視鏡的粘膜切除術ー透明プラスチックキャップを用いる方法(EMRC). Gastroenterol Endosc 35:600-607, 1993
12. 片山 修, 小黒康平, 大久保裕雄ほか:斜形透明フードを利用した胃癌の内視鏡的粘膜切除術の適応と限界. Prog Dig Endosc 41:154-157, 387, 1992
13. Yamamoto H, Sekine Y, Higashizawa T et al: Successful *en bloc* resection of a large superficial gastric cancer by using sodium hyaluronate and electrocautery incision forceps. Gastrointest Endosc 54:629-632, 2001
14. 片山 修, 廣多康光, 並木 薫ほか:先端斜形漏斗形透明キャップを用いた内視鏡的粘膜切除術. 消化器の臨床 6:464-468, 2003
15. 大浦通久, 細井薫三, 山村彰彦ほか:表層拡大型の胃型高分化型腺癌の1例. 胃と腸 38:733-738, 2003
16. Ida K, Nakazawa S, Yoshino J et al: Multicentric collaborative prospective study of endoscopic treatment of early gastric cancer. Dig Endosc 16:295-302, 2004
17. Yamano H, Matsushita H, Yamanaka K et al: A study of physical efficacy of different snares for endoscopic mucosal resection. Dig Endosc 16:S85-S88, 2004
18. 片山 修, 並木 薫, 廣多康光ほか:切開・剥離法によるEMRの現状と問題点ー切開・吸引法による早期胃癌のEMRー. 埼玉医会誌 39:288-291, 2004
19. 片山 修, 吉田行雄:治療内視鏡の習熟法、山中桓夫, 片山 修(編):食道・胃の治療内視鏡, 6-7, メジカルビュー社, 東京, 2002
20. 桑原一彰, 今中雄一:外科のあるべき診療報酬体系ー医師の立場からー. 日外会誌 106:44-49, 2005

Endoscopic Submucosal Dissection (ESD) in Japan from the Viewpoint of Cost-effectiveness

Osamu KATAYAMA*[1] et al.

*[1]*Department of Endoscopy, Saitama-ken Saiseikai Kurihashi Hospital, Saitama, Japan et al.*

In view of the prices of the IT knife® (35,000yen), Hook knife® (30,000yen), and Flex knife® (13,500yen), it is obviously unjust that the technical fee for EMR, including ESD method, is only 49,700yen in Japan. To study the economic problems, we sent out questionnaires on ESD to 69 hospitals and clinics, and got answers from 21 hospitals. The average cost for the equipment and drugs was 70,757yen for ESD for lesions within the indications set by the JGCA gastric cancer treatment guidelines, 80,787yen for ESD for lesions with ulcer scars, and 81,632yen for ESD for lesions over 2 cm.

key words: endoscopic mucosal resection (EMR), endoscopic submucosal dissection (ESD), cost-effectiveness

Legends to Figures and Tables

Figure 1 Cost of equipment for ESD.
Figure 2 Cost of drugs for ESD.
Figure 3 Time required for ESD.
Table 1 Questionnaire on equipment for ESD.
Table 2 Questionnaire on drugs, time, and staff for ESD.
Table 3 Hospitals and institutes.
Table 4 Equipment for ESD.
Table 5 Drugs for ESD.
Table 6 Times and staffs for ESD.
Table 7 Cost, time, and staff required for ESD for lesions within the indications set by the JGCA gastric cancer treatment guidelines.
Table 8 Cost, time, and staff required for ESD for lesions with ulcer scars.
Table 9 Cost, time, and staff required for ESD for lesions over 2 cm.

