second-line therapy for failure of initial Helicobacter pylori eradication therapy. Hepatogastroenterology. 2005; 52(62): 645-8.

⑭Inamori M, Togawa J, Iwasaki T, Ozawa Y, Kikuchi T, Muramatsu K, Chiguchi G, Matsumoto S, Kawamura H, Abe Y, Kirikoshi H, Kobayashi N, Shimamura T, Kubota K, Sakaguchi T, Saito S, Ueno N, Nakajima A. Early effects of lafutidine or rabeprazole on intragastric acidity: which drug is more suitable for on-demand use? J Gastroenterol. 2005; 40(5): 453-8.

⑮稲森正彦,阿部泰伸,中島淳:日常診療で診るGERD(胃食道逆流症) Ⅲ.GERDの治療 18. GERDの薬物療法(H2ブロッカー, 消化器運動機能改善薬の使用法). Medicina. 2005, 42巻1号 Page62-64(2005)

⑯稲森正彦:ようこそ健康塾 胸焼けがつらい人へ (逆流性食道炎). 暮らしと健康2006, 61巻4号Page46-48(2006)

⑰稲森正彦, 中島淳:悪心, 嘔吐. 講義録 消化器学. メジカルビュー社. 2005, Page137-139

⑱稲森正彦, 中島淳:胸やけ, げっぷ, 吃逆. 講義録 消化器学. メジカルビュー社. 2005, Page140-142

⑲前山史朗, 米田正人, 中島 淳. 非アルコール性脂肪性肝炎(NASH)(3)病理組織. 日本内科学会雑誌第2006, 95号巻1号

⑳米田正人, 中島 淳, 岩崎知之, 寺内康夫, 前山史朗. 2型糖尿病と肝 肝胆膵 2005, 51巻5号 アークメディア社

㉑米田正人, 中島 淳, 油谷浩幸, 前山史朗. 糖尿病と肝臓死 Medical Practice 2005, Vol. 22 No.10 文光堂

㉒米田正人, 藤田浩司, 中島 淳, 岩崎知之, 寺内康夫. NASH患者の肝臓内脂肪沈着, 線維化形成に関与する内臓脂肪, adipocytokinesの解析. アルコールと医学生物学2005, Vol. 25 東洋書店

㉓中島 淳, 米田正人, 藤澤 信隆, 高橋宏和, 和田孝一郎. 消化器疾患とPPARs. 日本臨床第65巻第4号(2005年4月) 日本臨床社

㉔米田正人, 中島 淳. 臨床検査ガイド2005 コリンエステラーゼ(ChE).臨床検査ガイド2005-2006(2005年2月) 文光堂

㉕Saito S, Heller T, Auerbach J, Williams T, Moreen TR, Jazwinski A, Cruz B, Jeurkar N, Sapp R, Luo G, Liang TJ An in vitro model of hepatitis C virion production. Proc Natl Acad Sci U S A 2005; (102): 2579-83

Characteristics of a Clinical Isolate of Urease-Negative *Helicobacter pylori* and its Ability to Induce Gastric Ulcers in Mongolian Gerbils

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ABSTRACT

Background. We clinically obtained urease-negative mutant strains of *Helicobacter pylori*. The goal of this study was to investigate the ability of the urease-negative strain to colonize and subsequently damage the gastric mucosa in Mongolian gerbils. In addition, the genes encoding the urease production in the test strain were analyzed, and other genes encoding the virulence factors, cytotoxin-associated protein and vacuolating-cytotoxin were evaluated.

Materials and methods. The character of urease-negative isolates of *H. pylori* was defined. The identification of *H. pylori* was confirmed by polymerase chain reaction (PCR). The *H. pylori* isolate was transfected into Mongolian gerbils as previously described, which were followed up to 42 weeks, and the changes in their gastric mucosa were examined histologically.

Results and conclusion. Fifteen Mongolian gerbils orally infected with 10^7 colony forming units of urease-negative *H. pylori* were killed at 4, 12, 24, 36 and 42 weeks (n = 3) after infection. Culture medium without urease-negative *H. pylori* was given to the Mongolian gerbils as control. *H. pylori* continued to exist in the subject's stomach and gastric ulceration was observed and compared with the control.

Clinically obtained urease-negative *H. pylori* continued to exist for at least 42 weeks in the subject's stomach and it induced gastric ulcers. These data demonstrated that the urease in *H. pylori* was not a necessary factor in the formation of gastric ulcers in the Mongolian gerbil model.

Keywords. *Helicobacter pylori*, urease-negative, Mongolian gerbils, clinical isolate, peptic ulcer.

Recent growing evidence indicate that *Helicobacter pylori* infection is also associated with the increased risk of developing gastric cancer [1]. *H. pylori* produces large amounts of urease that catalyzes the hydrolysis of urea to ammonia and carbon dioxide [2].

The production of high levels of urease by an organism is thought to be essential for the initiation and maintenance of gastric infection with *H. pylori* [3]. On the other hand, it is known that spontaneously urease-negative mutants exist at extremely low frequency in wild strains [4]. Facts suggest that the enzyme plays a major role in the ability of the organism to colonize the acidic environment of the stomach by providing an alkaline microenvironment, subsequently damaging the gastric mucosa [5]. Furthermore, urease-negative mutant strains of *H. pylori* failed to colonize in gnotobiotic piglets [6], suggesting that the enzyme plays a role in permitting survival of the organism in the gastric mucosa.

In this study, a clinically obtained urease-negative isolate of *H. pylori* was used. The isolate was from a patient with peptic ulcer whose clinical outcome was clearly defined. The goal of this study was to investigate the ability of the urease-negative strain to colonize and to subsequently damage the gastric mucosa in Mongolian gerbils. In addition, the genes encoding the urease production in this isolate were analyzed, and other virulence factors, cytotoxin-associated protein and vacuolating-cytotoxin production were evaluated.

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Materials and Methods

Sampling of Urease-Negative H. pylori

A urease-negative strain of *H. pylori*, consisting of the negative cells alone, was isolated from a

male patient with chronic relapsing gastric ulcer at the middle body of the stomach. The patient was 55 years old and presumed to have an *H. pylori* infection. During periodic upper gastrointestinal endoscopic examinations, several gastric mucosa specimens were taken from the antrum and corpus. The specimens were homogenized in sterile physiological saline and were cultured on both selective and nonselective agar media; which were Pourmedia HP Agar (Eiken Chemical Co. Ltd, Tokyo, Japan) and Columbia agar with 5% sheep blood (Becton Dickinson, Cockeysville, MD, USA), respectively. The agar plates were incubated at 35 °C under microaerophilic conditions for 5 days. Sixteen suspected *H. pylori* colonies were selected and identified by microaerophilic growth requirement, typical morphology, existence of Gram-negative curved rods and biochemical characteristics including catalase, oxidase and urease productions and motility. The identification of *H. pylori* was confirmed by the polymerase chain reaction (PCR), as described previously [7,8].

Extraction of DNA

H. pylori DNA was extracted using a standard phenol-chloroform procedure. After 3 days of culture on Columbia agar with 5% blood, the bacteria were harvested in 1 ml of Tris-EDTA buffer (TE) (10 mmol Tris-HCl, 1 mmol EDTA, pH 8.0). They were centrifuged at 8000 g for 15 minutes. The pellets were washed in TE and centrifuged. The bacterial pellets were resuspended in 1 ml lysis buffer (50 mmol Tris-HCl, 50 mmol EDTA and 0.1% Triton X-100). Lysozyme was added to a final concentration of 3 mg/ml, and the solution was incubated for 15 minutes at 35 °C. DNA was extracted in an equal volume of a phenol-chloroform mixture (1 : 1) and was precipitated overnight at −20 °C in the presence of 0.3 mol sodium acetate and 3 : 1 volumes of absolute ethanol. The DNA was then pelletted by centrifugation at 18,000 g for 10 minutes and was air-dried. The pellets were suspended in TE.

PCR Amplification for ureA-ureI *DNA Regions*

PCR amplification of the DNA regions contributing to the expression of *ureA-ureI* was performed with oligonucleotide primers designed according to GenBank accession no. M60398 X57132 (*ureA-ureD*) and M84338 (*ureE-ureI*) (Table 1). Amplification was performed in a total volume of 50 µl containing 4 µl of deoxynucleoside triphosphate (2.5 mol), 0.25 µl of TaKaRa *Taq* polymerase (5 U/µl), 2 µl of template DNA, and 0.5 µl of each primer. The temperature profile for amplification was as follows: predenaturation at 94 °C for 3 minutes, 40 cycles of denaturation at 94 °C for 0.5 minute, annealing at 55 °C for 1 minute, and 1 minute extension at 72 °C and 7 minutes post-extension at 72 °C. The PCR products were separated by agarose-gel electrophoresis and stained with ethidium bromide.

