patients with periodontal disease. On the other hand, few studies have successfully cultured this bacterium from oral specimens. It has also been observed that the periodontal-disease-associated bacteria *Porphyromonas, Prevotella* and *Fusobacterium* can produce butyric acid (Kurita-Ochiai *et al.*, 2006) and, recently, Imai *et al.* (2009) indicated that butyric acid is the major short-chain fatty acid (SCFA) produced by *P. gingivalis.* Our previous study demonstrated that the cell supernatants of *C. butyricum* strain MIYAIRI 588 inhibited survival of *H. pylori* (Takahashi *et al.*, 2000). This antibacterial activity is, in part, due to the production of butyric acid by this micro-organism. We demonstrated in the present study that the culture supernatants of *C. butyricum* strain MIYAIRI 588 and *P. gingivalis* strain ATCC 33277 exhibited antibacterial activity against *H. pylori* (Fig. 1b and c). In fact, these bacteria produce various other SCFAs in smaller quantities, such as formic, acetic, propionic and lactic acids, and therefore the butyric acid in the supernatant may not be solely responsible for the bactericidal activity against *H. pylori.*

The antimicrobial effects of these SCFAs have been well characterized. Chaveerach *et al.* (2002) reported that organic acids, such as formic acid, propionic acid and acetic acid have a strong bactericidal effect on *Campylobacter jejuni* and *Campylobacter coli* culturability at low pH. Midolo *et al.* (1995) reported the inhibition of the growth of *H. pylori* by lactic acid, acetic acid and hydrochloric acid in a concentration-dependent manner. These antimicrobial effects are dependent upon acidic pH conditions, which were affected by the organic acids. However, our previous report indicated that butyric acid has an antibacterial effect on *H. pylori* and that this property is independent of acidic pH (Takahashi *et al.*, 2000). In order to clarify this antibacterial action, we used sodium butyrate in the present study, since it does not change the medium pH. After treatment of the *H. pylori* strains with sodium butyrate, growth inhibition of *H. pylori* was detected, correlating with the concentration of sodium butyrate added (Fig. 2). In addition, the c.f.u. value decreased after 48 h of culture and treatment with sodium butyrate, suggesting that the antibacterial effect of butyrate on *H. pylori* was slowly induced. Our previous study indicated that 50 mM butyric acid had antibacterial activity against *H. pylori* within 5 h, but also indicated that *H. pylori* could survive after treatment with 12.5 mM butyric acid (Takahashi *et al.*, 2000). SEM analysis indicated that this antibacterial action causes damage of the cell envelope of *H. pylori* (Fig. 3a). The bacterial cell envelope is responsible for many essential functions such as transport, biosynthesis and cross-linking of peptidoglycans, and the synthesis of lipids, and envelope integrity is absolutely necessary for all of these functions. Disturbance of the cell envelope, directly or indirectly, causes a significant increase in permeability leading to a destabilization of the cell membrane and finally cell death. We analysed the amount of urease in the extracellular environment and the results indicated that the extracellular urease was elevated following treatment with sodium butyrate in a dose-dependent manner (Fig. 3b). Urease is a cytoplasmic protein and becomes associated with cell-surface proteins as well as extracellular proteins in the external environment following bacterial autolysis (Cao *et al.*, 1998). Similarly, the DNA of *H. pylori* was released into the extracellular space after treatment with sodium butyrate. With regard to the origin of the extracellular DNA detected, some reports suggest that cell lysis may be the main source, whereas others observed that DNA could be secreted by specific transport systems in *H. pylori* or in the presence of specific reagents in oral streptococci (Grande *et al.*, 2011; Kreth *et al.*, 2009). We analysed the DNA of *H. pylori* treated with sodium butyrate using random amplification of polymorphic DNA (RAPD)-PCR analysis as described previously (Grande *et al.*, 2011). The pattern and intensity of the bands in the <1 kb size range were identical between the extracellular DNA and control DNA extracted from the cells (data not shown). In the >1 kb size range, multiple attempts at detecting conventional PCR amplicons with *H. pylori* specific primers (*ureA, ureB, vacA* and *oipA*) were unsuccessful, suggesting that DNA fragments greater than 1 kb in size were digested by DNases in the medium. Nevertheless, these results suggested that the extracellular DNA was derived from disintegration of the cells. In addition, we demonstrated that there was a positive correlation between the amount of extracellular DNA and sodium butyrate concentration (Fig. 4). These findings strongly indicated that the antibacterial properties of butyrate on *H. pylori* are bactericidal and act through disintegration of the cell envelope.

