorbent assay for *Helicobacter pylori* needs adjustment for the population investigated. *J Clin Microbiol* 2004;42:627–30.
6 Khanna B, Cutler A, Israel NR, Perry M, Lastovica A, Fields PI, Gold BD. Use caution with serologic testing for *Helicobacter pylori* infection in children. *J Infect Dis* 1998;178:460–5.
7 Kim SY, Ahn JS, Ha YJ, Doh HJ, Jang MH, Chung SI, Park HJ. Serodiagnosis of *Helicobacter pylori* infection in Korean patients using enzyme-linked immunosorbent assay. *J Immunoassay* 1998;19:251–70.
8 Leung WK, Ng EKW, Chan FKL, Chung SCS, Sung JJY. Evaluation of three commercial enzyme-linked immunosorbent assay tests for diagnosis of *Helicobacter pylori* in Chinese patients. *Diagn Microbiol Infect Dis* 1999;34:13–7.
9 Matsuo K, Hamajima N, Tominaga S, Suzuki T, Nakamura T, Matsuura A. *Helicobacter pylori* IgG antibody test established in the United States showed a substantially lower sensitivity for Japanese population. *Am J Gastroenterol* 2000;95:1597–8.
10 Sunnerstam B, Kjerstadius T, Jansson L, Giesecke J, Bergstroem M, Ejderhamn J. Detection of *Helicobacter pylori* antibodies in a pediatric patient population: comparison of three commercially available serological tests and one in-house enzyme immunoassay. *J Clin Microbiol* 1999;37:3328–31.
11 Tindberg Y, Bengtsson C, Bergstrom M, Granstrom M. The accuracy of serologic diagnosis of *Helicobacter pylori* infection in school-aged children of mixed ethnicity. *Helicobacter* 2001; 6:24–30.
12 Okuda M, Miyashiro E, Koike M, Tanaka T, Bouoka M, Okuda S, Yoshikawa N. Serodiagnosis of *Helicobacter pylori* infection is not accurate for children aged below 10. *Pediatr. Int.* 2002;44:387–90.
13 Evans DJ, Evans DG, Graham DY, Klein PD. A sensitive and specific serologic test for detection of *Campylobacter pylori* infection. *Gastroenterology* 1989;96:1004–8.
14 Marchildon PA, Sugiyama T, Fukuda Y, Peacock JS, Asaka M, Shimoyama T, Graham DY. Evaluation of the effects of strain-specific antigen variation on the accuracy of serologic diagnosis of *Helicobacter pylori* infection. *J Clin Microbiol* 2003;41:1480–5.
15 Obata Y, Kikuchi S, Miwa H, Yagyu K, Lin Y, Ogihara A. Diagnostic accuracy of serological kits for *Helicobacter pylori* infection with the same assay system but different antigens in a Japanese patient population. *J Med Microbiol* 2003; 52:889–92.
16 Okuda M, Sugiyama T, Fukunaga K, Kondou M, Miyashiro E, Nakazawa T. A strain-specific antigen in Japanese *Helicobacter pylori* recognized in sera of Japanese children. *Clin Diagn Lab Immunol* 2005;12:1280–4.
17 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;15:680–5.
18 Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci* 1979;76:4350–4.
19 Shevchenko A, Wilm M, Vorm O, Mann M. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. *Anal Chem* 1996;68:850–8.
20 Gharahdaghi F, Weinberg CR, Meagher DA, Imai BS, Mische SM. Mass spectrometric identification of proteins from silver-stained polyacrylamide gel: a method for the removal of silver ions to enhance sensitivity. *Electrophoresis* 1999;20:601–5.
21 Sugiyama T, Yoshida H, Takayama Y, et al. A novel enzyme immunoassay for serodiagnosis of *H.pylori* infection. *Gastroenterology* 1991;101:77–83.
22 Graham DY, Evans DJ Jr, Peacock J, Baker JT, Schrier WH. Comparison of rapid serological tests (FlexSure HP and QuickVue) with conventional ELISA for detection of *Helicobacter pylori* infection. *Am J Gastroenterol* 1996;91:942–8.
23 Bodhidatta L, Hoge CW, Chumratanakul S, Nirdnoy W, Sampathanukul P, Tungtaem C, Raktham S, Smith CD, Echeverria P. Diagnosis of *Helicobacter pylori* infection in a developing country: comparison of two ELISAs and a seroprevalence study. *J Infect Dis* 1993;168:1549–53.
24 Yamaoka Y, Kodama T, Kashima K, Graham DY, Sepulveda AR. Variants of the 3′ region of the cagA gene in *Helicobacter pylori* isolates from patients with different *H. pylori*-associated diseases. *J Clin Microbiol* 1998;36:2258–63.
25 van Doorn LJ, Figueiredo C, Sanna R, Pena S, Midolo P, Enders KW.Ng, Atherton JC, Blaser MJ, Quint WGV. Expanding allelic diversity of *Helicobacter pylori* vacA. *J Clin Microbiol* 1998; 36:2597–603.
26 Hatakeyama M. *Helicobacter pylori* and gastric carcinogenesis. *J Gastroenterol* 2009;44:239–48.

Journal of Gastroenterology and Hepatology

JGHF

doi:10.1111/j.1440-1746.2012.07080.x

ORIGINAL ARTICLE

Effect of ecabet sodium treatment on urea breath test and stool antigen tests in volunteers with *Helicobacter pylori* infection

Masumi Okuda,* Noriyasu Yamamoto,* Nobuhisa Fukuda,* Kohei Maekawa,* Takeshi Kusaka,* Manabu Hashimoto,* Junichiro Kotake,* Hiromasa Koizuka† and Yoshihiro Fukuda*

*Department of General Medicine and Community Health Science, Sasayama Medical Center, and †Clinical Nutrition and Health Science, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan

Key words
ecabet sodium, *Helicobacter pylori*, stool antigen test, urea breath test.

Accepted for publication 7 September 2011.

Correspondence
Masumi Okuda, Department of General Medicine and Community Health Science, Sasayama Medical Center, Hyogo College of Medicine, Kuro-oka 5, Sasayama, Hyogo 669-2321, Japan. Email: okuda@naxnet.or.jp

Conflict of Interest
No potential conflict of interest has been declared by the authors.

Abstract

Background and Aim: Ecabet sodium is reported to have a bactericidal effect on *Helicobacter pylori* and inhibit urease activity *in vitro*.

Methods: Seven male volunteers (mean age, 51.3 years; range, 45–55 years) with *H. pylori* infection were medicated with 1 g ecabet sodium t.i.d. for 4 weeks. The urea breath test (UBT) was performed 10 times per person: before medication, seven times in 2 weeks, and once in the third and fourth weeks. Stool antigen tests (HpSA PLUS and Testmate pylori antigen) were performed five times per person: before medication and weekly during medication.