Transfection of Mongolian Gerbils with H. pylori

Male Mongolian gerbils (MGS/Sea, 6 weeks old), weighing 45–55 g, were supplied by Seac-Yoshitomi Ltd. (Fukuoka, Japan). Five groups of

Table 1 Oligonucleotide primer for PCR amplification of *Helicobacter pylori* urease genes

Primer		Position		Sequence (5′→3′)	PCR product size (bp)
ureA	forward	2477	2497	TAA ATG CAC TCC CAA TAA CGC	954
ureA	reverse	3430	3410	CTG TAG TAG GAC CAT ACA TAG	
ureB	forward	3333	3352	CGC TAA AAG CGA TGA CAA CT	1768
ureB	reverse	5100	5078	CTC TAA AAA ATC CTA GAA AAT GC	
ureC	forward	416	435	GCC ATA GCC TTG TTT TAA TC	1469
ureC	reverse	1884	1864	ACC CCT ATA AAA ACC AAC AGG	
ureD	forward	1760	1780	ACG AAT CCT TTT AGA AGC TAA	510
ureD	reverse	2269	2249	TTA ACA ATA AAA CGC CCA CAC	
ureE	forward	756	776	CTT GGA TCC CTG CTT GGT TAC	614
ureE	reverse	1369	1349	ACC CAC GCT TTT TTC AAT GCT	
ureF	forward	1280	1300	ACT GGC GAG CGA TTT TAA AGT	873
ureF	reverse	2152	2133	ACA GGA CCA CAA ACT CCA AT	
ureG	forward	2072	2092	TAC TCG CGC CTT TAT ATG TCT	685
ureG	reverse	2756	2736	ACC TGA GCT TGG ATT CTT GAG	
ureH	forward	2680	2700	ATG TGA TCG CTT GGA TCA AGC	822
ureH	reverse	3501	3481	GTT TGC GTG ATA AAG CGA GCG	
urel	forward	160	180	TTA TTC GTA AGG TGC GTT TGT	689
urel	reverse	848	828	CAA GGG GTT TAA ATC CCT TAG	

three animals was housed in five cages in an air-conditioned room (temperature is 24 ± 2 °C, humidity is 60 ± 5%) and provided with food and water *ad libitum*. As control, we used male Mongolian gerbils (MGS/Sea, 6 weeks old), weighing 45–55 g, which was given sterile physiological saline only.

Bacterial Inoculation and Tissue Sampling

After the gerbils had been fasted for 24 hours but with *ad libitum* water, 1 ml (10^7 colony forming units/ml) of the bacterial suspension was administered to the gerbils, followed by another 2 hours of fasting. Also, the suspension without *H. pylori* was administered to the gerbils as control. After keeping the subjects under normal conditions for 4, 12, 24, 36 and 42 weeks, their stomachs were removed. Each stomach was divided into two parts for bacteriological and pathological tests. For the bacteriological test, each stomach was lightly washed with sterilized saline and the lining of the stomach was aggressively scraped with a spatula. The scrapings were homogenized with 3-fold volumes of sterilized saline, followed by a series of 10-fold dilutions. One half ml aliquots of diluted suspension were spread each over M-BHM pylori agar (Nikken Bio Medical Laboratories, Kyoto, Japan) and incubated at 35 °C for 4 to 7 days under microaerophilic conditions. Visible growth of the bacteria was observed. For the pathological testing, tissue samples were embedded in paraffin wax, sectioned and stained routinely with hematoxylin, eosin and giemsa solution.

Western Blot of H. pylori *Urease*

Bacteria cells were harvested by centrifugation (11,000 g for 15 minutes at 4 °C), were and washed in 50 mmol sodium phosphate buffer (pH 6.8). A cell pellet was suspended in the same buffer, sonicated with an ultrasonic disruptor BH-200P (Tomy Seiko Co., Ltd, Tokyo, Japan) at an intensity of 4 for 3 minutes at 4 °C, and was centrifuged (11,000 g for 15 minutes at 4 °C). The supernatant was used as the urease sample.

Urease samples were denatured for 10 minutes at 100 °C in 10 mmol Tris-HCl (pH 6.8), containing 1% sodium dodecyl sulfate, 20% glycerol, 0.02% bromophenol blue, and 1% 2-mercaptoethanol. Samples were loaded onto a 12.5%-polyacrylamide gel and were electrophoresed for 90 minutes at 20 mA.

Proteins were transferred to polyvinylidene difluoride membranes (Immobilon™ PVDF Transfer Membrane, Nihon Millipore Ltd, Yonezawa, Japan) for 60 minutes at 8 V. The blotted membranes were blocked with 1% skim milk for 1 hour at room temperature. Whole membranes were exposed to MAb D12 (for the small subunit, 1 : 2000 dilution) and MAb A7 (for the large subunit, 1 : 100 dilution) for 1 hour at room temperature. After three washings in Tris-buffered saline with 0.2% Tween 20 (Kanto Chemical Co. Inc., Tokyo, Japan), the membranes were incubated in a 1 : 2000 dilution of horseradish peroxidase conjugated goat antimouse Ig's (Biosource, Camarillo, CA, USA) for 1 hour at room temperature. The membranes were then washed three times in Tris-buffered saline with 0.2% Tween 20 and once in Tris-buffered saline without Tween 20 for 5 minutes per washing. Immunoreactive proteins were detected using Konica Immunostaining HRP-1000 (Konica, Tokyo, Japan).

Results

Microbiological Characteristics

All the reference strains (ATCC43504, 43579 and 43629) turned pink at the inoculated sites as a result of the ammonia produced from urea in the medium by the bacterial urease, whereas the clinical urease-negative *H. pylori* isolate did not turn pink at the inoculated site (Fig. 1). A complete defect of urease production in the urease-negative isolate was also confirmed spectrophotometrically (data not shown). No difference in the biochemical characteristics except for urease activity was found between the urease-negative and positive strains of *H. pylori*. Furthermore, the presence of *cagA* and *vacA* genes in the urease-negative isolate was confirmed by PCR. The urease-negative phenotype of the isolate was very stable in transfers under both in vitro and in vivo conditions.

Analysis of Urease Gene Products

The genes encoding the urease of *H. pylori* consist of an enzyme cluster which contains nine genes, including the structural genes as well as those involved in regulation of urease synthesis and assembly of the holoenzyme. The PCR-amplified fragments of urease genes from a urease-negative isolate and a reference strain ATCC43504 were analyzed and compared with

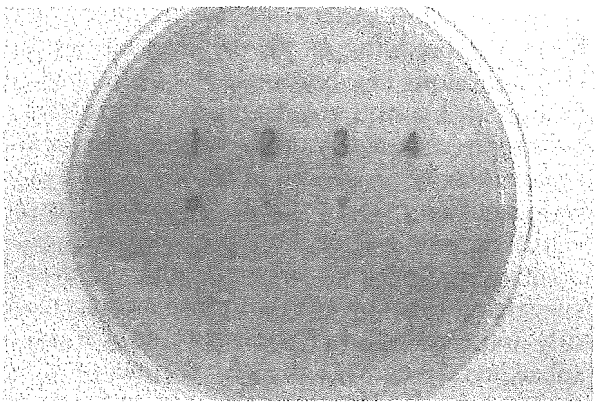

Figure 1 Urease reactions of *Helicobacter pylori* strains on urea agar. (1) *H. pylori* ATCC43504, (2) *H. pylori* ATCC43579, (3) *H. pylori* ATCC43629 and (4) urease-negative *H. pylori* isolate.

the two groups, the results of which are shown in Fig. 2. All the urease genes were detected in both the urease-negative isolate and the urease-positive reference strain; the predicted PCR products from both strains were identical.

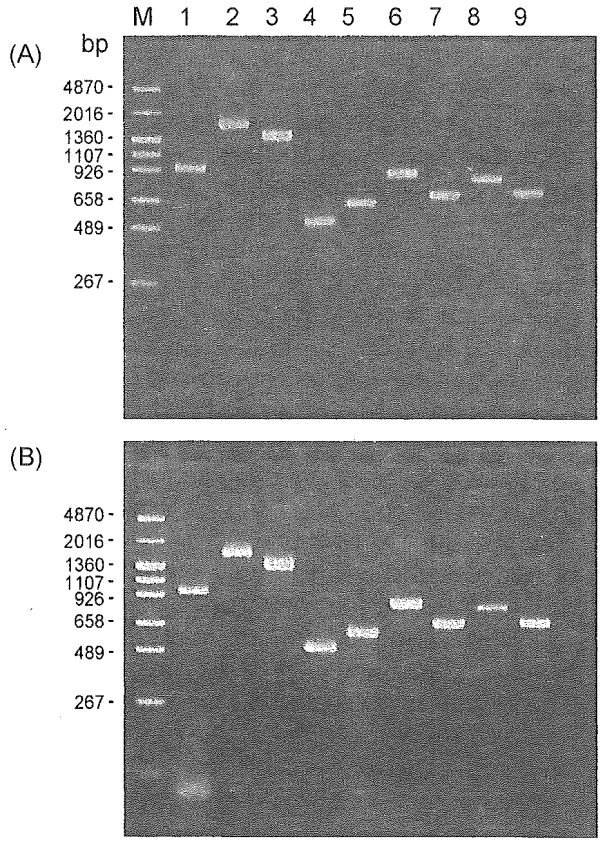

Figure 2 Ethidium bromide staining of PCR amplification of *ure* genes. *H. pylori* ATCC43504 (A) and urease-negative *H. pylori* isolate (B). M, size marker; lane 1, *ureA* gene; lane 2, *ureB* gene; lane 3, *ureC* gene; lane 4, *ureD* gene; lane 5, *ureE* gene; lane 6, *ureF* gene; lane 7, *ureG* gene; lane 8, *ureH* gene; and lane 9, *ureI* gene.