私の診療経験から

消化性潰瘍の過去・現在・未来

峯　　徹　　哉

は　じ　め　に

消化性潰瘍の過去・現在・未来と題して講演を行なった。何故講演のタイトルとして消化性潰瘍を選んだかというと，この20年間にその成因・治療に関して劇的にかわった疾患の最も代表するものであったからである。過去・現在・未来と大層な題名をつけたのは全て森羅万象は温故知新のくり返しによって解明されるという私の信念故のものであった。消化性潰瘍を客観的にみなおしてみると前述の如くまず消化性潰瘍の成因論と治療の変遷に驚かされる。次にそれらをふまえて最近の成因論および治療に辿りつかなければならない。さらに一昨年出版された胃潰瘍診療ガイドラインに基づいた治療について述べ，具体的な薬剤の選択についても述べたいと思う。

I．消化性潰瘍の成因論と治療の変遷

古くは1852～1853年のVirchowに代表されるように血管障害説が有名であった。この概念は血管がつまると組織に酸素がいかなくなり，その部位に壊死が生じ潰瘍形成するというものでありこの説は現在でも虚血再灌流の際にラジカルが形成される事実も加味されながら，成因の一つであると考えられている。更にCushingの中枢性潰瘍説，これは様々な脳血管障害後に臨床的には潰瘍が生ずるという事実に基づいた説である。最近はあまりやられていないが動物モデルもしきりに行なわれていた。その代表的な例が拘束浸水ラットモデルであったと思われる。その後に消化性潰瘍の考え方をリードしてきた有名な説がでてきた。1961年のShay and Sunの天秤説である。この説は防御因子と攻撃因子のバランスが重要であり，攻撃因子の割合が強くなると潰瘍が生ずるというものである。更に大井の二重規制説を忘れてはいけない。胃粘膜・筋による二重規制及び胃分泌・運動による二重規制により，消化性潰瘍が形成されるというものである。これらの概念が消化性潰瘍の本流と考えられていた。当然，攻撃因子を低下させる為の薬剤が開発されたり，単独ではあまり用いられないが防御因子を増強する薬剤も開発されていったわけである[1]。その第1弾がH2受容体拮抗薬である。シメチジンは1982年に本邦で発売された。これにより消化性潰瘍の臨床が180度変化したといっても過言ではない。これによって消化性潰瘍が治るという状態から治すという時代に変化したと思われる。しかし，同時に様々な変化を招くにいたった。ひとつは手軽に服用することにより，症状が簡単に消失するが，潰瘍は完全に治癒しない為消化性潰瘍の待機手術例は減少したが，緊急手術例は逆に増加することになった。また，一旦潰瘍は治癒するが，再発率はH2受容体拮抗薬の普及以前と比較してもあまり差がなかったわけである。潰瘍の再発を抑制する為に維持療法が広く行なわれたわけである。即ち，通常量の半量を長期的に服用しつづけるわけである。これにより，再発率は20%くらいまで低下したが服薬を止めるとまた潰瘍が再発する症例はやはり多かった。その為にcytoprotectionという概念が提唱され（この言葉は今でも研究会名として残っている)，プロスタグランディン製剤を中心に開発され市販された。しかし，残念ながらプロスタグランディン製剤は使用量の加減が難しく量が多いと流産や下痢を生じてしまう。少量であると効果が弱いというジレンマから遂に開放されなかったのではないかと思われる。しばらくすると消化性潰瘍の周囲の環境が劇的に変化してくることになる。1983～1984年にWarrenとMarshall[1]が，偶然にも*H. pylori*を発見したのである。それ以前にも胃内に細菌がいるという報告が多数あった