Results: The premedication UBT value ranged from 4.9‰ to 77.4‰ and from 2.9‰ to 44‰ at the end of the treatment period. Not one of the subjects had a negative UBT result during medication. The optical densities of the HpSA and Testmate pylori antigen tests ranged from 0.4 to > 3.0 premedication and from 0.0 to > 3.0 at the end of treatment. HpSA and Testmate pylori antigen were negative in two cases.

Conclusions: In this study, ecabet sodium did not effect the results of UBT in volunteers with *H. pylori* infection. Ecabet sodium may influence stool antigens because in two of seven cases the *H. pylori* stool antigen tests returned negative results.

Introduction

Helicobacter pylori infection is known to be associated with gastroduodenal or hematologic diseases, including gastritis, peptic ulcer disease, low-grade mucosa-associated lymphoid tissue lymphoma, gastric cancer and idiopathic thrombocytopenic purpura. Eradication of *H. pylori* is recommended for these diseases in Japan.[1] Ecabet sodium, which is a widely used cytoprotective agent for the gastric mucosa, is reported to have anti-pepsin activity and a bactericidal effect on *H. pylori*.[2,3] This antibacterial effect of ecabet sodium is observed even with clarithromycin- and metronidazole-resistant *H. pylori* strains.[3] Furthermore, ecabet sodium is reported to inhibit urease activity *in vitro*.[4,5] The aim of this study was to investigate the effect of ecabet sodium on urease activity and stool antigen tests in volunteers with *H. pylori* infection. We used the UBT and stool antigen tests in this study, because they are recommended as non-invasive tests in the guidelines of the Japanese Society for Helicobacter Research.[1]

Methods

Subjects

Forty-one volunteers (mean age, 43.5 years; range, 27–55 years; 38 men, 3 women) were evaluated for *H. pylori* infection using the urea breath test (UBT), which revealed that 10 were infected and of them, seven volunteers (mean age, 51.3 years; range, 45–55 years) were enrolled in this study.

This study was approved by the departmental ethics committee and informed consent was given by all subjects.

Protocol

The volunteers were administered 1 g ecabet sodium (Gastrom; Mitsubishi Tanabe Pharma Corp., Osaka, Japan) t.i.d. for 4 weeks. The UBT and stool antigen tests were performed as follows (Fig. 1).

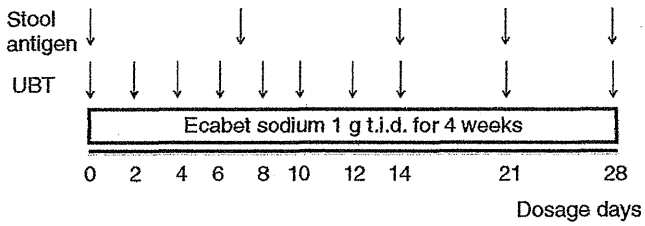


Figure 1 Protocol of administration of ecabet sodium and performance of urea breath test (UBT) and stool antigen tests.

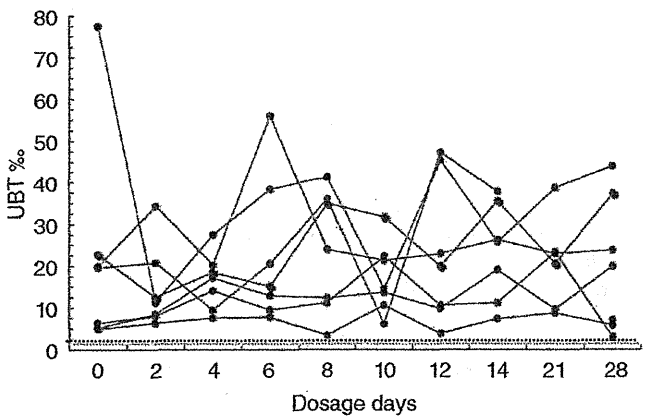


Figure 2 Urea breath test (UBT) values of individuals during medication of ecabet sodium.

The UBT was performed 10 times per person: before medication, seven times in 2 weeks, and once in the third and fourth weeks. After fasting for at least 4 h, subjects were administered 100 mg of ^{13}C-Urea (UBIT tablet; Otsuka Pharmaceutical, Tokyo, Japan) and instructed to remain in left lateral recumbency for 5 min, followed by a sitting position for 15 min. Breath samples before and 20 min after tablet administration were collected and the ^{13}C concentration was measured using an infrared spectrometer (UBiT-IR300; Otsuka Electronics Co., Hirakata, Japan). An increase of more than 2.5‰ was considered positive.

Stool antigen tests were performed five times per person: before medication and every week during medication. We used enzyme immunoassay kits (Premier Platinum HpSA PLUS [HpSA ELISA II: Meridian Diagnostics, Inc., Cincinnati, OH, USA] and Testmate pylori antigen [Wakamoto Pharmaceutical Co. Ltd, Tokyo, Japan]) according to the manufacturer's instructions. Absorbance was measured at wavelengths of 450 nm and 630 nm, with a cut-off value of 0.120.

Results

The premedication UBT value ranged from 4.9‰ to 77.4‰ and from 2.9%‰ to 44‰ at the end of medication (i.e. at 4 weeks). Not one subject had a negative result during medication (Fig. 2, Table 1). Using paired *t*-test, there is no statistical difference of before and last UBT values.

The optical densities of the stool antigen tests ranged from 0.38 to > 3.00 premedication and from 0.00 to > 3.00 at the end of medication (Table 2). In total, there were two cases of a negative stool antigen results, but the UBT remained positive (Fig. 3).

Table 1 Urea breath test (UBT) values before and after ecabet sodium administration in subjects infected with *Helicobacter pylori*

Case no.	UBT (%)		
	Before	Mean	Last
1	5.1	13.7	19.9
2	4.9	6.8	5.8
3	22.8	25.1	37.3
4	19.9	28.0	23.7
5	77.4	31.9	44.0
6	19.6	23.1	7.0
7	6.3	12.6	2.9

Table 2 Optical densities of stool antigen tests before and after ecabet sodium administration in subjects infected with *Helicobacter pylori*

Case no.	HpSA		Testmate	
	Before	Last	Before	Last
1	2.25	0.08	1.21	0.08
2	> 3.00	2.41	2.32	0.51
3	> 3.00	> 3.00	2.66	1.73
4	> 3.00	2.77	1.31	1.86
5	1.81	> 3.00	0.77	1.23
6	1.84	0.00	0.38	0.02
7	2.16	0.53	1.03	0.13

Discussion

Ecabet sodium is derived from pine resin and was used for the treatment of gastric disease in ancient China. It is very safe and widely used for peptic ulcer disease and gastritis in Japan. Ecabet sodium has a high affinity for the gastric mucus and has anti-pepsin activity and mucosal defensive factors.[2] We reported that a threefold increase in the dosage of ecabet sodium inhibited *H. pylori* infection in Japanese monkeys,[6] and several reports have demonstrated that treatment regimens including ecabet sodium increase the rate of eradicating *H. pylori*.[7–10] Usual quantity of ecabet sodium is 1 g b.i.d. in Japan, but in order to examine the effects more clearly, we used 1 g ecabet sodium t.i.d. which is safety.