In order to determine whether the antibacterial action of the cell supernatants was similar to that of sodium butyrate, extracellular DNA of *H. pylori* was analysed after treatment with the cell supernatants (Fig. 5) and it was found that the amounts of extracellular DNA detected also increased in a dose-dependent manner. These results were similar to the action of sodium butyrate, suggesting that the butyrate produced by these strains may be the principal product responsible for their antibacterial activity. However, we did not obtain any direct evidence of this, so purification and chemical characterization of the butyric acid in these supernatants will be required to resolve this issue. In addition, the antibacterial effects of other SCFAs produced by *P. gingivalis* or *C. butyricum* on *H. pylori* still remain to be determined.

Ishihara *et al.* (1997) found that *P. gingivalis* and *F. nucleatum* strongly coaggregated with *H. pylori.* This finding suggested that these resident oral bacteria might be effectively trapping newly transiting bacteria such as *H. pylori* in periodontal pockets of the oral cavity. Indeed, some reports indicated that *H. pylori* is a transient member of the oral microflora, since the growth of *H. pylori* in the oral cavity is influenced by various factors such as temperature, pH, oxidation–reduction potential, the availability of nutrients, flow of saliva and antimicrobial substances (Song *et al.*, 2000; Silva Rossi-Aguiar *et al.*, 2009;

Okuda *et al.*, 2000, 2003). Furthermore, Imai *et al.* (2009) indicated that *P. gingivalis* culture supernatant contained butyric acid in high concentrations, from 6.7 to 14.7 mM. Moreover, Margolis *et al.* (1988) indicated that the concentration of butyric acid in dental plaque was in the range of 14.4 to 20.0 mM. On the basis of our previous and present results, we have shown that 12.5 mM butyrate is sufficient to exhibit a bactericidal effect on *H. pylori.* Therefore, survival of *H. pylori* in the oral cavity, especially within the periodontal pocket which harbours butyrate-producing bacteria such as *P. gingivalis*, may be difficult based upon this observation. *H. pylori* DNA released from dying cells into the environment would therefore be increased in concentration and might contribute to periodontal bacterial biofilm formation, as it has been shown that bacterial DNA is a major component of the extracellular matrix. This would then explain the ease of detection of *H. pylori* DNA in the human oral cavity by PCR, and the difficulty of detection by culture methods in patients with periodontal disease.

In summary, we have characterized the antimicrobial effects of butyrate on *H. pylori.* This molecule exhibited bactericidal effects and reacted with the cell envelope of *H. pylori.* Similar properties were also detected with the culture supernatants of butyrate-producing bacteria, which suggests that *H. pylori* may have difficulty in colonizing sites which harbour butyric acid-producing inhabitants such as *P. gingivalis* in subgingival plaque. It is possible that *H. pylori* previously detected in the oral cavity may have originated from the stomach following regurgitation and as we have shown, the presence of *H. pylori* DNA in the oral cavity, as observed in other studies, may not represent the true viability of the organism in this site.

ACKNOWLEDGEMENTS

We thank Dr Ishihara and Dr Inagaki, Tokyo Dental College, for providing the *P. gingivalis* strain. This work was supported in part by the Ministry of Education, Culture, Sport, Science and Technology (MEXT) with grants for scientific research (20590455 and 21590492), and a grant from the Strategic Research Base Development Program for Private Universities, 2010-2014 (S1001024). This work was also supported in part by the U.S.–Japan Cooperative Medical Science Program, Cholera and Related Diarrheal Diseases Panel.