東海大学医学部内科学系消化器内科学　教授

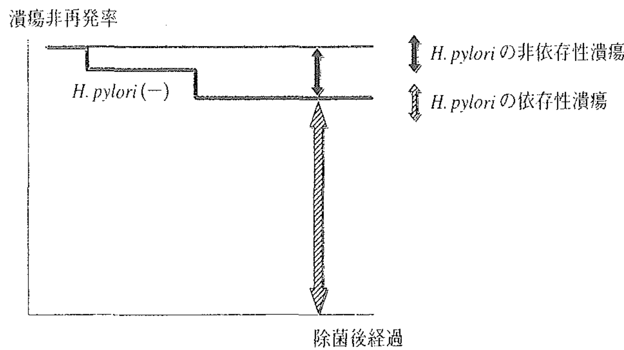

図 1　*H. pylori*(－)胃潰瘍の再発

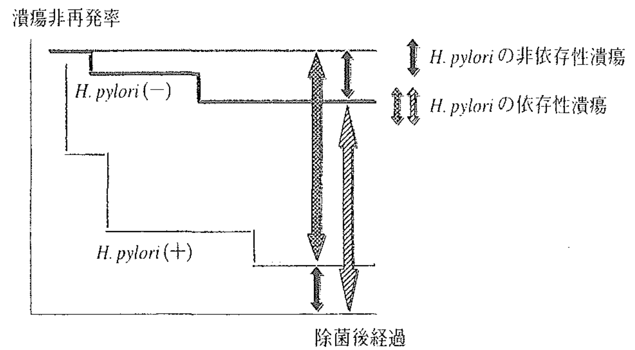

図 3　*H. pylori*(－), *H. pylori*(+)胃潰瘍の再発

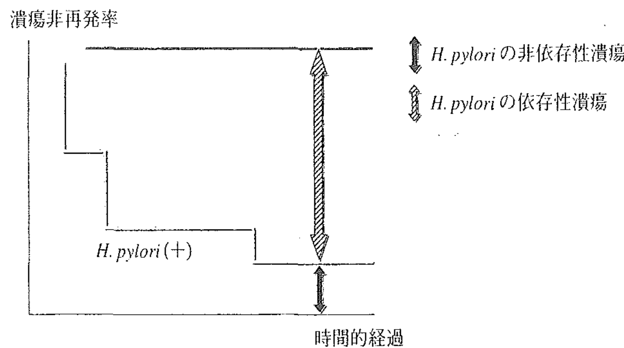

図 2　*H. pylori*(+)胃潰瘍の再発

が，いずれもpH2-3の状態の中で細菌が生息するはずはなく，contaminationであると一蹴されていた。*H. pylori*の意義については日本ではかなりの期間冷ややかにみられていた。そういう理由で1987年から海外で*H. pylori*の除菌療法が既に始まり，ようやく日本で保険が認可されたのが2000年である。この5年間の時間のtime lagは大きく消化性潰瘍の臨床については殆んど外国にイニシアティブがとられてしまった形となった。その間にH2受容体拮抗薬のみではなくプロトンポンプ阻害薬が登場してくることになる。これと2剤の抗生剤を併用することにより除菌率がかなり上昇し，現在80～90%といわれている。これにより*H. pylori*の除菌が日本でも一般的に行なわれるようになり消化性潰瘍の治療が激変したわけである。前述の維持療法でさえも20%を下まわれなかったのに*H. pylori*の除菌が完了すると約10%の再発しか認めないわけである。しかも除菌後はdrug freeの状態になりうるわけである。

これらのことを模式的に書いたものが図1と図2である。図1は*H. pylori*が完全に除菌されても再発する群が存在することを表わしており，図2は*H. pylori*が存在しても再発しない潰瘍があることがわかり，これらは*H. pylori*非依存性ということになる。それをあわせると図3となる。いずれにしても*H. pylori*非依存症の潰瘍が存在する。

Ⅱ. 潰　瘍　の　病　態

次に潰瘍の病態を考えてみると全て酸がかかわった状態であるが，それに加えて*H. pylori*が関与したりストレスが関与したりNSAIDsが関係して消化性潰瘍ができる。現在，慢性潰瘍の殆んどは*H. pylori*中心に考えられている。しかも説得力が比較的乏しい様々な説が唱えられている。例えば，十二指腸潰瘍形成の場合は胃底腺の萎縮がなく，高酸状態にあるので球部の胃上皮化生部分に潰瘍を形成する。一方，*H. pylori*により胃底腺に萎縮が生ずると胃潰瘍が形成されると言われている。しかし，前述の如くこの仮説も十分ではないと思われる。消化性潰瘍の次に多いのがNSAIDs潰瘍である。NSAIDs潰瘍の機序については様々なことがわかり始めている。Cox（シクロオキシゲナーゼ）というPG産生の律速段階の酵素がありこの酵素はCox1とCox2にさらに分類されるが，Cox1は胃粘膜障害防御に作用し，Cox2は炎症を惹起する方に作用していると思われている。最近，NSAIDsの中でもCox1あるいはCox2を選択的に阻害するものができており特にCox2を抑制するNSAIDsは消化性潰瘍を生じにくいとされている。しかし，NSAIDsと*H. pylori*との感染との関係は必ずしも明白ではない。少し話はそれるが潰瘍治療の機序に対する様々な展開もある。ひとつが我々が研究を行なっていた細胞間接合装置である。細胞間接合装置にタイト結合，アドヘレンス結合，ギャップ結合といろいろあるがいずれも重要であり，その制御している