It is reported that ecabet sodium irreversibly inhibits the coarse urease derived from *H. pylori* at pH 5, which is the optimal pH for urease, but there was no inhibition at pH 8.0.[4,5] However, in the present study using human volunteers, the medication of 1 g ecabet sodium t.i.d. did not affect the UBT values. Murakami *et al.* reported that UBT values showed a tendency to decrease after administration of ecabet sodium to volunteers of *H. pylori* infection.[11] Background of the subjects or number of subjects may effect the results, and further studies are needed.

In two of the seven volunteers, stool antigen tests turned negative during medication and both volunteers had persistence of *H. pylori* infection after discontinuation of ecabet sodium. Orally administered ecabet sodium is distributed mainly in the gas-

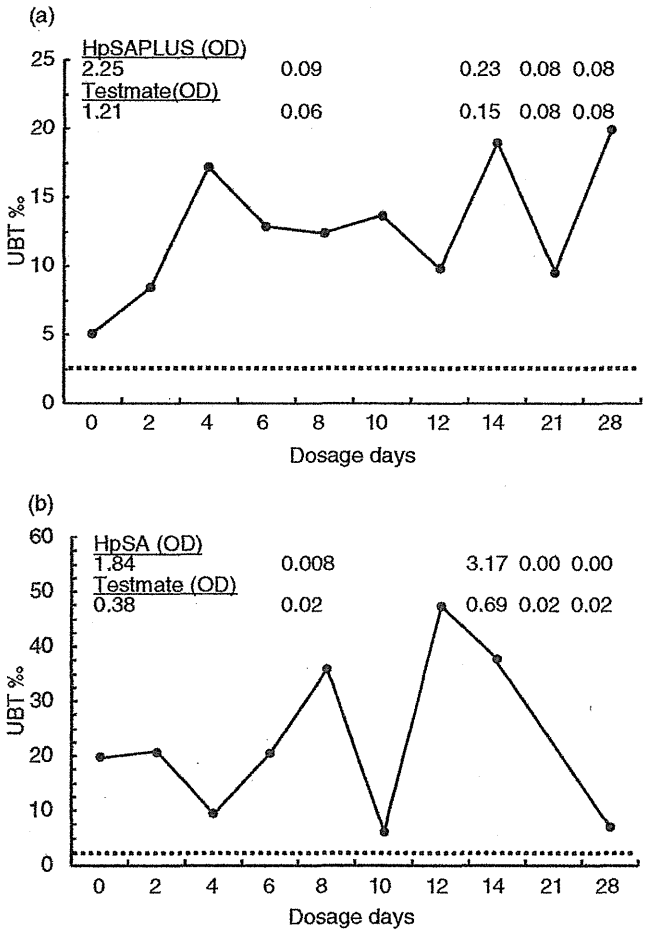


Figure 3 Urea breath test (UBT) and stool antigen tests (HpSA PLUS and Testmate) of (a) case 1 and (b) case 6. OD, optical density.

trointestinal tract, and the intestinal absorption rate in rats has been estimated to be only 3.4–7.0% of the dose.[12] We examined the influence of ecabet sodium on chromogenic reactivity in Testmate pylori antigen *in vitro*. A control *H. pylori* antigen was added to sample dilution buffer containing ecabet sodium, and the reactivity of the sample dilution buffer was examined by Testmate pylori antigen. The reactivity of Testmate pylori antigen was not influenced by ecabet sodium. The high affinity of ecabet sodium for the gastric wall[12] may contribute to the reduction of *H. pylori* from the stomach to the intestine, represented by low optical densities of the stool antigen tests, or it may directly inhibit *H. pylori* in the stomach. But, as there are no reports about the effects of ecabet sodium on stool antigen tests, we are going to have a further investigation.

In conclusion, ecabet sodium did not affect the UBT values in volunteers with *H. pylori* infection. In two of the seven volunteers, *H. pylori* stool antigen tests turned negative. Ecabet sodium may influence the stool antigens during medication.

References

1 Asaka M, Kato M, Takahashi S *et al.*; Japanese Society for Helicobacter Research. Guidelines for the management of *Helicobacter pylori* infection in Japan: 2009 revised edition. *Helicobacter* 2010; **15**: 1–20.
2 Ito Y, Nakamura S, Onoda Y *et al.* Effects of the new anti-ulcer drug ecabet sodium (TA-2711) on pepsin activity. I. Inactivation of enzyme protein. *Jpn. J. Pharmacol.* 1993; **62**: 169–74.
3 Shibata K, Ito Y, Hongo A *et al.* Bacterial activity of a new antiulcer agent, ecabet sodium, against *Helicobacter pylori* under acidic conditions. *Antimicrob. Agents Chemother.* 1995; **39**: 1295–9.
4 Ito Y, Hongo A, Kinoshita M *et al.* Mechanism of anti-urease action by the anti-ulcer drug ecabet sodium. *Biol. Pharm. Bull.* 1995; **18**: 850–3.
5 Ito Y, Shibata K, Hongo A *et al.* Ecabet sodium, a locally acting antiulcer drug, inhibits urease activity of *Helicobacter pylori*. *Eur. J. Pharmacol.* 1998; **345**: 193–8.
6 Fukuda Y, Mizuta T, Yamamoto I *et al.* The novel anti-ulcer agent ecabet sodium (TA-2711) eradicates *Helicobacter pylori* colonizing on the gastric mucosa of Japanese monkeys. *Scand. J. Gastroenterol.* 1994; **29**: 1055–6.
7 Fukuda Y, Yamamoto I, Okui M *et al.* Combination therapies with a proton pump inhibitor for *Helicobacter pylori*-infected gastric ulcer patients. *J. Clin. Gastroenterol.* 1995; **20** (Suppl. 2): S132–5.
8 Ohkusa T, Takashimizu I, Fujiki K *et al.* Prospective evaluation of a new anti-ulcer agent, ecabet sodium, for the treatment of *Helicobacter pylori* infection. *Aliment. Pharmacol. Ther.* 1998; **12**: 457–61.
9 Kagaya H, Kato M, Komatsu Y *et al.* High-dose ecabet sodium improves the eradication rate of *Helicobacter pylori* in dual therapy with lansoprazole and amoxicillin. *Aliment. Pharmacol. Ther.* 2000; **14**: 1523–7.
10 Kim HW, Kim GH, Cheong JY *et al. H. pylori* eradication: a randomized prospective study of triple therapy with or without ecabet sodium. *World J. Gastroenterol.* 2008; **14**: 908–12.
11 Murakami K, Sato R, Okimoto T *et al.* Influence of anti-ulcer drugs used in Japan on the result of (13)C-urea breath test for the diagnosis of *Helicobacter pylori* infection. *J. Gastroenterol.* 2003; **38**: 937–41.
12 Ito Y, Sugawara Y, Takaiti O *et al.* Metabolic fate of a new anti-ulcer drug (+)-(1R,4aS,10aR)-1,2,3,4,4a,9,10,10a-octahydro-1, 4a-dimethyl-7-(1-methylethyl)-6-sulfo-1-phenanthrenecarboxylic acid 6-sodium salt pentahydrate (TA-2711). II: distribution in the rat stomach. *J. Pharmacobio-dyn.* 1991; **14**: 547–54.