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Helicobacter pylori の環境中での生存条件についての検討

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要　旨：　*Helicobacter pylori* の感染ルートを明らかにする研究の一端として，生体から環境に暴露された本菌が水中でどの程度生存するかを調べた。さらにマウスへの投与を行って２次感染の原因となるかを検討した.

感染３週後のマウスから採取した胃粘膜懸濁液中の *H. pylori* はハンクス液にて１日放置後に約10分の１の菌数が培養可能であり，３日後には検出限界以下となった．また，滅菌水に懸濁したサンプルでは，１日後には培養されなかった．定量的リアルタイム PCR 法による解析の結果，１日後および２日後の生存率はそれぞれ50％および20％であった.

懸濁液の一部を投与したマウス６匹中２匹に感染成立が認められ，懸濁液を１日放置後に投与したマウスでは本菌の定着を認めなかった．感染マウスの胃内から回収した直後の菌は，培養した菌と比べて，少ない菌数であっても定着した個体が認められた．本結果より懸濁された菌は培養菌と比べてより感染を起こしやすい状態にある可能性が示された.

キーワード：*Helicobacter pylori*，感染ルート

Ⅰ　目的

H. pylori の感染源および感染ルートは家族由来および環境由来の説にわかれるが，現在本邦で最も多い感染ルートは小児期の母子感染・家族感染と集団保育等による水平感染と考えられる．しかし，そのルートについては依然として明らかにされていない．今回は，感染ルートを明らかにする研究の一端として，生体から環境に暴露された本菌が水中でどの程度生存するかを明らかにし，２次感染の原因となるかどうかを検討した．

Ⅱ　方法

H. pylori TK1402株を７％馬血清添加ブルセラ寒天培地で２日間培養し，その5.0×10^{8} CFU を C57/BL-He J マウスに0.5 mLHBSS に懸濁して，連日２回投与した[1]．投与３週後にマウスの胃粘膜を掻きとり，ハンクス液または滅菌水に懸濁し，室温にて保存した．０－３日後の生菌数を比較した．生菌数の評価には，ヘリコバクター選択培地（ニッスイ）を用いた培養法と *H. pylori* 16SrRNA 遺伝子に対する定量的リアルタイム PCR 法を実施した．PMA（propidium monoazide）処理後に DNA 抽出してたサンプルと無処理サンプルから抽出した DNA を用いて定量的リアルタイム PCR 法を行い，生存率についても併せて検討した[2]．

また，同じサンプルをマウスに投与して，２次感染成立の有無を観察した．すなわち，マウス胃粘膜を調整後，同日中に別のマウスに投与した群をグループA，24時間 HBSS 中室温に置いた，胃粘膜を投与した群をグループ B，ブルセラ培地で培養した *H. pylori* TK1402株10^{4-8}CFU を投与した群をコントロール（グループ C，D，E）とした．

Ⅲ　結果

感染３週後のマウスの胃内菌数は，2×10^{2}から1×10^{4}CFU であった．胃粘膜懸濁液作成後，ハンクス液にて１日後に約10分の１の菌数が培養可能であり，３日後には検出限界以下となった．一方，滅菌水に懸濁したサンプルでは，１日後に *H. pylori* は培養されなくなっていた．定量的リアルタイム PCR 法による解析の結果，１日後および２日後の生存率は50％およ

Table 1. Detection of *H. pylori* in gastric mucosa of infected mouse

	Inoculation (CFU /500μL)	number of *H. pylori* (Average CFU /stomach)	Infection rate
Stomach specimen with *H. pylori*			
Group A	5.6×10^3	2.2×10^3	2 / 6
Group B	2.0×10^3	N.D.	0 / 6
Cultured *H. pylori*			
Group C	3.2×10^8	6.6×10^6	6 /6
Group D	3.2×10^6	3.2×10^3	1 / 4
Group E	3.2×10^4	N.D.	0 / 3

び20%であった.