カテニンなども様々な作用をしている。我々もギャップ結合におけるコネキシン蛋白の重要性を指摘している。またカプサイシンを中心とした粘膜防御機構も新たな展開を認めつつある。カプサイシン投与はバニロイド受容体に作用しCGRPの増加を生じ，PGの増加を生ずるという経路を有していることが解明されている。即ち，*H. pylori* の感染及びNSAIDs服用が同時にあり，潰瘍が生じている場合 *H. pylori* を除菌するかどうかは意見がわかれている。

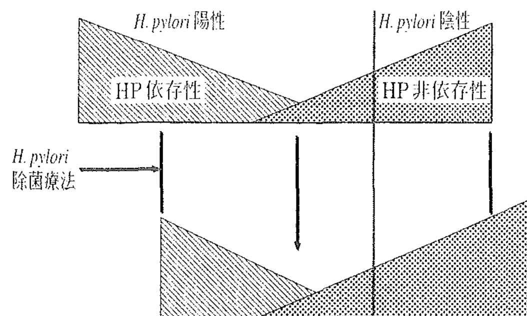

図 4　*H. pylori* 感染と胃潰瘍

Ⅲ．胃潰瘍診療ガイドライン

次に胃潰瘍診療ガイドラインについて述べることにする。最近，日本ではガイドラインがさかんに作成されているが必ずしも満足いくのは少ない。その中でも胃潰瘍診療ガイドラインはかなりの時間を費やし，作成されたものであり，その努力は推測するにあまりあるが，やはり，英語の論文を中心に構成されている。その論文の多くは残念ながら外国の論文であり，日本人が書いた論文は少ない。そこから発生するEBMが果たして日本人に通じるかということも十分に考えなければいけない。更にガイドラインは論文を中心に構成されており，相反する論文も引用されているので結論も出しにくい点もあったように思われる。実際どう書かれているかというと慢性胃潰瘍で *H. pylori* 陽性の場合は除菌をする。出血性潰瘍の場合はとりあえず，内視鏡で止血を行ない，その後潰瘍の治療については *H. pylori* 陽性であれば除菌を行なう。NSAIDsの潰瘍の場合はどうしても除菌により胃潰瘍の治療が遷延する可能性があるのでNSAIDsの服用を中止して *H. pylori* の除菌を行なうことになる。

Ⅳ．潰瘍治療に用いられる薬剤

これらのことをまとめてみると薬剤としては胃潰瘍診療ガイドラインでは現在その殆んどがPPIとH2受容体拮抗薬が中心となることになり，粘膜保護剤の影がかなりうすい。しかし，論文を調べていくとこのような粘膜保護剤により潰瘍の治癒が促進されたという論文もかなりある。やはり，一部の潰瘍では *H. pylori* だけではなくShay and Sunの天秤説に基づく機序を有しているのではないかと思われる。またPPIとH2受容体拮抗薬の使い分けについても様々な報告がある。まず，PPIは酸で活性化され H^+-K^+ATPaseと結合できる形態になりその効果を発揮するので酸分泌抑制効果が生ずるまで多少時間がかかる。またPPI投与の一部に夜間のみ抑制が効かないnocturnal acid breakthrough（NAB）が存在する。さらにPPIにはCYP2C19という代謝酵素があり代謝速度が人によって異なる。H2受容体拮抗薬についても酸分泌抑制が80%～90%でありPPIは95%以上であることを考えると両者にはかなりの差があることも事実である。