Helicobacter Research

別　刷

発行：株式会社　先端医学社
〒103-0007　東京都中央区日本橋浜町2-17-8　KDX浜町ビル

特集

小児・青年期の *Helicobacter pylori* 感染症：
胃癌予防を考慮した診療方法を考える

小児・若年者の *Helicobacter pylori* 感染率と感染経路：胃癌予防のために小児科医がすべきこと

奥田真珠美[1)]　坊岡美奈[2)]　辻　知見[3)]　檜皮谷朋子[4)]
前川講平[1)]　菊地正悟[5)]　福田能啓[1)]

小児の *Helicobacter pylori*（*H. pylori*）感染率は地域差があるが，2～5%と急速に低下している．生活環境が良くなったことに加え，親世代の感染率の低下が影響している．*H. pylori* 感染は乳幼児期早期に成立した後は新たな感染は少なく，感染の多くが家族内感染であり，親から子への感染が主であると考えられるが，感染様式は不明であり，感染予防をすることは困難である．親になる前の若年者の除菌治療をおこなうことで小児への感染を予防し，すでに感染している小児については適切な時期に除菌治療をおこなうことにより胃癌を予防することが可能になると考える．

KEY WORDS
小児，*Helicobacter pylori*（*H. pylori*），感染率，感染経路，胃癌予防

はじめに

Helicobacter pylori（*H. pylori*）感染は小児期に成立し，持続感染する．感染者は未感染者にくらべて胃癌のリスクが高くなるが，感染既往がないと胃癌発生はきわめてまれであることから最大の胃癌予防策は *H. pylori* 感染阻止である．

本稿では，小児・若年者の感染率・感染時期と感染経路を検討し，感染予防と胃癌予防における小児科医の役割を考える．

1．日本人の *H. pylori* 感染率

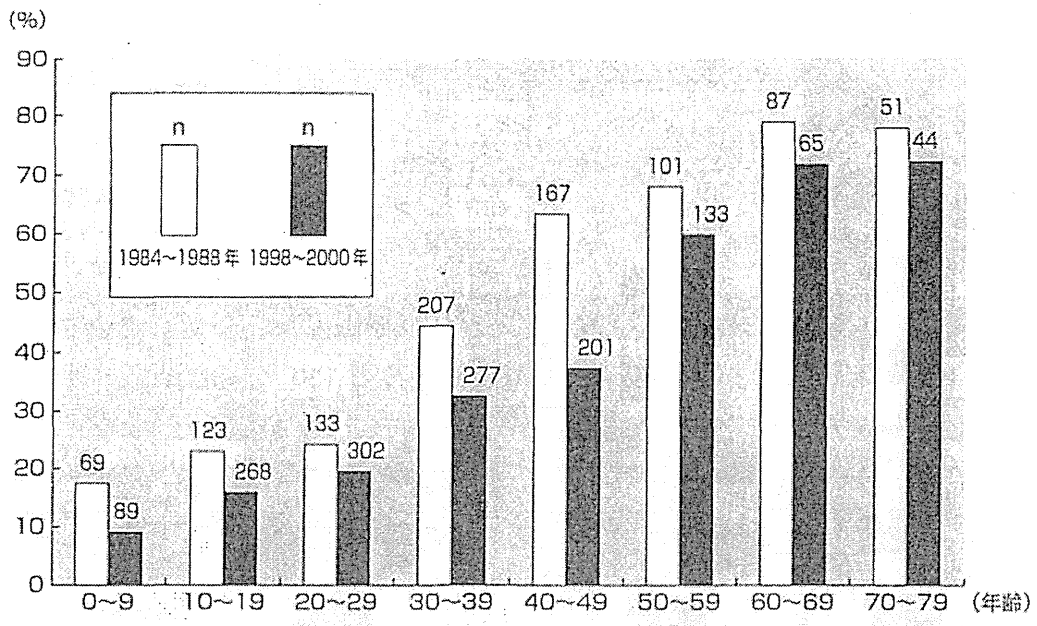
1）兵庫県における *H. pylori* 抗体保有率[1)]

兵庫県の 0～79 歳の無症状のボランティア 2,317 名の血清抗 *H. pylori* 抗体を測定した（1984～1988 年 938 名，1998～2000 年 1,379 名）（図❶）．いずれの年齢層でも抗体保有率が低下してきている．

2）小児・若年者の *H. pylori* 感染率と地域差

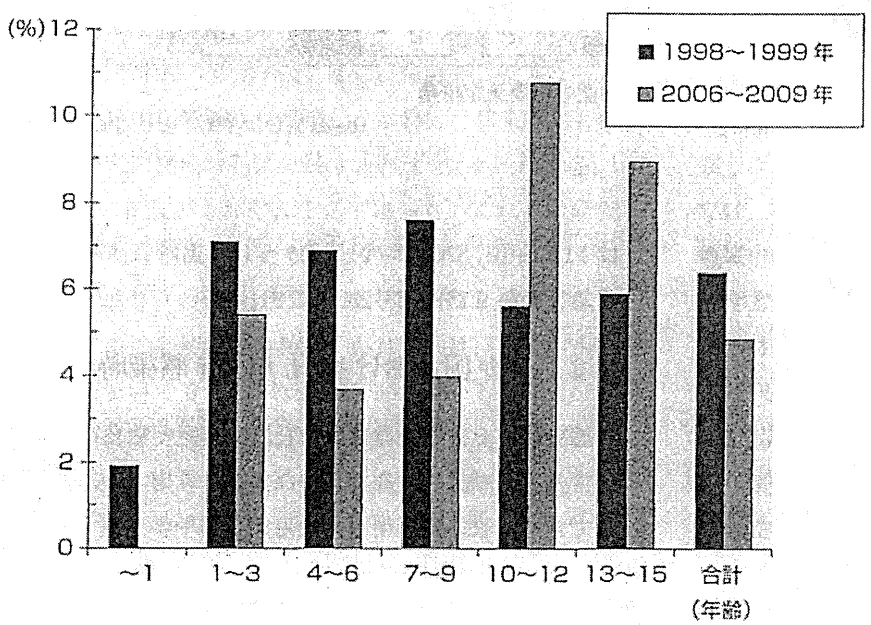
われわれは 1998～1999 年[2)]と，2006～2009 年に和

1) Okuda Masumi, Maekawa Kohei, Fukuda Yoshihiro/兵庫医科大学地域総合医療学
2) Booka Mina/和歌山県立医科大学小児科
3) Tsuji Tomomi/公立那賀病院小児科
4) Hiwatani Tomoko/和歌山労災病院小児科
5) Kikuchi Shogo/愛知医科大学公衆衛生学