懸濁液の一部（5.6×10^3CFU）を，作成直後に感染させたマウス（グループA）は6匹中2匹に *H. pylori* の定着が認められ，24時間後の投与(グループB)（2.0×10^3CFU）では定着を認めなかった．同時に行った液体培養の *H. pylori*（3.2×10^4CFU）（グループE）を用いた投与では，定着しなかった.

マウスの胃内感染成立は，培養菌を約10^6CFU投与した場合に，4匹中1匹（グループD)，10^8 CFU投与した場合に6匹中6匹（グループC）に認められた.

Ⅳ 考察

感染マウスの胃内から回収した直後の *H. pylori* は，培養した *H. pylori* と比べて少ない菌数でも定着が成立したマウスが検出されたことから，より感染を起こしやすい状態にある可能性が示された．しかし，定着が成立しない例も認められ，全マウスの定着成立にはさらに多い菌数または繰り返しの投与が必要だと考えられた．感染ルートの解明のためには，水中での本菌の生存条件と感染成立の条件の検討を行うことが重要である．加えて，環境由来の感染についての詳細な条件を解明してくことが期待される.

Analysis of surviving conditions of *Helicobacter pylori* in environments.

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The major route of transmission of *Helicobacter pylori* is not clearly determined. This study was performed to clarify a transmission route of *H. pylori* in the environments. Gastric mucosa was collected from mice infected with *H. pylori* in three weeks infection period and suspended in Hanks' balanced salt solution or sterilized water. $2\times10^{2-4}$ CFU of *H. pylori* was cultured at 0 hr. After one day incubation at room temperature in HBSS, 2×10^{3} CFU of *H. pylori* was detected by cultivation. As a result of analysis by the quantitative real-time PCR method using Propidium monoazide stained DNA, a survival rate was detected as 50% and 20% after one and two days, respectively.

The gastric mucosa from the mice infected with isolated *H. pylori* and liquid culture of *H. pylori* were used for inoculation to other mice. The infection ability of the isolated *H. pylori* was higher than that of cultured *H. pylori*. This study suggests that *H. pylori* from infected host may have an important role in the transmission of *H. pylori* in drinking water.

Keywords: *Helicobacter pylori,* transmission route

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一般演題3

スナネズミ胃内細菌とヘリコバクター・ピロリとの微生物生態学に関する研究

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要旨

スナネズミへの *Helicobacter pylori* 感染モデルを用いて、胃粘膜での *H. pylori* の定着性を調べた結果、糞便からの本菌の検出頻度および胃内細菌数が各個体により異なることが明らかとなった。そこで、胃内正常細菌叢と *H. pylori* の相互作用が *H. pylori* の定着や長期持続感染に関与している可能性について検討した。糞便中の *H. pylori* の検出状況をもとに、高頻度検出群スナネズミの中から最も胃内 *H. pylori* 菌数の高いスナネズミと、低頻度検出群から、*H. pylori* 陰性のスナネズミの胃粘膜懸濁液を別のスナネズミに投与した。これらの胃内菌叢修飾スナネズミに *H. pylori* の投与を行って、投与８週後に胃粘膜における *H. pylori* の感染状況と各細菌を分離培養し、16SrRNA 遺伝子の塩基配列により菌種同定を行い、本菌の持続感染の成立には *Lactobacillus* 属菌の菌種構成が関与している可能性が示された。

背景

H. pylori は WHO により胃癌の確実性発癌因子グループ１として認定されているが、日本国内では中高年齢者の感染率は依然として高い。*H. pylori* のスナネズミ感染モデルは、ヒトの慢性持続性胃炎および胃癌発症モデルとして汎用されている。しかし、スナネズミ胃内の持続感染は、１回の *H. pylori* の投与では全例には成立しないこと、感染の長期化により時に *H. pylori* が自然消失することが知られている[1,2]。そこで、*H. pylori* の持続感染に影響する胃内細菌叢を明らかにするために、本研究を実施した。