Western Blotting of Urease-Negative H. pylori

Identical main bands were detected at the 29 kilodalton (kDa) region with the subunit A-antibody (MAb D12) and at the 68 kDa region with the subunit B-antibody (MAb A7) in both crude enzymes from the urease-negative and positive strains of *H. pylori*. These results closely accorded with the bands at 31 kDa for the subunit A and 66 kDa for the subunit B of *H. pylori* urease, respectively, as reported by Evans et al. [9]

The result indicates that the urease-negative isolates, like the urease-positive strains, produce subunits A and B of urease.

Change in Gastric Mucosa Inoculated with Urease-Negative H. pylori

Fifteen Mongolian gerbils orally infected with *H. pylori* were killed at 4, 12, 24, 36 and 42 weeks (n = 3) after the bacterial challenge. The stomachs of the infected animals were opened along the longitudinal axis from cardiac area to pyloric area and were observed macroscopically. At 42 weeks, in contrast to control, ulcers were widely observed in the gastric tissues of Mongolian gerbils infected with the urease-negative isolate, as shown in Fig. 3. Histological findings of ulcerated gastric tissues showed mucosal defects, with the depth of the ulcer extending to the submucosal layer, penetrating serial layers, or both (Fig. 4, hematoxylin and

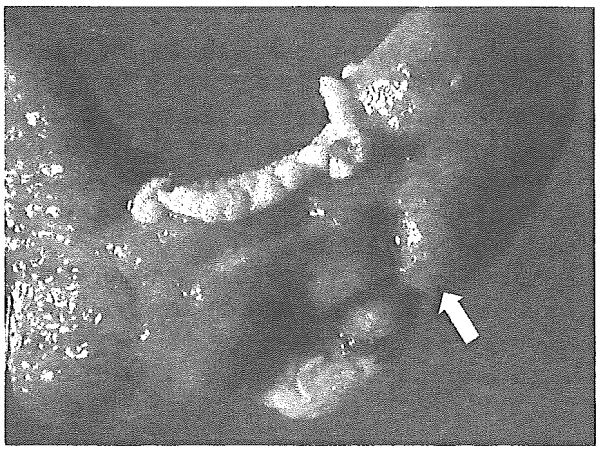

Figure 3 Gastric tissue of Mongolian gerbils infected with urease-negative *H. pylori* isolate at 42 weeks. The arrow indicates the gastric ulcer.

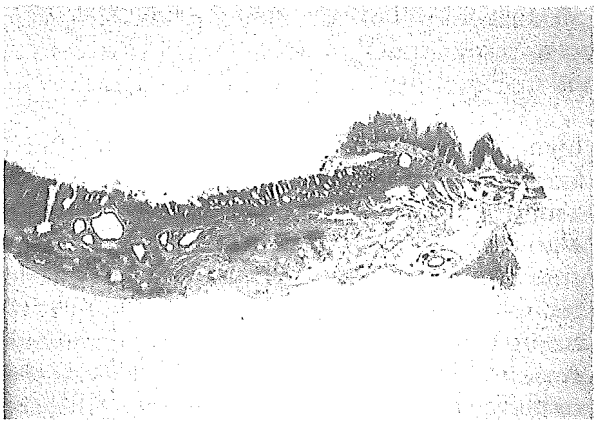

Figure 4 Histological findings of a gastric ulcer and surrounding tissues (hematoxylin and eosin staining, × 20).

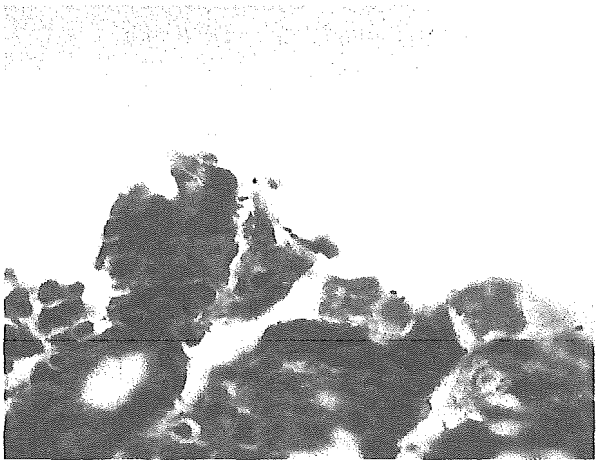

Figure 5 *H. pylori* exists in the gastric mucosa (giemsa-staining, × 400).

eosin staining). Neutrophil and lymphocyte infiltrations are seen in the gastric tissues around the ulcers in Fig. 4. Figure 5 shows that giemsa-stained *H. pylori* existed in the gastric mucosa. Viable *H. pylori* at levels of 6.0×10^4–8.0×10^5 colony-forming units per gram tissue were detected in gastric tissues associated with ulcer formation. All strains failed to show any urease activity, when they were conventionally cultured using the urea agar method.

Ulcer was not observed macroscopically in any animals of the negative control group which were given sterile physiological saline only (data not shown).

Discussion

H. pylori is recognized as an etiological factor in chronic gastritis and contributes to peptic ulceration [10–12]. A variety of virulence factors have been proposed for *H. pylori*, such as motility [13], adhesion [14], cytotoxin-associated protein (encoded by the *cagA* gene), vacuolating-cytotoxin (encoded by the *vacA* gene) [15,16] and urease activity [6,17–19]. Most of the wild-type isolates of *H. pylori* from patients with chronic active gastritis and peptic ulceration are urease-positive, and produce large quantities of urease, suggesting that the enzyme plays an important role in the ability of *H. pylori* to colonize and to subsequently damage the gastric mucosa of the patients, while also protecting *H. pylori* against the acidic environment of the stomach by acting as a cytotoxin and disrupting tight cell–cell junctions [17,19]. In earlier studies, distinct urease-deficient kinds of mutagenic agents and spontaneous urease-negative mutants were reported [5]. The N-nitrosoguanidine-induced urease-deficient mutant strains (0.4% activity of that of the wild-type strain) did not differ in biochemical characteristics from the wild-type strain other than in urease activity [6]. Gnotobiotic piglets were challenged with the wild-type strain or the mutant strain [6]. *H. pylori* was recovered in the gastric specimens from all the piglets subjected to the wild-type strain but not recovered from those subjected to the mutant strain. The study revealed that seven mutant species did not live in the gastric mucosa. Gastritis was histologically observed in all piglets subjected to the wild-type strain. In a study on the ethylmethanesulfonate-derived *ureI* gene deficient mutant of *H. pylori*, the mutant cells were quickly killed when exposed to low pH and exhibited no cytotoxicity to eukaryotic cells [4]. Pérez-Pérez et al. [5] found that urease-negative variants spontaneously arose at a frequency of 10^{-5} to 10^{-6} from a wild-type strain of *H. pylori*, and were consisted of three-type mutant cells in the profiles obtained by the whole-cell sodium dodecyl sulfate-PAGE. In our survey on *H. pylori* infection in 1996, about 2300 patients were diagnosed as having chronic active gastritis or peptic ulcers. The survey revealed that seven of the 1602 isolates consisted of only the urease-negative *H. pylori*. Various biochemical characteristics including the presence of the *vacA* and *cagA* genes were identical except the urease activity in these urease-negative and positive isolates of *H. pylori*, and the phenotype of urease-negative isolates was stable even in the in vitro transfers and in the stomach of transfected animals. In urease-negative *H. pylori* of this study, nine

ureA-ureI genes participating in urease-production were detected by PCR, and the production of subunit A and subunit B was confirmed by Western blotting. This type of urease-negative strain was not observed in the previous study. These findings suggest one possibility that in this isolate, mutation occurs in the *ureE-ureI* (urease activity regulatory genes) and the other possibility that *H. pylori* has no or has an extremely low transcription activity in *ureE-ureI* genes. The data in Fig. 2 strongly supported the latter. On the basis of the findings on the urease negative-variants of *H. pylori* reported thus far, it may not be commonly argued that the urease-negative strains colonize the acidic environment of the stomach and subsequently damage the gastric mucosa. However, the fact exists that the urease-negative strain possesses the virulent ability to induce gastric lesions in Mongolian gerbils, showing a similar state and course as the gastric lesions induced by *H. pylori* infection in humans. These results were confirmed in this study by microbiological and histological tests of the gastric tissues from the animals infected with the urease-negative strain. Despite the advances made in the understanding of functions of urease activity in *H. pylori*, many questions still remained unanswered. Although there are hypotheses that *H. pylori* urease plays an important role in the initiation of infection or permitting survival of the organism in the gastric mucosa [17], there is the undeniable possibility that the urease-negative isolates or cells from patients can survive in the acidic milieu of the stomach and are associated with pathogenic processes of gastritis and ulcer formation. The disagreement between the present and the previous results from other authors as to the ability of urease-negative isolates of *H. pylori* to survive in the gastric mucosa remains unexplained. It is probable that the disagreement has arisen from the differences in the kind of animals used as host, the strain, or both used as the infector. Furthermore, a following hypothesis exists as to why urease-negative strains of *H. pylori* colonize in the gastric mucosa of humans. Infection may be somehow established in the gastric pH environment of the host which approximates to the neutral state and there is a very strong possibility that other factors, such as high chemotaxis and motility, and the intake of components acting as a barrier to gastric acid in the outer membrane of the bacterial cells, participate greatly in the infection.