お　わ　り　に

消化性潰瘍の過去，現在，未来を振り返ってみるとその時々にエポロックメイキングなことがある。ひとつはShay and Sunの説であり，これは消化性潰瘍の仮説として長く君臨してきた。その次がH2受容体拮抗薬の登場である[1]。その後は *H. pylori* の発見であり，同時にPPIの使用という経過ではないかと思われる。図4で示したように今後 *H. pylori* 非依存性の潰瘍やそれに付随して逆流性食道炎が増加する時代になってくると思われる。

文　　献

1) Brimblecombe, R. W., Duncan, W. A., Durant, G. J., Ganellin, C. R., Parsons, M. E., Black, J. W.：Proceedings：The pharmacology of cimetidine, a new histamine H_2-receptor antagonist. Br J Pharmacol., 53：435-436, 1975.
2) Marshall, B. J., Warren, J. R.：Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet, 16：1311-1315, 1984.

An *in vitro* model of hepatitis C virion production

Theo Heller*†, Satoru Saito*†, Jonathan Auerbach*, Tarice Williams*, Tzivia Rachel Moreen*, Allison Jazwinski*, Brian Cruz*, Neha Jeurkar*, Ronda Sapp*, Guangxiang Luo‡, and T. Jake Liang*§

*Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892; and ‡University of Kentucky College of Medicine, Lexington, KY 40506

Communicated by Reed B. Wickner, National Institutes of Health, Bethesda, MD, December 23, 2004 (received for review November 29, 2004)

The hepatitis C virus (HCV) is a major cause of liver disease worldwide. The understanding of the viral life cycle has been hampered by the lack of a satisfactory cell culture system. The development of the HCV replicon system has been a major advance, but the system does not produce virions. In this study, we constructed an infectious HCV genotype 1b cDNA between two ribozymes that are designed to generate the exact 5′ and 3′ ends of HCV. A second construct with a mutation in the active site of the viral RNA-dependent RNA polymerase (RdRp) was generated as a control. The HCV-ribozyme expression construct was transfected into Huh7 cells. Both HCV structural and nonstructural proteins were detected by immunofluorescence and Western blot. RNase protection assays showed positive- and negative-strand HCV RNA. Sequence analysis of the 5′ and 3′ ends provided further evidence of viral replication. Sucrose density gradient centrifugation of the culture medium revealed colocalization of HCV RNA and structural proteins in a fraction with the density of 1.16 g/ml, the putative density of HCV virions. Electron microscopy showed viral particles of ≈50 nm in diameter. The level of HCV RNA in the culture medium was as high as 10 million copies per milliliter. The HCV-ribozyme construct with the inactivating mutation in the RdRp did not show evidence of viral replication, assembly, and release. This system supports the production and secretion of high-level HCV virions and extends the repertoire of tools available for the study of HCV biology.

assembly | cell culture | infection | ribozyme | viral replication

The hepatitis C virus (HCV) is an important cause of human illness worldwide (1). Although it has proven to be a difficult public health problem, it has been no easier to study in the laboratory. A major impediment has been the lack of robust model systems to study the complete viral life cycle. HCV is a member of the Flaviviridae family of ≈9.6 kb, and it has a central ORF flanked by the 5′ and 3′ noncoding regions. The ORF is divided into the coding sequences for the structural proteins at the 5′ end and the nonstructural proteins at the 3′ end. Study of the biology of hepatitis C at a molecular level focused initially on expression and manipulation of individual viral proteins in tissue culture.

The development of the subgenomic and genomic replicons is a major breakthrough to understanding viral replication and viral-cell interactions and provides a means to test therapeutic targets (2, 3). However, as yet, none of these systems produce viral particles, nor do they produce infectious virions. Although some infectious tissue culture systems have been described; in general, these systems have not been robust enough to study the complete viral life cycle (4, 5).