方法

*H. pylori*TK1402 株 1 x 10^7 CFU をスナネズミ(MGS/sea、雌、５週齢、九動)に投与した。翌日から週１度、糞便を採取して、ビーズフェノール法にて全 DNA を抽出し、*H. pylori ureA* 遺伝子特異的プライマー[3] (UreA2F2：
5′-ATATTATGGAAGAAGCGAGAGC-3′,
UreA2R ：5′-ATGGAAGTGTGAGCCGATTTG-3′,
UreA 2F35′-CATGAAGTGGGTATTGAAGC-3′)を用いた。nested PCR により、*H. pylori* の検出頻度をスナネズミ個体間で比較した。感染８週後にすべてのスナネズミの胃粘膜懸濁液を作成し、凍結保存するとともに、胃内 *H. pylori* の菌数を測定した。*H. pylori* の菌数の定量には、*H. pylori* 選択培地（日水）を用いた 37℃微好気培養法（三菱ガス）と、16SrRNA 遺伝子プライマーを用いた定量的リアルタイムＰＣＲ法[4]を実施した。

次に、別のスナネズミ（5 週齢、雌）を 2 群にわけ、*H. pylori* 陰性（低頻度検出群）または陽性(高頻度検出群)の胃粘膜懸濁液を経口投与した。投与１週後に、*H. pylori*TK1402 株 1 x 10^9 CFU を 2 日間連続投与した。感染８週後に *H. pylori* の感染率、胃内細菌叢の解析、体重、胃重量の測定を実施した。*H. pylori* と *Lactobacillus* 属菌については 16SrRNA 遺伝子プライマーを用いた PCR 法により定量的に解析した。

さらに、胃内細菌叢の解析のために、GAM 培地（ニッスイ）および５％血液添加 Phenylethyl alcohol agar、DHL 培地（ニッスイ）を用いて、嫌気培養（三菱ガス）、好気培養を実施した。通性嫌気性菌の同定には API20E(ビオメリュー)を用いた（腸内細菌科）。偏性嫌気性菌の解析は 16SrDNA 遺伝子ユニバーサルプライマー
27F: 5′-AGAGTTTGATCMTGGCTCAG-3′、
1492R:5′-TACGGYTACCTTGTTACGACTT-3′　、
518F: 5′-CCAGCAGCCGCGGTAATACG-3′、
800R: 5′-TACCAGGGTATCTAATCC-3′)

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。

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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

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

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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)

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[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

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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.