The alteration of phenotypes of various pathogens is sometimes found in both in vitro results and in clinical isolates from infected patients. In the case of chronic infections with *H. pylori*, contact of the pathogen in the infected loci continues with various factors including antibiotics at sub-inhibitory levels and various endogenous of human defense mechanisms. The emergence of urease-negative isolates from the gastric mucosa of patients infected with *H. pylori* may also be explained by the same consideration as previously discussed. In the future, there is the possibility that the isolation frequency of urease-negative strains will increase among clinical isolates of *H. pylori* from patients with chronic active gastritis and peptic ulcers. If such a tendency is generally recognized, it is suggested that the urease-negative strains of *H. pylori* have adapted to survive in the acidic milieu of the infected loci of the patients. Studies will thus be directed at the determination of the early kinetics of colonization by urease-negative *H. pylori* and the effect of modulation of the host gastric pH will further elucidate the role of urease in colonization.

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References

1 Forman D, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, Sltas F. Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigator. *Br Med J* 1991;302:1302–5.
2 Turbett GR, Hag PB, Horne R, Mee BJ. Purification and characterization of the urease enzymes of *Helicobacter* species from humans and animals. *Infect Immun* 1992;60:5259–66.
3 Mobley HLT, Cortesia MJ, Rosenthal LE, Jones BD. Characterization of urease from *Campylobacter pylori. J Clin Microbol* 1988;26:831–6.
4 Segal ED, Shon J, Tompkins LS. Characterization of *Helicobacter pylori* urease mutants. *Infect Immun* 1992;60:1883–9.
5 Pérez-Pérez GI, Olivares AZ, Cover TL, Blaser MJ. Characterization of *Helicobacter pylori* variants selected for urease deficiency. *Infect Immun* 1992;60:3658–63.
6 Eaton KA, Brooks CL, Morgan DR, Krakowka S. Essential role of urease in pathogenesis of gastritis

induced by *Helicobacter pylori* in gnotobiotic piglets. *Infect Immun* 1991;59:2470–5.
7 Tummuru MKR, Cover TL, Blaser MJ. Cloning and expression of a high-molecular-mass major antigen of *Helicobacter pylori*: evidence of linkage to cytotoxin production. *Infect Immun* 1993;61:1799–809.
8 Phadnis SH, Ilever D, Janzon L, Normark S, Westblom TU. Pathological significance and molecular characterization of the vacuolating toxin gene of *Helicobacter pylori*. *Infect Immun* 1994;62:1557–65.
9 Evans DJ Jr, Evans DG, Kirkpatrick SS, Graham DY. Characterization of the *Helicobacter pylori* urease and purification of its subunits. *Microb Pathogen* 1991;10:15–26.
10 Blaser MJ. Gastric *Campylobacter*-like organisms, gastritis and peptic ulcer disease. *Gastroenterology* 1987;93:371–83.
11 Marshall BJ, Royce H, Annear DI, Goodwin CS, Perman JW, Warren JR, Armstrong JA. Original isolation of *Campylobacter pyloridis* from human gastric mucosa. *Microbiol Lett* 1984;25:83–8.
12 Warren J, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983;?:1273–5.
13 Eaton KA, Morgan DR, Krakowka S. *Campylobacter pylori* virulence factors in gnotobiotic piglets. *Infect Immun* 1989;57:1119–25.
14 Evans DG, Evans DJ, Moulds JJ, Graham DY. N-Acetylneuraminyllactose-binding fibrillar hemagglutimin of *Campylobacter pylori*: a putative colonization factor antigen. *Infect Immun* 1988;56:2896–906.
15 Cover TL, Puryear W, Perez-Perez GJ, Blaser M. Effect of urease on HeLa cell vacuolation induced by *Helicobacter pylori* cytotoxin. *Infect Immun* 1991;59:1264–70.
16 Leunk RD, Johnson PT, David BC, Kraft WG, Morgan DR. Cytotoxic activity in broth-culture filtrations of *Campylobacter pylori*. *J Med Microbiol* 1988;26:93–9.
17 Ferrero RL, Lee A. The importance of urease in acid protection for the gastric-colonising bacteria *Helicobacter pylori* and *Helicobacter felis* sp. *Nov Micro Ecol Hlth Dis* 1991;4:121–34.
18 Smoot DT, Mobley HLT, Chippendale GR, Lewison JF, Resau JH. *Helicobacter pylori* urease activity is toxic to human gastric epithelial cells. *Infect Immun* 1990;58:1992–4.
19 Xu JK, Goodwin CS, Cooper M, Robinson J. Intracellular vacuolization caused by the urease of *Helicobacter pylori*. *J Infect Dis* 1990;161:1302–4.

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Effect of CYP2C19 polymorphism on the safety and efficacy of omeprazole in Japanese patients with recurrent reflux oesophagitis

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SUMMARY

Background: The polymorphic enzyme cytochrome P450 2C19 affects omeprazole metabolism. This influence on metabolism might affect serum gastrin levels, and safety, during long-term treatment of reflux oesophagitis.

Aim: To examine the relationship between cytochrome P450 2C19 genotype and the safety profile of long-term omeprazole treatment.

Methods: A total of 119 Japanese patients with recurrent reflux oesophagitis underwent cytochrome P450 2C19 genotyping prior to receiving daily omeprazole 10 mg or 20 mg for 6–12 months, during which adverse event frequency, serum gastrin levels and endoscopic findings were monitored.

Results: The incidences of adverse events, serious adverse events and adverse events leading to withdrawal did not differ between homozygous extensive metabolizer ($n = 46$), heterozygous extensive metabolizer ($n = 53$) or poor metabolizer ($n = 20$) groups. In all genotype groups, serum gastrin increased during the first 3 months of dosing but stabilized thereafter. No significant differences were seen either in the rate of reflux oesophagitis healing or symptom improvement among genotype groups.

Conclusions: Long-term treatment with omeprazole was well-tolerated in Japanese patients, irrespective of their cytochrome P450 2C19 metabolic genotype, indicating that dose adjustment depending on metabolic genotype is not required during treatment with omeprazole.

INTRODUCTION

In recent decades, omeprazole and other proton-pump inhibitors (PPI) have emerged as the first choice for gastro-oesophageal reflux disease because of their high healing rate,[1] and the effectiveness of long-term maintenance treatment in maintaining symptomatic and endoscopic remission from disease.[2] Studies in predominantly Caucasian populations have shown extensive short-term and long-term use of omeprazole to be well-tolerated and safe.[3–5]

Omeprazole is hydroxylated in the liver by cytochrome P450 2C19 (CYP2C19),[6] an enzyme that is encoded

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either by the predominant 'wild-type' gene, or by one of two polymorphic alleles, CYP2C19*2 and CYP2C19*3,[7, 8] which can encode less effective metabolizers of omeprazole. The proportions of poor metabolizers (PM; exhibiting two mutated alleles), heterozygous extensive metabolizers (hetero EM; exhibiting one mutated allele) and homozygous extensive metabolizers (homo EM) in Caucasian populations are reported as being 3%, 30% and 67%, respectively,[9] while the Japanese population is reported to consist of approximately 20% PM,[10, 11] with the majority (about 60%) of most Asian populations being either PM or hetero EM.[9, 12] The higher frequency of CYP2C19 polymorphism in the Japanese population raises the question of whether there may be increased adverse events (AEs) with omeprazole use in Asian populations, especially long-term.

In Caucasian populations, an increase in serum gastrin levels (generally two- to fourfold)[13] has been associated with repeated (8 days) or short-term maintenance (up to 6 months) omeprazole treatment,[14–16] but thereafter serum gastrin levels appear to plateau and further marked increases have not been observed, even with continuous omeprazole therapy for over 5 years.[14] Because Asians demonstrate a high percentage of PM (20%) relative to Caucasians, assessment of such data in Asian populations is worthwhile. This study is the first to examine the effects of long-term omeprazole treatment on serum gastrin levels, to establish the safety profile of long-term omeprazole treatment in Japanese patients with recurrent reflux oesophagitis, and to correlate these measures with CYP2C19 genetic polymorphism.

MATERIALS AND METHODS

Patients

Patients were eligible for the study if they exhibited reflux oesophagitis with a history of at least one recurrence of reflux oesophagitis requiring long-term therapy. All patients were 20 years or older and were Japanese. The main exclusion criteria were: PPI treatment within 28 days before dosing; gastrointestinal (GI) tract resection or vagotomy; Zollinger–Ellison syndrome or pernicious anaemia; serious hepatic, renal, cardiac or haematological disease; malignant tumour or severe dysplasia; pregnancy or lactation; allergy to omeprazole; and concomitant diazepam, phenytoin, warfarin, digoxin, methyldigoxin or itraconazole treatment.

Study design

This multicentre, parallel group, non-randomized open comparative study was performed in 16 hospitals in Japan. The study, approved by the ethics committee at each hospital, was conducted in accordance with the Declaration of Helsinki and all patients gave written informed consent before participating in the study. Omeprazole was initially administered to all patients for a 6-month period, with the possibility to continue treatment for an additional 6 months in any patient requiring further treatment. Symptoms and serum gastrin levels were assessed at entry and again 3, 6, 9 and 12 months after treatment, although patients were encouraged to report any AE on occurrence at any time during the study. Reflux oesophagitis status was assessed by endoscopy at entry, 6 and 12 months after treatment.

Treatments

At each participating centre, patients deemed to be eligible for study participation were openly allocated to treatment with either omeprazole 10 mg or 20 mg once daily oral administration, the dose being decided at the investigator's discretion based on the patient's condition. During the study, patients were prohibited from taking other PPI therapies, H_2-blockers, antigastrin drugs, sodium alginate, sucralfate and protease inhibitors. Other medications were permitted at the discretion of the investigator.