Why virion production has been such an elusive goal remains unclear; however, the promise of a system that produces authentic virions is clear. Not only would more of the biology of the virus become accessible for study, but also such a system would provide a means to screen a wider range of potential therapeutic compounds. There is evidence for an inverse relationship between viral replication in tissue culture and virulence in the host organism. This relationship is true for hepatitis A, and there is evidence that it may be true for HCV as well (6, 7). Regardless of the reason for this difficulty, there is an urgent need to establish such a system if improved therapies are to be developed, particularly given the absence of a simple small-animal model of HCV infection. This need is especially true for genotype 1, given that this genotype is the major genotype of human infections worldwide and is the type most resistant to current therapies (8, 9).

In this study, we describe an *in vitro* HCV replication system that is capable of producing viral particles in the culture medium. A full-length HCV construct, CG1b of genotype 1b, known to be infectious (10), was placed between two ribozymes designed to generate the exact 5′ and 3′ ends of HCV when cleaved. By using this system, we showed that HCV proteins and positive and negative RNA strands were produced intracellularly, and viral particles that resemble authentic HCV virions were produced and secreted into the culture medium. This system provides a unique opportunity to further study the life cycle and biology of HCV and to test potential therapeutic targets.

Materials and Methods

Plasmid Construction. The ribozymes were constructed by means of three pairs of overlapping primers that were based on a described ribozyme pair that was functional in hepatocytes (11). The innermost set (5′-CGG TAC CCG GTA CCG TCG CCA GCC CCC GA and 3′-ACG GAT CTA GAT CCG TCA CAT GAT CTG CA) was used to amplify pHCVGFP2. The pHCVGFP2 was derived from an infectious full-length HCV CG1b clone (10) and was constructed by replacing the HCV sequences between nucleotide 709 (*Cla*I) and 8935 (*Bgl*II) by the sequence coding for the GFP. The middle (5′-TCC GTG AGG ACG AAA CGG TAC CCG GT and 3′-CAC GGA CTC ATC AGG ACG GAT CTA GA) and outermost (5′-GGC TGG CCT GAT GAG TCC GTG AGG A and 3′-GAT CAT GTT CGT CCT CAC GGA CTC A) sets were then added on to this sequence by PCR. This fragment was cloned into the *Srf*I site of pCMV-Script (Stratagene) and in turn subcloned into pcDNA3.1 (Invitrogen) by using *Not*I and *Hin*dIII sites to generate the pHt plasmid. pcDNA has both a CMV and a T7 promoter. The GFP was then removed, and the missing part of the HCV sequence was reinserted to generate the pHr plasmid. The pHr was used to generate the HCV-ribozyme RNA by T7 polymerase to assess the efficiency of the ribozymes. The HCV-ribozyme fragment was subcloned into pTRE2hyg+ (Clontech) under the control of a tetracycline-responsive promoter. This construct was named pTHr. In all the experiments described in this study, pTHr transfection always refers to cotransfection with pTet-Off (Clontech) expressing the tetracycline-responsive transactivator. A mutation in the GDD motif of the polymerase (GDD→GND)

This work was presented in part at the 55th Annual Meeting of the American Association for the Study of Liver Disease, Boston, October 30–November 2, 2004.

Abbreviation: HCV, hepatitis C virus.

†T.H. and S.S. contributed equally to this work.

§To whom correspondence should be addressed at: Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Building 10, Room 9B16, 10 Center Drive MSC 1800, Bethesda, MD 20892-1800. E-mail: jliang@nih.gov.