図❶ 日本人の抗 *H. pylori* 抗体保有率の変遷

（奥田真珠美ら，2010[1]より引用）

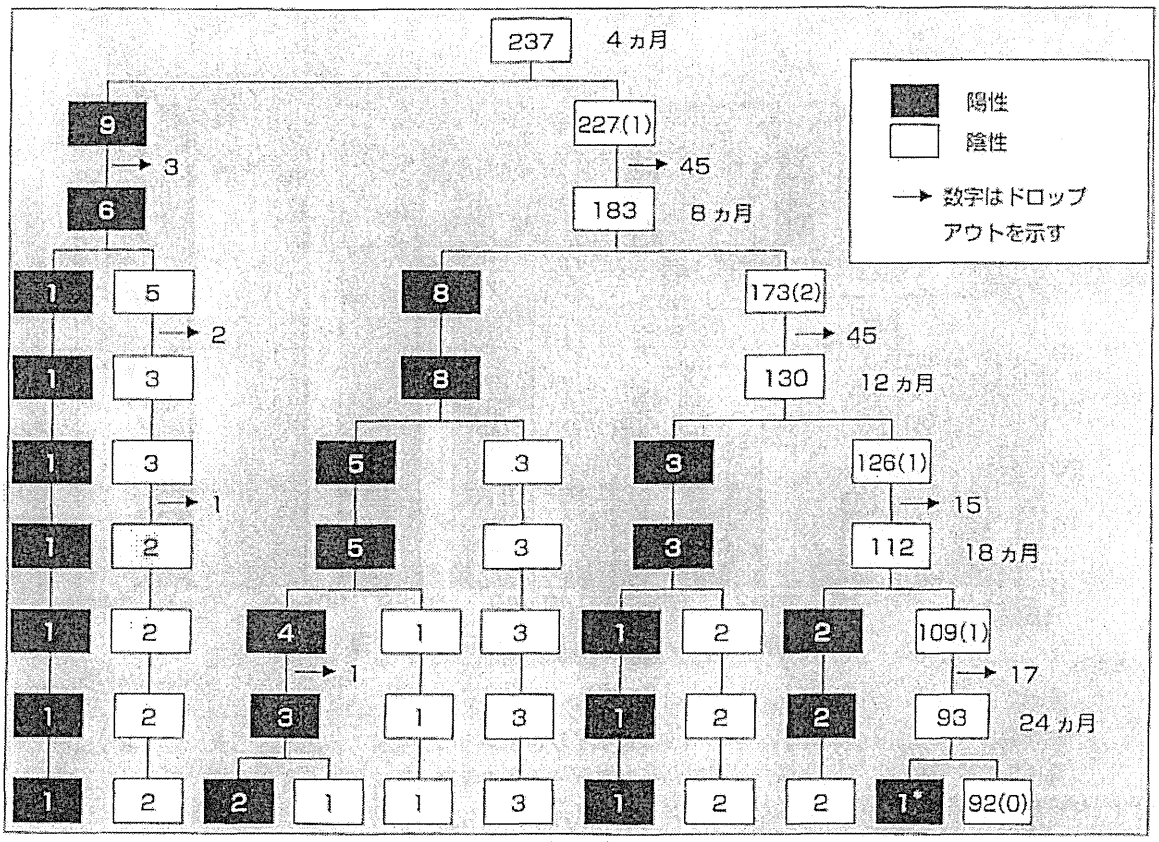


図❷ 和歌山県における小児の便中抗原陽性率

1998～1999年（n＝501）*，2006～2009年（n＝1,000）

（＊：Okuda M *et al*, 2001[2]より引用）

山県下の小児の便中 *H. pylori* 抗原を測定した．前者は和歌山市内を中心とし，便中抗原はメリディアン HpSA ELISA を用いたもので，後者は和歌山市，御坊市周辺，田辺市の小児も含み，便中抗原はテストメイトピロリ抗原を用いたものである．約 10 年間隔の検討であるが，2006～2009 年には 0～9 歳の小児で感染率の低下を認めた（図❷）．和歌山県の山間部にある M 地区で 2006 年におこなった調査であるが，同地区に住む 15 歳以下の小児 231 名のうち 181 名（78.3%）で便中抗原検査をおこない，陽性率は 9.4%であった．和歌山県下の感染率の推移で，2006～2009 年の 10～15 歳で M 地区の小児が占める割合が大きいためこの年齢層の陽性率が前期より



図❸ 生後 4〜24 カ月までの便中抗原測定経過

＊：後に陰性

(Okuda M *et al*, 2007[3] より引用)

後期のほうで高くなっている．筆者が勤務する篠山市の0〜9 歳の小児で 2010 年に調査した便中抗原陽性率は約 2%であった．赤松ら[3]は長野県の高校 2 年生（16〜17 歳）の尿中抗体（ラビラン®）陽性率を検討し，2007 年 3.4%（14/409），2008 年 7.6%（28/370），2009 年 4.9%（22/445），2010 年 4.8%（23/478）で全体では 5.1%（87/1702）であった．小児の *H. pylori* 感染率には地域差があるが，いずれの地域でも顕著に低下してきている．小児・青年期の *H. pylori* 感染率は地域によって若干差はあるものの 2〜5%（高い地域では 10%）であると推定できる．

若年者の感染率であるが，Konno ら[4]は 1995〜1999 年に調査した妊婦 350 名の抗 *H. pylori* 抗体保有率は 19.7%であったと報告しており，約 15 年前であるが若年者の感染率は低下してきていることが明らかである．われわれは H 医科大学学生（主として 5 年生）の *H. pylori* 感染率を検討したが，2001〜2006 年までの抗体保有率は 11.7%(61/520)，2009〜2011 年は尿素呼気試験(UB でおこない 9.7%（23/238）であった．

2．わが国における *H. pylori* 感染時期

感染時期を知ることは感染経路の特定と除菌時期を定するうえで重要である．すなわち，乳児から幼児期期であれば家族内感染が，幼児期後期や学童期であれ家族外感染が疑われる．感染診断や除菌治療は新たな染リスクがなくなる時期におこなう必要がある．