Genotyping

The CYP2C19*2 allele was identified by polymerase chain reaction (PCR)-based allele-specific amplification of exon 5 of CYP2C19 followed by digestion with the restriction enzyme *Sma*I. Similarly, the CYP2C19*3 allele was analysed by PCR amplification of exon 4 of CYP2C19 followed by digestion with the restriction enzyme *Bam*HI.[8] Based on the results of these assays, patients were classified as being homo EM, hetero EM or PM. Collection and assay of the samples was performed in a blind fashion by Mitsubishi Chemical BCL (Itabashi-ku, Tokyo, Japan).

Serum gastrin analysis

Serum gastrin concentrations were determined by radioimmunoassay using Gastrin-Riakit (Abbott,

Minato-ku, Tokyo, Japan). Collection and assay of the samples was performed by Mitsubishi Chemical BCL.

H. pylori *tests*

Upon entry into the study, the *Helicobacter pylori* status of each patient was determined with immunoglobulin G (IgG) antibody of serum blood sample using the EPlate 'Eiken' *H. pylori* Antibody (Eiken Chemical Co Ltd, Bunkyo-ku, Tokyo, Japan). These tests were performed by Mitsubishi Chemical BCL.

Outcome measures

The primary outcome measure was to determine if any significant differences in the omeprazole safety profile, in terms of type, severity and frequency of AE and changes in serum gastrin levels, existed between the CYP2C19 genotype subgroups. All AEs occurring during the study were recorded, and assessment was made as to whether an AE was serious or resulted in treatment discontinuation. The secondary outcome measure was to assess the efficacies of 6- and 12-month long-term treatments, via endoscopic findings and reflux oesophagitis symptom rates, to determine if any differences existed between the CYP2C19 genotype subgroups. The severity of reflux oesophagitis was graded according to the revised Los Angeles (LA) classification.[17] Symptoms of epigastric pain, heartburn, nausea and belching, assessed by response to active questioning, were assigned a score based on severity (0–3) and frequency (0–3). Symptom improvement rating was assessed as; remission maintained or resolved if no symptom score; improved if improved compared with baseline score; no change if similar to baseline score; recurrence or aggravation if increased from baseline score.

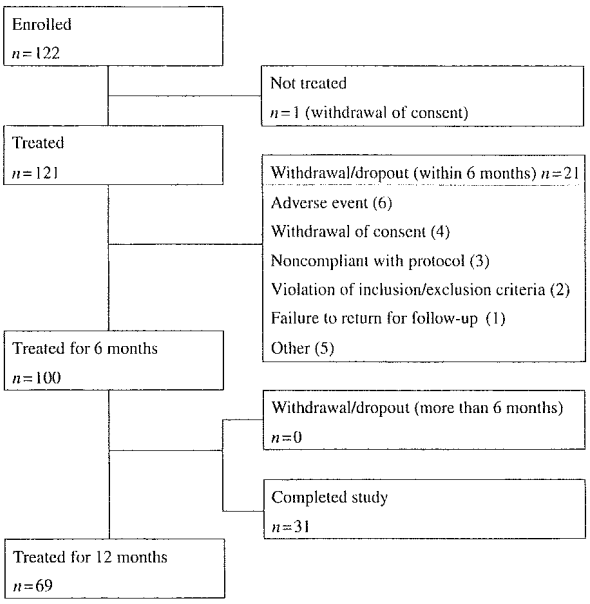
Statistical methods

In a study involving 100 subjects, one or more AE occurring with a frequency of 3% or greater can be detected with 95% probability. Assuming that there would be some discontinuations, the sample size for the study was set at 120 patients. As the proportion of PM in the Japanese population is about 20%,[11] the sample size should contain 20 PM and 80 EM patients. AE data were classified according to MEDDRA/J Version 6.0. The number of cases and events, frequency and two-sided 95% confidence intervals were calculated for all AE data. Data on AE frequencies, serum gastrin levels, endoscopic findings and symptom improvement rate have been presented descriptively in subgroups based on CYP2C19 genotype group (homo EM, hetero EM, PM). The statistical analyses were performed by the Wilcoxon rank sum test and Fisher's exact test using SAS, Version 8.2 (SAS Institute, Cary, NC, USA). *P*-value of <0.05 was considered to be statistically significant.

RESULTS

Patients

A total of 122 patients entered the study, of whom 121 (46 homo EM, 53 hetero EM, 20 PM and two of unknown genotype) received the study drug (Figure 1). A total of 21 patients (19 EM, one PM, one of unknown genotype) discontinued treatment within 6 months for various reasons. The remaining 100 patients were treated for more than 6 months, including 69 who received long-term treatment for a 12-month period. The mean duration of treatment was 34.8 and 42.3 weeks in EM and PM groups, respectively. A total of 103 patients (84 EM, 17 PM and two of unknown genotype) started treatment at omeprazole 20 mg/day, while 18 (15 EM and three PM) started treatment at omeprazole 10 mg/day. Safety data were evaluated for



[a]: Other reasons: unable to return for follow-up within the specified time (3), job transfer to a distant place (1)and improper acquisition of informed consent (1)

Figure 1. Flow of patients.

all patients who received the study drug at least once (121 patients), whereas efficacy data were evaluated in the Full Analysis Set (FAS) population. Two patients were excluded (one for whom recurrent reflux oesophagitis was not confirmed, and one with whom the informed consent procedure was violated). Demographic characteristics at study entry were similar across the three genotype groups (Table 1).

Adverse events

Omeprazole long-term treatment was well-tolerated and no marked differences were found in the safety profiles of the homo EM, hetero EM and PM groups (Table 2). The proportion of patients experiencing an AE was 65.2%, 67.9% and 75.0% in the homo EM, hetero EM and PM groups, respectively. The most commonly

Table 1. Demographic characteristics according to genotype groups

		EM	
	PM	Homo	Hetero
	20	46	53
Sex			
Male	7 (35.0)	26 (56.5)	25 (47.2)
Female	13 (65.0)	20 (43.5)	28 (52.8)
Age (years)			
20–49	1 (5.0)	5 (10.9)	7 (13.2)
50–64	12 (60.0)	12 (26.1)	21 (39.6)
65+	7 (35.0)	29 (63.0)	25 (47.2)
Serum gastrin level (pg/mL)			
Mean	52	89	67
s.d.	34	96	38
Symptom score			
Mean	10	8	6
s.d.	10	6	5
Pre-treatment drug			
H_2RA	16 (80.0)	36 (78.3)	48 (90.6)
Drugs other than H_2RA	1 (5.0)	1 (2.2)	1 (1.9)
None	3 (15.0)	9 (19.6)	4 (7.5)
Smoking status			
Non-smoking	17 (85.0)	36 (78.3)	40 (75.5)
<20 cigarettes/day	1 (5.0)	4 (8.7)	5 (9.4)
≥20 cigarettes/day	2 (10.0)	6 (13.0)	8 (15.1)
Alcohol intake			
None	11 (55.0)	23 (50.0)	38 (71.7)
Occasional	6 (30.0)	8 (17.4)	11 (20.8)
Everyday	3 (15.0)	15 (32.6)	4 (7.5)
Helicobacter pylori result			
Negative	14 (70.0)	31 (67.4)	30 (56.6)
Positive	6 (30.0)	15 (32.6)	23 (43.4)
Classification of reflux oesophagitis			
Healing	1 (5.0)	5 (10.9)	5 (9.4)
Grade A	11 (55.0)	18 (39.1)	24 (45.3)
Grade B	3 (15.0)	9 (19.6)	13 (24.5)
Grade C	3 (15.0)	11 (23.9)	6 (11.3)
Grade D	1 (5.0)	1 (2.2)	4 (7.5)
Unknown	1 (5.0)	2 (4.3)	1 (1.9)

PM, poor metabolizer; EM, extensive metabolizer; H_2RA, histamine 2-receptor antagonist.

Table 2. Summary of adverse events and adverse drug reactions according to genotype groups

		EM	
Category	PM	Homo	Hetero
	20	46	53
Adverse event			
Number of patients*	15	30	36
Frequency (%)	75.0	65.2	67.9
95% CI (lower–upper)	50.9–91.3	49.8–78.6	53.7–80.1
Number of events	33	74	100
Serious adverse event			
Number of patients*	1	4	3
Frequency (%)	5.0	8.7	5.7
95% CI (lower–upper)	0.1–24.9	2.4–20.8	1.2–15.7
Number of events	1	4	3
Serious adverse event leading to death			
Number of patients*	0	0	1
Frequency (%)	0.0	0.0	1.9
95% CI (lower–upper)	0.0–16.8	0.0–7.7	0.0–10.1
Number of events	0	0	1
Serious adverse event other than death			
Number of patients*	1	4	2
Frequency (%)	5.0	8.7	3.8
95% CI (lower–upper)	0.1–24.9	2.4–20.8	0.5–13.0
Number of events	1	4	2
Adverse event leading to treatment discontinuation			
Number of patients*	1	2	3
Frequency (%)	5.0	4.3	5.7
95% CI (lower–upper)	0.1–24.9	0.5–14.8	1.2–15.7
Number of events	1	2	3
Adverse event neither serious nor mild			
Number of patients*	1	4	7
Frequency (%)	5.0	8.7	13.2
95% CI (lower–upper)	0.1–24.9	2.4–20.8	5.5–25.3
Number of events	1	5	10

* Subjects with multiple events in the same category are counted only once in that category. Subjects with events in more than one category are counted once in each of those categories.
No statistically significant difference was observed in the frequency of adverse events between homo EM and PM groups ($P = 0.569$) or between hetero EM and PM groups ($P = 0.776$); Fisher's exact test.
PM, poor metabolizer; EM, extensive metabolizer; CI, confidence interval.

reported AE (incidence ≥3%) were nasopharyngitis (14.0%), upper respiratory tract inflammation (9.1%), diarrhoea (8.3%), headache (5.0%), arthralgia (4.1%), back pain (3.3%), insomnia (3.3%) and cystitis (3.3%). The proportions of patients experiencing serious AEs or AEs leading to treatment withdrawal in the homo EM, hetero EM and PM groups were: 8.7%, 5.7% and 5.0%; and 4.3%, 5.7% and 5.0%, respectively.