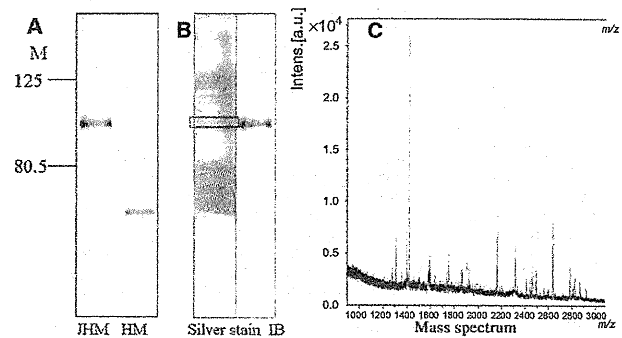

Figure 1 (*A*) Immunoblot of Japanese strain–derived high-molecular-weight proteins (JHM-CAP) antigen (JHM) and HM-CAP antigen (HM) with JHM-CAP-positive patient's serum. JHM (0.03 μg) and HM (0.03 μg) were separated by SDS-PAGE, followed by immunoblot. M: molecular size markers (kD), (*B*) Silver stain 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 R**YQIFTSWVS** **LQKDPSK**INT QQIRNFMENI
151 IQPPISDDKE KAEFLRSAKQ SFAGIIIGNQ R**SDEKFMGV** **FDESLKER**QE
201 AEKNAEPAGG DWLDIFLSFV FNKKQSSDLK ETLNQEPRPD FEQNLATTTT
251 DIQGLPPESR **DLLDERGNFF** **KFTLGDVEML** **DVEGVADKDP** **NYK**FNQLLIH
301 NNALSSVLMG GHSNIEPEKV SLLYGDNGGP EARHDWNATV GYKNQQGNNV
351 ATLINAHLNN GSGLVIAGNE NGIKNPSFYL YKEDQLTGLK **QAMSQEEIQN**
401 **KVDFMEFLAQ** **NNAK**LDNLSE KEKEK**FQTEI** **ENFQKDR**KAY LDALGNDHIA
451 FVSKKDPK**HL** **ALVTEFGNGE** **VSYTLKDYGK** KQDKALDGET K**TTLQGSLKY**
501 **DGVMFVNYSN** **FK**YTNASKSP DKGVGATNGV SHLEANFSK**V** **AVFNLPNLNN**
551 **LAITNYIRR**D 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** **ALAELK**SLSL DLGKNSDLQK SVKNGVNGTL VGNGLSKTEA
851 TTLTKSFSDI RKELNEKLFG NSNNNNNGLK NNTEPIYAKV NKKKTGQVAS
901 PEEPIYAQVA KKVSAKIDQL NEATSAINRK IDRINKIASA GKGVGGFSGA
951 GQSASPEPIY ATIDFDETNQ AGFPLRR**SAA** **VNDLSKVGLS** **R**EQELTRRIG
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).

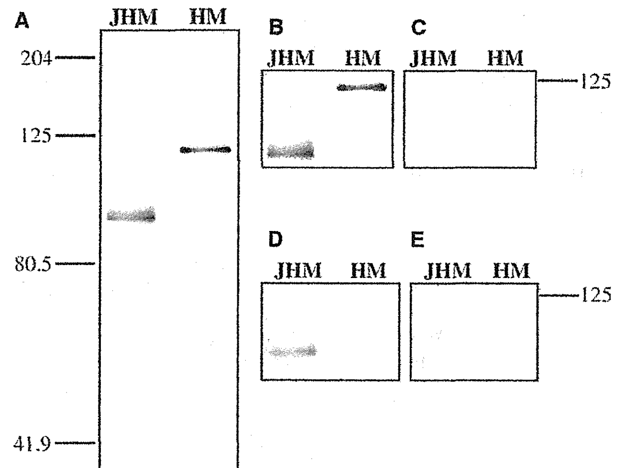

Figure 3 (*A*) Immunoblot of Japanese strain–derived high-molecular-weight proteins (JHM-CAP) antigen (JHM) and high-molecular-weight cell-associated proteins (HM-CAP) antigen (HM) with anti-CagA specific antibody. (*B–D*) Inhibition assays of JHM-CAP and HM-CAP antigens. (*B*) Anti-CagA antibody was 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.

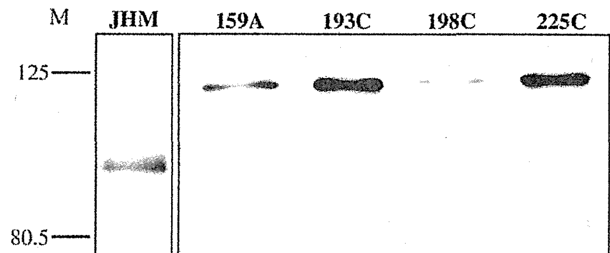

Figure 4 Immuoblot of the Japanese strain–derived high-molecular-weight proteins (JHM-CAP) antigen and the ultrasonicated antigens extracted from four Japanese *Helicobacter pylori* strains with anti-CagA antibody.

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).

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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.

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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

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

Accepted for publication 7 September 2011.

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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).