感染の多くは小児期に成立すると報告されている．きに示した兵庫県のデータ[1]からもわかるように，抗保有率はいずれの年齢層でも低下してきており，右側のシフトが認められ，成人での新たな感染の頻度はきわめて低いことがわかる．

わが国で便中抗原を用いて乳児から幼児期の感染時期を前向きに検討した二つの報告がある．Konno ら[4]が，本

表❶ 生後4ヵ月～6歳までの便中抗原経過

	4ヵ月	8ヵ月	12ヵ月	18ヵ月	24ヵ月	30ヵ月	3歳	4歳	5歳	6歳
検査総数	237	189	142	126	108	80	61	40	33	21
陽性数	9	9	9	8	5	3	1	2	1	2
陽性率	3.8	4.8	6.3	6.3	4.1	3.8	1.6	5.0	3.0	9.5
陽転	9	8	3	2	1	1	0	1	0	1
陽転率	3.8	4.4	2.3	1.7	1.0	1.3	0	2.6	0	5.0
陽転率/月	0.95	1.1	0.58	0.28	0.17	0.22	0	0.22	0	0.42
感染成立	1	2	1	0	0	1	0	0	0	1

観市で抗 *H. pylori* 抗体陽性の母親から生まれた44名の便中 *H. pylori* 抗原を5年間追跡調査した研究では，5名の感染を確認し，感染時期は1歳4名，4歳1名であった．われわれ[5]は，和歌山市で2001年2月～2002年4月に出生した乳児237名の感染時期を前方視調査した（図❸）．この検討はポリクローナル抗体のHpSAを用いた検討であるが，陽性になっても陰転する症例がまれではなかった．1ヵ月あたりの陽転率は生後4～8ヵ月において最も高く1.1%，ついで生後4ヵ月までの0.95%，生後8～12ヵ月の0.58%で，その後陽転率は低くなった（表❶）．便中抗原陽性が持続し，最終的に感染成立と判断した小児の感染時期は生後4ヵ月1名，8ヵ月2名，1歳，2歳，6歳がそれぞれ1名であった．これらの研究で感染時期を確認できた11名のうち8名（73%）が2歳未満であり，わが国における感染時期は2歳までが高頻度であることが示唆された．われわれは，和歌山市と篠山市において0～9歳の小児約500名を1年以上の間隔で便中抗原で追跡検査したが，陽転児を見出せなかった．このことからも多くの感染は乳児期あるいは幼児期早期に成立していると考えられる．

3．感染経路

家族内感染，とくに母-子感染が重要であることはこれまでも多くの国からの報告があり主要な感染経路のひとつである．一方，途上国では家族外感染がメインというものや，水からの感染が重要であるという報告も多い．Konno ら[4]の報告では感染がある母親の子どもを5歳まで観察したときの感染率は11.3%であった．このことから，感染リスクが一番高い小児でも感染は約1/10となるが，図❶からもわかるようにわが国で少なくとも30～40年間は高い感染率を維持してきたことは何らかの環境因子や水系感染があったと推測され，現在に至っては感染経路や感染様式が変化してきていると考えられる．さらに，小児をとりまく成人の感染率の低下は小児の感染率の急速な低下の要因となっている．

1）家族内感染

家族内はおもな感染経路である．Konno ら[6]によると，*H. pylori* 感染が確認された子どもの家族の検討では *H. pylori* DNA パターンは約7割が母親と一致し，家族と一致しなかったのは約2割であった．篠山市における検討では，小児の感染状況は父母ともに同率であった．この地域では共働きが多いためか，父親が子どもの病院受診に付き添う場面が多く，育児への貢献度が感染経路に関与していると考えられた．

2）家族外感染

家族外として，保育施設や障害児（者）施設での感染があげられる．重症心身障害児施設に入園1年後の抗体陽転率がきわめて高い[7]ことが報告されており，施設内感染が重要であることが示されている．胃チューブの挿入者が多く，吸引を頻回におこない，胃・食道逆流，嘔吐の頻度も高いため感染の機会が相当に高い環境であることは推測できる．

4．感染様式・感染に関与する要因

H. pylori が経口感染であることは一致した見解であるが，感染を媒介するものについては十分解明されておら

ず，具体的に感染予防策を講じることは困難である．

唾液や扁桃，歯垢などからPCR法で*H. pylori*が分離された報告が多くあり，口-口感染経路は重要と考えられている．母の悪心・嘔吐が頻繁であると子の感染率が高くなる[8]というものや家族の胃腸炎症状はとくに2歳未満の乳幼児の感染リスクとなる[9]という報告がある．嘔吐物や下痢便から*H. pylori*が検出されるかどうかの検討でも下剤，催吐剤投与前後の便，唾液，嘔吐物の*H. pylori*培養をおこなったところ，催吐剤で嘔吐後の唾液や下剤投与後の下痢便で培養が可能であった[10]と報告されている．感染者が嘔吐・下痢を伴う病態では通常より感染源になりやすいと考えられる．飲用水については，小児期に飲用した井戸水が同じであると感染菌株のパターンが同一で，井戸水からの感染が示唆された[11]という報告があるが，水からの*H. pylori*検出は困難であり，先進国の主たる感染経路ではないと考えられる．産道感染については，新生時期に便中抗原あるいはPCR法で*H. pylori*が検出される[12]報告があるが，いずれも追跡調査で自然消失し持続感染はなく否定的である．

5．胃癌予防のために小児科医がすべきこと

*H. pylori*は乳幼児期早期に成立した後は新たな感染はきわめて少ないと考えられる．まず，*H. pylori*感染の多くが家族内感染であり，親から子への感染が主であると考えられるが，感染様式は不明であり，感染予防をすることは困難である．したがって，感染源となる大人の除菌治療をおこなうことで感染予防ができるが，乳幼児期に感染するため親になる前の除菌が必要となるだろう．祖父母からの感染があるのかはこれからの検討課題である．すでに感染している小児については小児科医がさらに明らかにすべきことがある．再感染のリスクがなく，かつ胃粘膜変化が可逆的である最適な除菌の時期，また，クラリスロマイシン耐性率が高いことが報告されているため除菌レジメンの検討も必要である．

小児・青年期の*H. pylori*感染率は低下している．小児・青年期において胃癌予防のために*H. pylori*の感染診断・除菌治療をすることは現実的だと思われる．さらに，除菌治療は次世代への伝搬防止となり，いずれ*H. pylori*感染診断さえ必要なくなるであろう．

おわりに

わが国における小児・青年期の*H. pylori*感染率域差があるが2〜10%程度ときわめて低くなってお胃癌予防のための若年者に対する除菌治療も現実的のとなってきた．現在の小児が胃癌に苦しむことが未来をめざすとともに，*H. pylori*感染なしに成長すども達が将来罹患しやすくなる疾患をも考慮してい要があるだろう．