A total of eight serious AEs occurred: four in the homo EM group (abdominal pain, hyperglycaemia, aggravated allergic granulomatosis angiitis and nasopharyngitis), three in the hetero EM group (cellulitis and a fractured sternum, death from acute cardiac disorder) and one in the PM group (colorectal cancer diagnosis). Of these eight serious AEs, three resulted in treatment discontinuation (aggravated allergic granulomatosis angiitis, colorectal cancer, death from acute cardiac disorder) and none reported causality because of omeprazole treatment.

Treatment was also discontinued as a result of a further three non-serious AEs, including neurosensory deafness in one homo EM patient, and pruritis and exanthema in two hetero EM subjects. All subjects were confirmed to have recovered from these AEs after treatment discontinuation. The majority of reported AEs in all groups were judged as being either 'mild' or 'moderate' in severity, and dosage adjustment was not required in any case. No further incidence of AEs was found during continuation of the study for 12 months.

Serum gastrin levels

Serum gastrin levels and changes from baseline are summarized in Figure 2. In all genotype groups, serum gastrin levels increased from prestudy levels following 3 months of omeprazole treatment. This increase was most pronounced in the PM group. Further marked serum gastrin increases were not observed in any genotype group when monitored after 6, 9 and 12 months of therapy. Although serum gastrin levels were highest at 3, 6 and 9 months of therapy in the PM group, no marked further increase in this group was observed on continuation of treatment beyond 3 months. In the 21 patients who demonstrated serum gastrin levels of 400 pg/mL or above, no severe AEs were observed. Furthermore, there was no marked difference in serum gastrin levels or in the degree of increase from baseline between patients who did or did not experience AEs.

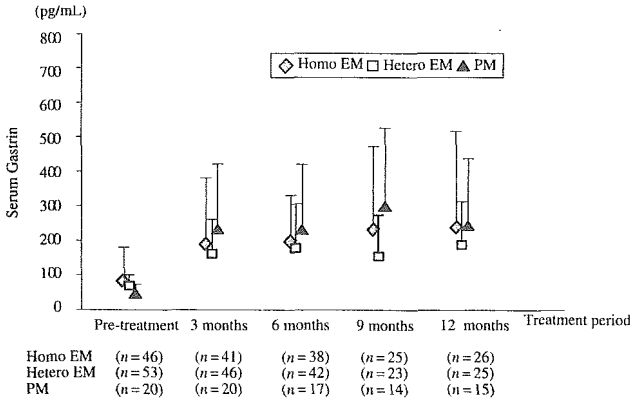


Figure 2. Mean serum gastrin levels with standard deviation by treatment duration and genotype group.

Assessment of changes in gastrin levels from baseline in *H. pylori*-positive and *H. pylori*-negative patients revealed they were greater in the *H. pylori*-positive group than in the *H. pylori*-negative group. The mean changes from baseline at 3, 6, 9 and 12 months were 134.7, 141.4, 159.8 and 184.2 pg/mL for the *H. pylori*-positive group, and 103.2, 114.6, 127.8 and 117.0 pg/mL for the *H. pylori*-negative group, respectively.

Endoscopic findings and symptom improvement rate

As measured by endoscopic findings, healing rates of the homo EM, hetero EM and PM genotype groups were high at all assessment times (Figure 3). Patients demonstrating healing in the homo EM, hetero EM and PM groups after 6 and 12 months of treatment accounted for: 85.0%, 83.3% and 83.3%; and 73.1%, 84.0% and 80.0% of the groups, respectively.

Symptom improvement rates corresponded well with healing observed during endoscopy, and high improvement rates were measured in all genotypes (Table 3). Patients showing improved or remission maintained/resolved in the homo EM, hetero EM and PM groups after 3, 6, 9 and 12 months of treatment accounted for: 88.1%, 97.9% and 95.0%; and 92.5%, 100.0% and 94.4%; and 96.3%, 88.0% and 100.0%; and 96.2%, 96.0% and 100.0% of the respective groups.

Investigation of the influence of *H. pylori* status on the efficacy results revealed that at 6 and 12 months, 89.7 and 89.7% of patients in the *H. pylori*-positive group, and 80.6 and 71.1% of patients in the *H. pylori*-negative group, were grade O on the revised LA classification. In terms of symptom improvement rating at 6 and 12 months, 70.0 and 86.2% of patients in the *H. pylori*-positive group and 64.5 and 73.7% of patients

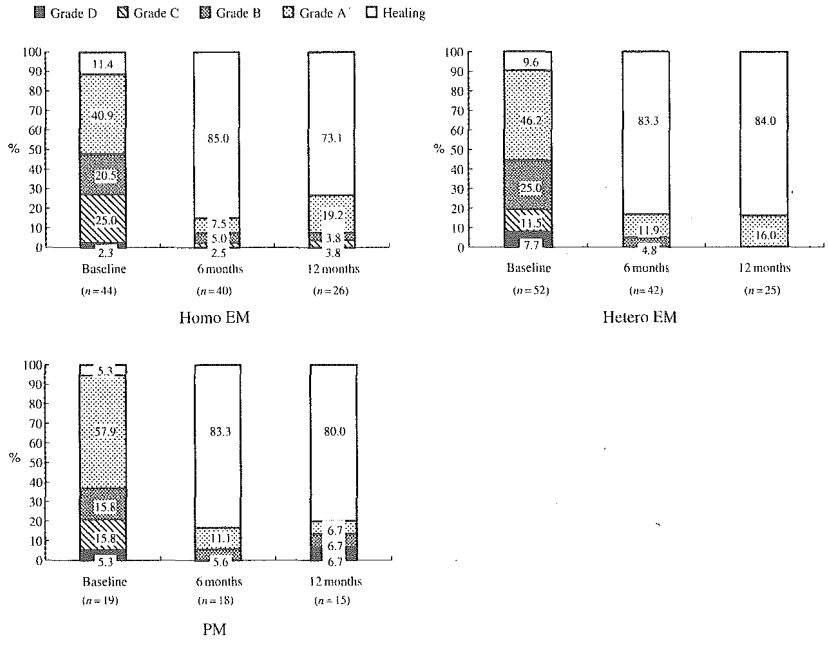


Figure 3. The percentage of poor metabolizer (PM) and extensive metabolizer (EM) patients with Los Angeles (LA) classification (healing – grade D) at baseline and following 6 and 12 months of omeprazole treatment. No statistically significant difference was observed in the healing rate between PM and EM groups ($P = 0.658$–1.000); Fisher's exact test.

Table 3. Symptom improvement rating (Full Analysis Set, FAS)

	Symptom improvement rating				
	Remission maintained or resolved	Improved	No change	Recurrence or aggravation	Total
PM (months)					
3	11 (55.0)	8 (40.0)	1 (5.0)		20
6	10 (55.6)	7 (38.9)	1 (5.6)		18
9	13 (81.3)	3 (18.8)			16
12	13 (86.7)	2 (13.3)			15
EM					
Homo (months)					
3	26 (61.9)	11 (26.2)	1 (2.4)	4 (9.5)	42
6	28 (70.0)	9 (22.5)	1 (2.5)	2 (5.0)	40
9	23 (85.2)	3 (11.1)	1 (3.7)		27
12	19 (73.1)	6 (23.1)		1 (3.8)	26
Hetero (months)					
3	34 (72.3)	12 (25.5)	1 (2.1)		47
6	29 (67.4)	14 (32.6)			43
9	18 (72.0)	4 (16.0)	2 (8.0)	1 (4.0)	25
12	20 (80.0)	4 (16.0)		1 (4.0)	25
Total (months)					
3	60 (67.4)	23 (25.8)	2 (2.2)	4 (4.5)	89
6	57 (68.7)	23 (27.7)	1 (1.2)	2 (2.4)	83
9	41 (78.8)	7 (13.5)	3 (5.8)	1 (1.9)	52
12	39 (76.5)	10 (19.6)		2 (3.9)	51

No statistically significant difference was observed in the rate of 'remission maintained or resolved' and 'improved' between PM and EM groups ($P = 0.268$–1.000); Fisher's exact test.
PM, poor metabolizer; EM, extensive metabolizer.

in the *H. pylori*-negative group were classed as 'remission maintained and resolved'.