© 2005 by The National Academy of Sciences of the USA

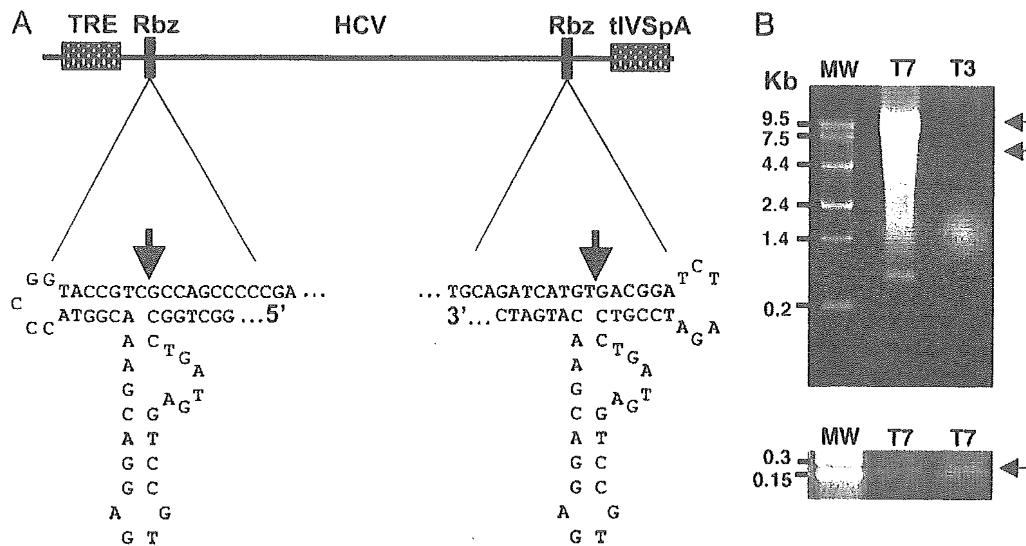

Fig. 1. Construction of HCV-ribozyme plasmid. (*A*) The design of the construct is shown with the positions and sequences of the ribozymes (Rbz) flanking the 5′ and 3′ ends of the HCV CG1B sequence. The cleavage sites are indicated by arrows. The boxes shown 5′ and 3′ to the construct represent the promoter sequence (5′ end) and the simian virus 40 small T antigen intron and polyadenylation signal (3′ end). (*B*) An RNA gel with *in vitro* transcription products from pHr. The first lane shows molecular weight (MW) markers, and the second lane shows a sense transcript beginning at the 5′ end under the control of the T7 promoter. (*Upper*) The expected fragments at ≈9,500 and 5,400 nucleotides are indicated by arrows. The third lane shows an antisense transcript from the 3′ end under the control of a T3 promoter showing bands representing the full length of the plasmid and a population of RNA ≈1,400 bp long that possibly represents a termination sequence or difficult secondary structure at that region. (*Lower*) The expected 150-nt fragment can be seen on this gel with longer exposure (both lanes labeled T7).

was introduced into this construct, and the mutated construct was named pTHrGND. The plasmid pTREhyg2+, without any insert, was also used as a control and is hereon referred to as pTRE.

Tissue Culture and Transfection and RNase Protection Assay. A human hepatoma cell line (Huh7) was maintained at 37°C in Dulbecco's modified Eagle's medium containing 10% FBS with 5% CO_2. Transfection was carried out by using Lipofectamine (Invitrogen) according to the manufacturer's instructions. RPA 111 ribonuclease protection assay kits (Ambion) were used according to the manufacturer's directions. The probe used was transcribed from a construct containing the core region from nucleotide 342 to nucleotide 707 of HCV CG1b strain flanked by the T3 and T7 promoters.

Immunofluorescence and Western Blot. Huh7 cells were grown on glass coverslips and transfected as described. Cells were fixed with acetone/methanol on ice at different time points after transfection. Cells were washed with PBS three times, incubated with primary antibody for 1 h, washed with PBS, incubated with secondary antibody, and washed again with PBS. Monoclonal antibodies against the core (C1) and E1 (A4) were from H. Greenberg (Stanford Medical School, Palo Alto, CA) (12). The anti-E2 monoclonal antibodies AP33 and ALP98 were from A. Patel (Medical Research Council, Glasgow, Scotland) (13). The NS5A monoclonal antibody was obtained from J. Lau (ICN). The Cy3-labeled donkey anti-mouse IgG was obtained from Kirkegaard & Perry Laboratories. The same primary antibodies were used for Western blotting. The peroxidase-labeled goat anti-mouse IgG used as the secondary antibody was obtained from Kirkegaard & Perry Laboratories.