文　献

1) 奥田真珠美，福田能啓：ヘリコバクターピロリ—小の感染時期と感染経路．綜合臨牀 59：1987-1988,
2) Okuda M, Miyashiro E, Koike M *et al*：Breast-fe prevents *Helicobacter pylori* infection in early childl *Pediatr Int* 43：714-715, 2001
3) 赤松泰次，市川真也，奥平貞英ほか：ヘリコバクタピロリ感染症の学校検診への導入．日本ヘリコバク学会誌 14：7-11, 2012
4) Konno M, Fujii N, Yokota S *et al*：Five-year follov study of mother-to-child transmission of *Helicob pylori* infection detected by a random amplified poly phic DNA fingerprinting method. *J Clin Microbiol* 2246-2250, 2005
5) Okuda M, Miyashiro E, Booka M *et al*：*Helicobacter p* colonization in the first 3 years of life in Japa children. *Helicobacter* 12：324-327, 2007
6) Konno M, Yokota S, Suga T *et al*：Predominance of M er-to-child transmission of *Helicobacter pylori* infec detected by random amplified polymorphic DNA fin printing analysis in Japanese families. *Pediatr Infect L* 27：999-1003, 2008
7) 蓮井正樹，本家一也，辻春江ほか：重症心身障害児における血清抗 *Helicobacter pylori* 抗体価の1年後の動．脳と発達 30：352-353, 1998
8) Ito LS, Oba-Shinjo SM, Shinjo SK *et al*：Communi based familial study of *Helicobacter pylori* infection am healthy Japanese Brazilians. *Gastric Cancer* 9：208-2 2006
9) Perry S, Sanchez ML, Yang S *et al*：Gastroenteritis transmission of *Helicobacter pylori* infection in househo *Emerg Infect Dis* 12：1701-1708, 2006

10) Parsonnet J, Shmuely H, Haggerty T : Fecal and oral shedding of *Helicobacter pylori* from healthy infected adults. *JAMA* 282 : 2240-2245, 1999

11) Karita M, Teramukai S, Matsumoto S : Risk of *Helicobacter pylori* transmission from drinking well water is higher than from infected intrafamilial members in Japan. *Dig Dis Sci* 48 : 1062-1067, 2003

12) Stray-Pedersen A, Gaustad P, Stray-Pedersen B *et al* : Detection rate of *Helicobacter pylori* stool antigen in newborn infants and small children. *J Perinat Med* 35 : 155-158, 2007

＜消化管＞

Helicobacter pylori 感染症の診断と治療

奥田真珠美*1,2 坊岡美奈*3 辻 知見*4 西岡隆文*1,2
前川講平*1,2 高木信明*5 福田能啓*2

はじめに

胃内に細菌が認められることは19世紀末から観察されていたがその後，否定的な報告が多く，一過性の細菌を除き胃内は無菌の環境であると考えられてきた。しかし，1982年にオーストラリアのMarshallとWarrenによって初めてヒトの胃粘膜からグラム陰性のらせん状桿菌（*Helicobacter pylori*：*H. pylori*）が分離培養され，胃に存在する細菌が世に認められるようになった。

Ⅰ．*H. pylori* の生物学的特徴，感染率，感染時期と感染経路

1．生物学的特徴

H. pylori はグラム陰性のらせん菌で複数の鞭毛をもつ微好気性菌である。胃内の強力な酸から逃れるためによく発達した鞭毛を回転させ，酸度が中性になっている胃粘膜下層に侵入し，胃粘膜上皮細胞や細胞間隙あるいは粘液内に存在する。強いウレアーゼ活性を有し，尿素を二酸化炭素とアンモニアに分解し，このアンモニアが酸を中和し菌の周囲のpHを上昇させて棲息する環境をつくっている。

2．感染率

日本人の感染率は二相性で，60歳台以降は60%以上と非常に高率であるが，60歳未満では30～40%と低くなっている。感染の多くは小児期に成立し，高い感染歴は第二次世界大戦による衛生環境が悪かった時代に成立したものと推測されている。便中抗原を用いた検討では小児の感染率は2009年に行った和歌山県の保育園・幼稚園児で4.3%（20/469），2010年に兵庫県篠山市で小学校3年生以下の小児を対象としたもので1.9%（13/689）であった。

3．感染時期と感染経路

乳幼児期は感染のリスクが最も高い時期と考えられている。急性感染の経過をとることはきわめてまれで，ほぼ無症状のうちに感染が成立する。日本で便中 *H. pylori* 抗原を用いて前方視的に感染時期を検討した報告[1,2]では，計11名の小児の感染時期（便中抗原陽転年齢）を同定し，0歳3名，1歳5名，2歳，4歳，6歳が各1名であり初感染は就学前の小児，とくに2歳以下に多いと考えられる。

感染経路は家族内が主であり，とくに母から子への感染が重要であると報告されている[1]が，子育てへの貢献度によるのか，父と子の感染が一致する場合もまれではない。

Okuda Masumi Bouoka Mina Tsuji Tomomi
Nishioka Takafumi Maekawa Kohei Takagi Nobuaki
Fukuda Yoshihiro
*1 兵庫医科大学ささやま医療センター小児科
〔〒669-2321 兵庫県篠山市黒岡5〕
TEL 079-552-1181 FAX 079-552-5343
E-mail：okuda@naxnet.or.jp
*2 兵庫医科大学地域総合医療学
*3 和歌山県立医科大学小児科
*4 公立那賀病院小児科
*5 兵庫医科大学小児科

表 1　生検組織を用いた診断法の感度・特異度（小児）

報告者	対象数	年　齢	検査法	感度（%）	特異度（%）
Roma-Giannikou E ら[8] （ギリシア）	1590 名	陽性平均 10.4 歳 陰性平均 7.3 歳	培養法 迅速ウレアーゼ 検鏡法	84.6 83.4 93.2	100 99 100
Guarner J ら[9] （Review）	Review	小児	培養法 迅速ウレアーゼ 検鏡法	55～96 75～100 66～100	100 84～100 94～100

Ⅲ．感染診断法

内視鏡による生検組織を必要とするものと必要としない診断法がある。診断法が複数であれば精度はさらに高くなる。それぞれの診断法には特徴があるため，理解したうえで選択する。

1．生検組織を必要とする診断法

生検組織を用いた診断法の感度，特異度（表 1）であるが，いずれの検査法も特異度は悪くないが感度はやや劣ることに注意が必要である。

1）培養法

唯一の直接的証明法であり，薬剤感受性試験もできる。感度がやや落ちるが特異性は 100%であり，陽性であれば感染ありと確定できる。専用培地が必要なため，外注検査となる医療機関が多い。組織を送付する際には *H. pylori* 用の保存輸送用培地が必要であり，検査前に取り寄せておく必要がある。