DISCUSSION

The safety of omeprazole, reported in a review of data from more than 19 000 individuals receiving mostly short-term administration of omeprazole,[5] indicated that the overall incidence of AE was low and generally in the same range as comparative drugs, and that these AE were generally mild, transient and did not necessitate dose reduction or cessation. A similar profile was reported in 859 patients with peptic ulcer or reflux disease who received omeprazole for up to 6 years, a study which further reported that no serious AE was causally related to omeprazole.[4] The majority of safety data with omeprazole, however, has been collected from studies in Caucasian populations. The question remained whether the safety profile would differ in other populations, such as the Japanese, in whom higher plasma levels of omeprazole may be predicted, because of the larger proportion of slower metabolizers. Indeed, the proportion with lower metabolic capacity was determined to be 60.3% (16.5% PM, 43.8% hetero EM) in the Japanese patients recruited into the current study, figures which agree with previous reports.[9–11] In this study, there were no obvious differences between the homo EM, hetero EM or PM groups in the incidences of AEs, serious AEs or AEs leading to treatment withdrawal (Table 2). The most commonly reported AEs were of a similar nature and frequency to those reported in Caucasian populations.[4, 5] These results clearly indicate that long-term treatment with omeprazole is well-tolerated in Japanese patients, irrespective of their CYP2C19 metabolic genotype.

In Caucasian populations, repeated short-term dosing with omeprazole has been associated with a two- to fourfold increase in serum gastrin levels, which appear to plateau when dosing is continued for a longer period, even over many years.[14–16] Several reports have indicated that the capacity of an individual to metabolize omeprazole influences the magnitude of serum gastrin response during treatment, and that higher serum gastrin levels were observed in both hetero EM and PM patients, compared with homo EM patients following omeprazole dosing.[7, 9, 18] Although the mean values at different visits are not directly comparable because some patients did not continue the treatment after 6 months, the increase in serum gastrin levels observed in this study are consistent with these reports, in that levels increased during the first 3 months of dosing, but increases thereafter were relatively minor in all genotype groups. Overall, the serum gastrin levels in all genotype groups of this study were similar to those reported in Caucasian populations.[14, 15, 18]

Changes in gastrin levels from baseline were greater in patients in the *H. pylori*-positive group, compared with those in the *H. pylori*-negative group. Klingenberg-Knol *et al.* have previously reported gastrin levels to be higher in *H. pylori*-positive patients compared with *H. pylori*-negative patients.[16] They also reported that very high gastrin levels were observed mainly in older patients, who had developed moderate or severe corpus mucosal atrophy. In addition to the expected increases of gastrin levels due to inhibition of acid secretion, inflammatory changes in the gastric mucosa of patients infected with *H. pylori* may also have affected gastrin level in this study.

It has been suggested that serum gastrin values five times above the upper normal limit may be an indication for dose reduction,[15] however, no AE judged to be clinically significant was observed in the 21 patients who demonstrated a serum gastrin level of 400 pg/mL or above. Furthermore, higher serum gastrin levels in the PM group were not associated with any clinical sequelae, and there were no evident differences or increases in serum gastrin levels between patients who did or did not experience an AE.

In this study, a cancerous colonic polyp was reported in the PM group, and a benign gastric polyp and a benign colonic polyp were reported in the EM group. The cancerous polyp was found in a 63-year-old (*H. pylori*-negative) female patient after 55 days dosing of the study drug. The patient subsequently had an operation and recovered: colonic cancer was confirmed by histopathological examination during the operation. The doctor in charge assessed there was no causal relationship with the study drug, as the polyp was considered to exist before study entry, and had been documented as a concomitant disease. There has previously been a discussion whether long-standing hypergastrinaemia might increase the risk of colonic cancer. In order to assess this, studies have been performed in patients with pernicious anaemia as they are known to have high gastrin values most often over decades. Talley *et al.* followed a group of patients with pernicious anaemia over a 20-year period and found that over the whole period there was no excess risk of colon cancer.[19] In another study, Elsborg and Mosbech found that the

occurrence of colon cancer in all patients in Denmark with pernicious anaemia (0.17%) was not different from that in the general population (0.13%).[20] In the long-term study referred to above by Klinkenberg-Knol *et al.* in 230 patients who had been treated continuously with omeprazole for a mean period of 6.5 years, there was no development of gastric or colonic cancer.[16]

It has been hypothesized that changes in gastric mucosal morphology related to increased serum gastrin levels during omeprazole treatment might be dependent on the degree of an individual's capacity to metabolize omeprazole.[7] With this in mind, it has been proposed that CYP2C19 genotyping could guide individual dosage adjustment to maximize clinical effect.[9] However, we did not observe any differences in efficacy between the CYP2C19 genotype groups. Furthermore, no evident differences were observed in the AE profiles of the genotype groups in this study. In PM patients, serum gastrin increases were similar to those observed in the other patient groups, and increased serum gastrin did not result in any greater incidence of AE. These data clearly indicate that genotype determination for the purpose of dose adjustment is not necessary in Japanese patients undergoing long-term therapy with 10 mg or 20 mg omeprazole daily.

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REFERENCES

1 Caro JJ, Salas M, Ward A. Healing and relapse rates in gastroesophageal reflux disease treated with the newer proton-pump inhibitors lansoprazole, rabeprazole, and pantoprazole compared with omeprazole, ranitidine, and placebo: evidence from randomized clinical trials. Clin Ther 2001; 23: 998–1017.

2 Tytgat GN. Review article: Long-term use of proton pump inhibitors in GORD – help or hindrance? Aliment Pharmacol Ther 2001; 15 (Suppl 2): 6–9.

3 Maton PN, Vinayek R, Frucht H, *et al.* Long-term efficacy and safety of omeprazole in patients with Zollinger-Ellison syndrome: a prospective study. Gastroenterology 1989; 97: 827–36.

4 Joelson S, Joelson I-B, Lundborg P, *et al.* Safety experience from long-term treatment with omeprazole. Digestion 1992; 51 (Suppl. 1): 93–101.

5 Sölvell L. The clinical safety of omeprazole. Digestion 1990; 47 (Suppl. 1): 59–63.

6 Andersson T. Pharmacokinetics, metabolism and interactions of acid pump inhibitors. Focus on omeprazole, lansoprazole and pantoprazole. Clin Pharmacokinet 1997; 31: 9–28.

7 Sagar M, Bertilsson L, Stridsberg M, *et al.* Omeprazole and CYP2C19 polymorphism: effects of long-term treatment on gastrin, pepsinogen I, and chromogranin A in patients with acid related disorders. Aliment Pharmacol Ther 2000; 14: 1495–502.

8 De Morais SM, Wilkinson GR, Blaisdell J, *et al.* Identification of a new genetic defect responsible for the polymorphism of (S)-mephenytoin in Japanese. Mol Pharmacol 1994; 46: 594–8.

9 Sagar M, Tybring G, Dahl M-L, *et al.* Effects of omeprazole on intragastric pH and plasma gastrin are dependent on the CYP2C19 polymorphism. Gastroenterology 2000; 119: 670–6.

10 Kimura M, Ieiri I, Mamiya K, *et al.* Genetic polymorphism of cytochrome P450s, CYP2C19, and CYP2C9 in a Japanese population. Ther Drug Monit 1998; 20: 243–7.

11 Horai Y, Nakano M, Ishizaki T, *et al.* Metoprolol and mephenytoin oxidation polymorphisms in Far Eastern Oriental subjects: Japanese versus mainland China. Clin Pharmacol Ther 1989; 46: 198–207.

12 Ishizaki T, Sohn DR, Kobayashi K, *et al.* Interethnic differences in omeprazole metabolism in the two S-mephenytoin

hydroxylation phenotypes studied in Caucasians and Orientals. Ther Drug Monit 1994; 16: 214–5.
13 Freston JW, Borch K, Brand S, *et al.* Effects of hypochlorhydria and hypergastrinemia on structure and function of gastrointestinal cells. A review and analysis. Dig Dis Sci 1995; 40: 50S–62S.
14 Brunner G, Creutzfeldt W, Harke U, *et al.* Efficacy and safety of long-term treatment with omeprazole in patients with acid related diseases resistant to ranitidine. Can J Gastroenterol 1989; 3 (Suppl. A): 72A–6A.
15 Lamberts R, Creutzfeldt W, Stöckmann F, *et al.* Long-term omeprazole treatment in man: effects on gastric endocrine cell populations. Digestion 1988; 39: 126–35.
16 Klingenberg-Knol EC, Nelis F, Dent J, *et al.* Long-term omeprazole treatment in resistant gastroesophageal reflux disease: efficacy, safety, and influence on gastric mucosa. Gastroenterology 2000; 118: 661–9.
17 Armstrong D, Bennett JR, Blum AL, *et al.* The endoscopic assessment of esophagitis: a progress report on observer agreement. Gastroenterology 1996; 111: 85–92.
18 Chang M, Tybring G, Dahl M-L, *et al.* Interphenotype differences in disposition and effect on gastrin levels of omeprazole – suitability of omeprazole as a probe for CYP2C19. Br J Clin Pharmacol 1995; 39: 511–8.
19 Talley NJ, Chute CG, Larson DE, *et al.* Risk for colorectal adenocarcinoma in pernicious anaemia. Ann Intern Med 1989; 111: 738–42.
20 Elsborg M, Mosbech J. Pernicious anemia as a risk factor for gastric cancer. Acta Med Scand 1979; 206: 315–8.