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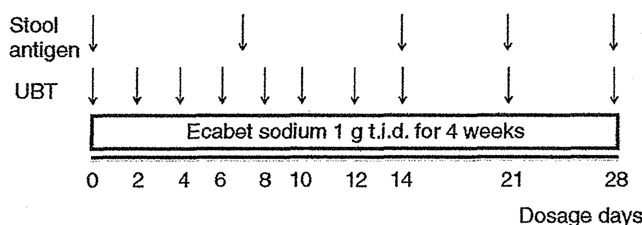

Figure 1 Protocol of administration of ecabet sodium and performance of urea breath test (UBT) and stool antigen tests.

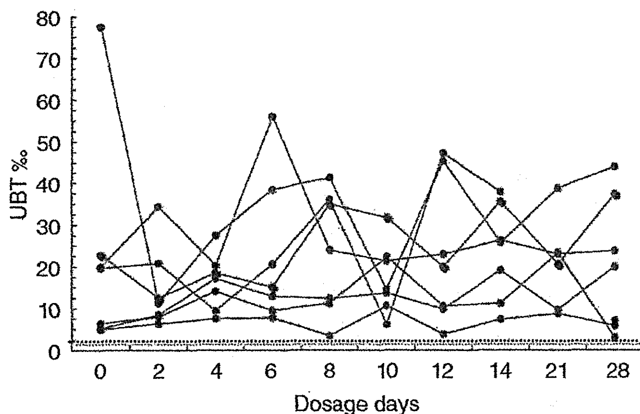

Figure 2 Urea breath test (UBT) values of individuals during medication of ecabet sodium.

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-

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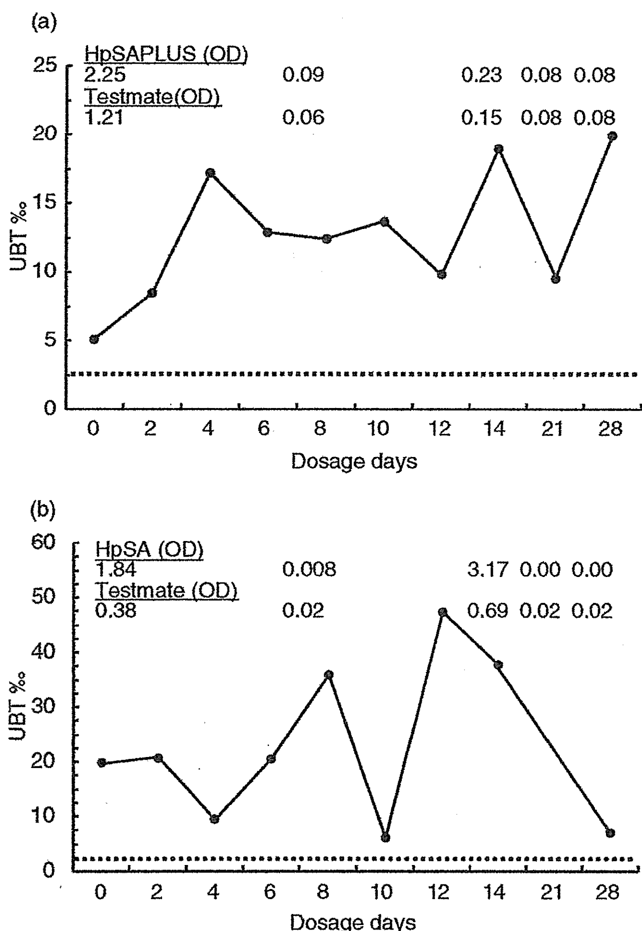

Figure 3 Urea breath test (UBT) and stool antigen tests (HpSA PLUS and Testmate) of (a) case 1 and (b) case 6. OD, optical density.

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.