2）迅速ウレアーゼ試験

胃生検組織中に含まれる菌のウレアーゼ活性を検出することにより，間接的に *H. pylori* の存在を確認する。試薬は尿素と pH 指示薬を利用したもので，アンモニアが生じることによって pH が上昇し，pH の変化に伴う指示薬の変化で診断する。陰性を確認するための時間は 30 分から 3 時間である。薬物などで胃内 pH が上昇すると菌のウレアーゼ活性が低下し偽陰性となる可能性がある。

3）検鏡法

胃生検組織標本上で菌による組織変化と併せて形態学的にらせん状菌を検出し，同時に組織診断も可能であるが熟練が必要である。

2．生検組織を必要としない診断法

1）尿素呼気試験（UBT）（図）

内視鏡を使わない検査法のゴールドスタンダードとされ，*H. pylori* がもつ強いウレアーゼ活性を間接的に測定する方法である。呼気の採取と錠剤が飲めない場合は "うがい" が必要となり，乳児では検査が困難である。^{13}C 尿素製剤を服用し，胃内に *H. pylori* が存在すれば尿素はただちに胃内でアンモニアと $^{13}CO_2$ に分解され，$^{13}CO_2$ は呼気に排出される。服用前後の呼気を採取し，前に採取した呼気中の $^{13}CO_2$ を服用後と比較し増加率から存在を診断する。服用した ^{13}C 尿素がウレアーゼ活性を有する口腔内細菌と接触し，偽陽性の原因となるため，^{13}C 尿素の除去目的で "うがい" が必要である。ユービット錠®とピロニック錠®（いずれも 100 mg）が市販されているが，表面がフィルムコーティングされた錠剤（ユービット錠®）ではうがいの必要はない。錠剤を飲めない場合は約 100 mL の水で溶解する。投与量は 12 歳未満 75 mg，12 歳以上は 100 mg を大まかな目安としていたが，全年齢 100 mg としても構わない。服用後の呼気はユービット錠®では 20 分後に，ピロニック錠®では服用 10 分後（質量分析法）あるいは服用 15 分後（赤外分光法）に採取する。小児では体位変換は必要ない。日本人小児の多施設研究結果では，カットオフ値を 3.5‰とすると感度 97.8%，特異度 98.5%と報告されている[3]。しかし，成人ではカットオフ値 2.5‰が推奨されているので，われわれは 2.5～3.5‰は gray zone として他の診断法を追加している。抗菌薬や酸分泌抑制薬，とくにプロトンポンプ阻害薬などの内服で偽陰性になるため，最低 2 週間は休薬した後に検査する。

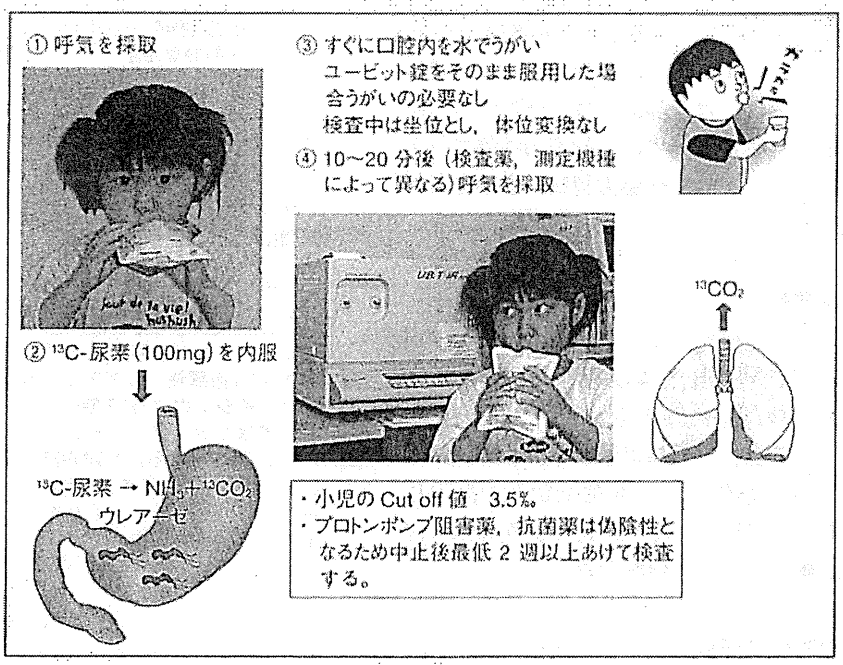


図　小児の UBT（奥田[10]，2012）

2）便中抗原測定法

便を採取するだけというきわめて非侵襲的，簡便な方法は乳幼児，重度の障害児も同様に検査ができる。小児においても成人と同じカットオフ値が用いられるが，抗菌薬の投与の影響については充分な検討はなく，UBT と同様に 2 週間程度の休薬後に検査することが望ましいと考えられる。現在販売されているものはいずれもモノクローナル抗体を用いたもので，小児において 90%以上の感度，特異度が報告されている。イムノクロマト法もあるが，ELISA 法（HpSA plus Meridian，テストメイトピロリ抗原 EIA）が推奨される。

3）抗 *H. pylori* 抗体測定

血清や尿を用いて測定できる。小児では抗原として用いられている菌株によってキット間で感度と特異度に差違がみられ，日本人から分離された菌株を使用したキットでは感度が良い[4,5]。乳児やγグロブリン投与後では受動抗体による偽陽性，除菌後は陰性となるまで長期間を要し偽陽性となる。また，感染の早期や年少児では偽陰性となるため注意が必要であり，診断法として単独では用いない。一方，抗菌薬の影響は受けず，特異度は良好であるため，これらの特性を知っていれば感染の目安となる。

3．その他の検査

H. pylori 感染児では非感染児と比較して血清ペプシノゲンⅠ，Ⅱ値，ガストリン値が上昇する。われわれの検討では血清抗体陽性の 20 歳以下の小児・青年 26 名のうち 8 名（31%）がガストリン 200 pg/mL 以上で，このうち 3 名が 800 pg/mL 以上（800 以上で定量なし）であった。ペプシノゲン，ガストリンが高値の場合，*H. pylori* 感染を疑う必要がある。

Ⅳ．*H. pylori* が関連する疾患，除菌治療が考慮される疾患

H. pylori 感染児の多くは無症状である。「小児期ヘリコバクター・ピロリ感染症の診断・治療，および管理指針[6]」に記載された以下の疾患について除菌治療が考慮される。

1．胃潰瘍，十二指腸潰瘍

日本の小児では，十二指腸潰瘍の約 80%，胃潰瘍の約 40%に *H. pylori* 感染が証明される[7]。