診断の疑問に答える

肝疾患における身体診察の重要性

峯　徹哉

ポイント
- 触診だけではなく，視診，打診，聴診も重要である．
- 身体診察は診断・治療に有益である．

身体診察は視診，触診，打診，聴診に分けられるので，おのおの重要な点を述べることにする．

視　診

腹壁自体の状態，腹部全体の陥没(retraction)，腹部全体の膨隆(abdominal distension)，局所的な腹部膨隆の存在を確める．腹部の所見の位置を示すためには，種々の分類があるが，臍を通る水平線および2本の垂直線により腹部を6分割して示す方法が一般的である．

このほか，肋骨弓，正中線，臍の高さ，左右腸骨稜，前腸骨棘などを基準として，その視診に際しては，腹部を十分露出させることが必要であり，通常，仰臥位で観察する．

1．腹壁自体の状態

1）貧血，黄疸，色素沈着，発疹

貧血，黄疸，色素沈着，発疹をみることがある．黄疸では瘙痒感のため皮膚に引っ掻き傷を認めることがある．肝硬変では出血傾向の存在を認めることがある．

手術瘢痕を認める場合には，大きさなどより，過去にどのような手術が行われたかが推定でき，患者の現在の愁訴と既往症との因果関係を推測できる．

2）腹壁静脈の怒張

肝硬変や下大静脈血栓症で，門脈，下大静脈に血行障害がある場合には，腹壁静脈を通じて側副路が発達するため，腹壁静脈の怒張をみる．この場合，血流の方向を必ずみるようにする．これには両手の示指あるいは左右どちらかの示指，中指を揃えて，静脈を圧迫し，次いで少ししごき，静脈の血流を圧排する．次いで一方の指で圧迫したまま他方の指を離し，血液の流入状態をみる．この際，血液が流入しなければ，血液は圧迫した指の側から，離した指の方向に流れていることになる．

2．腹部膨隆

腹部全体の膨隆は，主として鼓腸(meteorism)，腹水(ascites)でみられるが，そのほか，急性胃拡張，気腹，腹腔内の大きい腫瘤などでもみられ，また肥満者でも腹壁の脂肪沈着のため腹部全体の膨隆をみる．局所的な膨隆は肝癌などでみられる．

触　診

腹部疾患の診断において，触診は最も重要な診断手技の一つで，この手技により腹部各臓器の位置，性状を明らかにすることができ，また腹部全体あるいは限局性の緊張亢進，抵抗(resistance)，圧痛(tenderness)および腫瘤(tumor)の存在を認めることができる．

1．患者の腹壁を弛緩させる

腹部の触診を行うためには，患者の腹壁が十分弛緩していなくてはならない．患者の体位は通常仰臥位として触診を行うが，腹壁を弛緩させるため，両下肢を股関節および膝関節で屈曲させ，腹式呼吸を行うようにさせる．それでも患者は無意識に腹筋を緊張させることが多いので，深呼吸を

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行わせるか，あるいは適当に問診を行うことにより患者の注意をそらせる配慮が必要であり，疼痛がある場合には，疼痛のない部位から触診を開始するなどの注意も重要である．また，冬季には，検者の手指が冷たいと反射的に腹筋の緊張が生ずるので，触診の前に検者の手指を温めるなどの配慮を忘れてはならない．

2．腹壁の緊張亢進を探る

触診に際しては，原則として検者は患者の右側に位置し，検者の手掌を患者の腹壁に平行に当て，触診の初めには手掌全体で，力を入れることなく腹壁全体を軽く触診し，腹部全体または限局性の腹壁の緊張状態，各臓器の表面の性状などを把握し，次いでやや指頭に力を入れ，指先で探るようにして各臓器の性状，腫瘤の存在などを明らかにしていくが，この際あまり力を入れるとかえって腹筋の反射的緊張が起こり，所見が不明瞭になるおそれもあるので十分注意が必要である．

まず腹壁の緊張亢進(spasm, spasticity, tension, rigidity)であるが，腹部全体にみられる場合と，限局性にみられる場合とがある．腹部全体の緊張亢進は潰瘍穿孔，虫垂炎などに基づく汎発性腹膜炎に際してみられ，腹壁は板状に硬くなり(muscular rigidity)，強い圧痛(tenderness)が認められる．

3．肝を触知し，性状を探る

肝を触診するには，できるだけ患者の腹壁を弛緩させて，検者の手指が右肋骨弓に平行，あるいは体の長軸に平行になるようにして，腹壁に平らに当てる．吸気のときに腹部を膨隆させ，呼気のときに腹部を凹ませるように，ゆっくりと腹式呼吸を行わせ，腹壁の動きに合わせて，検者の手指先端を，腹部が陥凹するときは圧迫しつつ深く肋骨弓下に向かって進め，腹部が膨隆するときは軽く圧迫しつつ，腹壁とともに上昇させれば，肝下縁を触知できる．この腹式呼吸がうまく行えないと肝を触れえないので十分な注意が必要である．肝が触知できたときは，さらにその大きさ，辺縁の性状(鋭または鈍：sharp or rounded)，表面の性状(平滑：smooth, 凹凸不整：uneven)，硬度〔consistency(軟：soft，弾性硬：elastic firm，硬：hard)〕，圧痛の有無，その程度などを十分調べていく．なお表面が凹凸不整の場合，さらにその性状を程度により細結節状(fine nodular)，粗大結節状(coarse nodular)などと表現する．

4．肝の腫大と下垂を探る

肝下縁は，正常でも腹壁の軟らかい場合にはしばしば触れるものであり，肝を触知すること自体は，必ずしも病的な意味をもつものではない．正常で触れる場合には，右乳腺上，肋骨弓下にわずかに触れ，その辺縁は鋭，硬度は軟，表面は平滑である．肝が腫大を示す場合には，肋骨弓からの幅が大きくなるので，臨床上，肝の大きさをこの幅で表現し，何横指触れるというように示すのが普通である．また，肝の下垂があれば腫大がなくても肋骨弓からの幅が大きくなるので，肝の腫大と下垂とを区別しなければならない．肝の上縁では打診で容易に決定できるので，打診による肝上縁の決定と触診による肝下縁の触知とにより肝の大きさを決定することができる．

腫大した場合には多く硬度は増し，辺縁も鈍となり，その表面は肝炎では平滑であり，肝硬変では顆粒状を示し，肝癌などでは腫瘤状の凹凸を示すことが多い．また圧痛は，急性肝炎ではほとんど毎常みられるが，肝硬変，肝癌では通常これを欠く．

5．脾の触診

脾を触診するには熟練を必要とする．患者は仰臥位または右半側臥位とし，できるだけ腹壁の緊張を除いておく．検者の手指を平らに揃えて，左肋骨弓下に置き，深呼吸を行わせながら肋骨弓内に押し入れるようにして触診する．この際，検者の左手は左胸部下部，脾の存在部のあたりに置き，圧迫を加える必要がある(双手診)．脾を触れるときには大きさ，硬さ，表面の性状に注意する．

正常の脾は全く触れないが，チフス，敗血症などの感染症，肝硬変などに際して軽度の脾腫がみられる．感染症に際してみられる脾腫は軟らかく，触れにくい．特発性門脈圧亢進症，白血病，

溶血性黄疸では時に非常に大きい脾腫を認める．大きい脾腫の場合，触れる腫瘤が脾であることは，脾腫では内方に切痕のあること，移動の方向が下内方であることより診断される．

打　診

腹部疾患においては，打診の意義は胸部におけるほど大きくはない．補助的に使われるのみである．

肝上縁は打診により容易に決定できるが，これと触知による肝下縁の位置とにより，肝の大きさを知ることができる．肝腫大があっても肝下縁が触診でわかりにくいときは，打診で肝の大きさを推定できる．脾腫のある場合，その大きさによって脾濁音界は増大を示すので，打診は脾腫の大きさの決定に使われる．なお，腹部膨隆をみた場合，その主な原因は鼓腸と腹水である．鼓腸と腹水の鑑別は打診で行う．鼓腸では打診で腹部は全般に鼓音を示すが，腹水では鼓音とともに濁音を示す部位がみられ，かつ体位による変化が認められる(体位変換現象)．すなわち，仰臥位では臍部周辺に鼓音，側腹部に濁音，坐位では上腹部に鼓音，下腹部に濁音，かつ仰臥位で波動(fluctuation)を証明する．これは仰臥位において一方の側腹部に手掌をあてて，反対側の側腹部を叩くと腹水によって伝播されあてていた手掌にそれを感ずることができるというものである．この場合，腹壁によっても伝達される可能性があるので，遮断する目的で臍中央部に別に手を置いたほうがより確かである．そのほか，大きい腫瘤，例えば巨大な卵巣嚢腫などでも打診で濁音を証明するが，体位変換による変化がみられない．

聴　診

腹部の聴診は胸部に比べると診断的価値は少ない．腸管の蠕動運動により腸管内のガスと内容物が移動し発する音をグル音(腹鳴：borborygmus)という．グル音が著明な場合は聴診器を使用しなくてもよく聞こえるが，弱い場合は聴診器の使用が必要である．

●おわりに　このように身体診療はかなり重要であり，26％の患者の診断・治療に不可欠であったと報告している論文もある[1](☆☆☆)．このような身体診察と解剖とが同等度の所見を呈していたとの報告もある[2,3](☆☆)．

文献

1) Reilly BM：Physical examination in the care of medical inpatients；An observational study. Lancet 362：1100-1105, 2003
2) Goldman L, et al：The value of the autopsy in three medical eras. N Engl J Med 308：1000-1005, 1983
3) Landefeld CS, et al：Diagnostic yield of the autopsy in a university hospital and a community hospital. N Engl J Med 318：1249-1254, 1988