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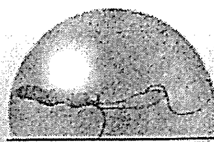
発行：株式会社　先端医学社
〒103-0007　東京都中央区日本橋浜町2-17-8　KDX浜町ビル



〈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

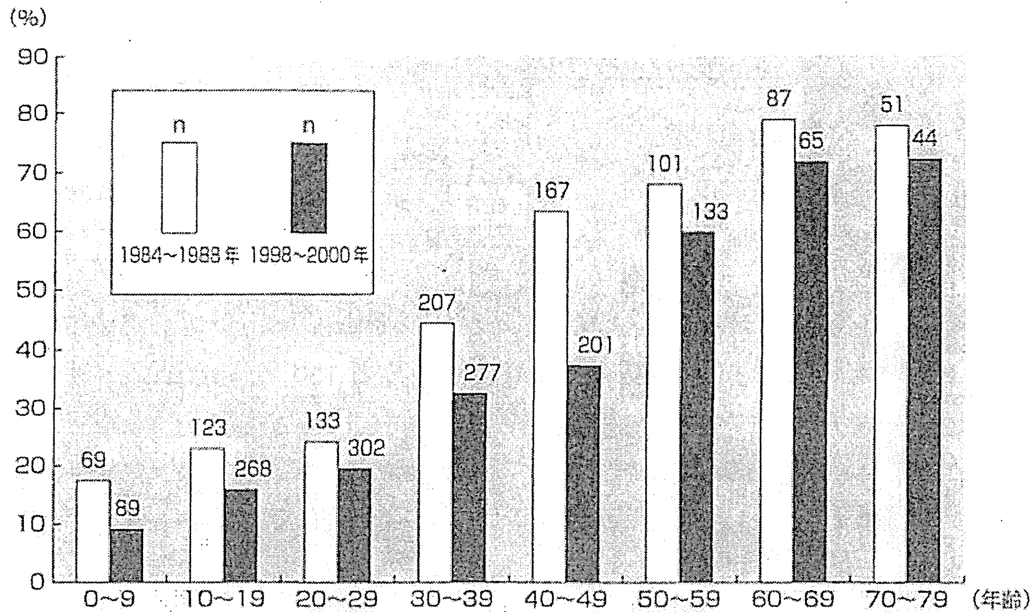
1）兵庫県における *H. pylori* 抗体保有率[1]

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

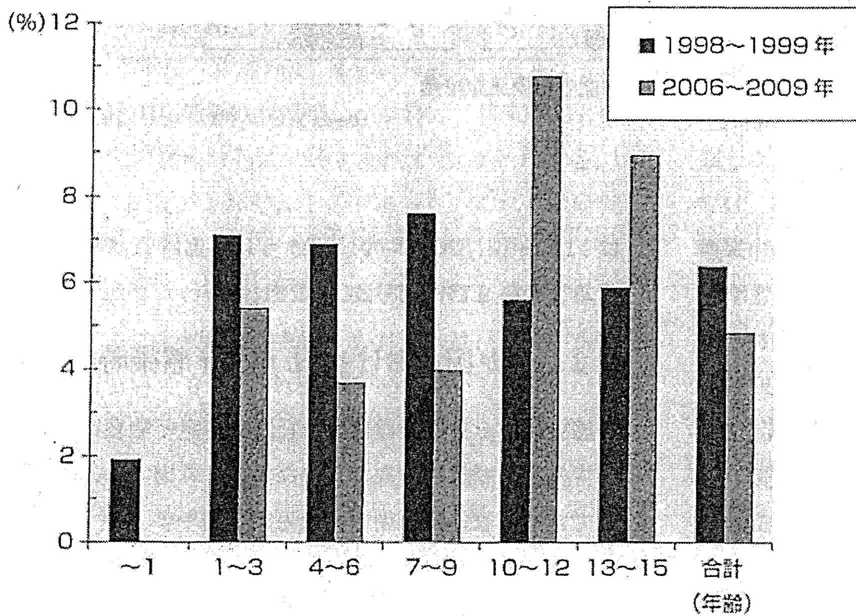
われわれは 1998～1999 年[2]と，2006～2009 年に和歌

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