Sucrose Gradient Density Centrifugation. The tissue culture medium was centrifuged to remove cellular debris, and the supernatant was pelleted over a 30% sucrose cushion. The pellet was resuspended in TNC buffer (10 mM Tris·HCl, pH 7.4/1 mM $CaCl_2$/150 mM NaCl) with EDTA-free protease inhibitors (Roche Applied Science) and applied onto a 20–60% sucrose gradient (10.5-ml volume) in SW41 tubes (Beckman Coulter) and centrifuged at 100,000 × *g* for 16 h at 4°C. We collected 1-ml fractions from the top of the gradient. The fractions were tested for HCV proteins and viral RNA as described below. Cryoelectron microscopy was performed by using standard techniques.

HCV RNA, Protein Quantitation, and RACE. HCV RNA level was quantitated by using the TaqMan real-time PCR method as described in ref. 10. RNA was extracted from 100 μl of the sucrose gradient fractions or tissue culture media by using TRIzol (Invitrogen) and resuspended in 20 μl of double-filtered RNase-free water. Samples were tested in duplicate. The core protein was quantitated by using the HCV core ELISA kits, which were provided by S. Yagi (Advanced Life Technology, Saitama, Japan) and used as described in ref. 14. Samples were tested in 50- or 100-μl aliquots. RNA was extracted by using TRIzol (Invitrogen), reverse-transcribed, and amplified by RNA ligase-mediated RACE (RLM-RACE, Ambion). The 5′ and 3′ RACE procedure was performed as described in ref. 15.

Results

Ribozyme Activity. To prove that the ribozymes function properly in the context of HCV genome, the HCV-ribozyme RNA was generated by *in vitro* transcription of pHr and analyzed by formamide gel electrophoresis. The results are shown in Fig. 1*B*. A band corresponding to the full-length HCV genome of ≈9,587 nt was detected. Also seen were bands corresponding to the vector (5,400 nt), a 150-nt fragment corresponding to the RNA between the T7 transcription initiation and the cleavage site of the 5′ ribozyme, and other molecular weight fragments probably representing uncleaved or prematurely terminated transcripts. A similarly expected pattern of cleavage was also observed with the pHt, which is the precursor construct of the pHr and contains the GFP sequence in place of the HCV polyprotein sequence (data not shown). Further proof of the ribozymes cleaving correctly is discussed later with the RACE results.

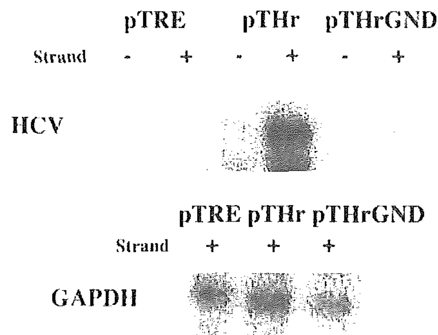

Fig. 2. Detection of HCV positive- and negative-strand RNAs. (*Upper*) The experiment. Shown are the total cellular RNA probed for the HCV core sequence, either positive or negative strand, and the findings when cellular RNA from pTRE-, pTHr-, or pTHrGND-transfected cells were probed for either positive- or negative-strand core sequence. (*Lower*) The control. Shown is the total cellular RNA probed for GAPDH messenger RNA. Note that the amounts are roughly comparable in the three lanes.

HCV RNA and Protein Production in Transfected Cells. Both positive- and negative-strand HCV RNAs were detected in cells transfected with pTHr (Fig. 2). The level of positive-strand HCV RNA was at least 10-fold higher than the level of negative-strand HCV RNA in multiple experiments. The GND mutant pTHrGND produced a small amount of positive-strand RNA but did not produce any detectable negative-strand RNA. The positive-strand RNA produced with the GND mutant was less than that produced with pTHr. No viral RNA was detected in cell lysates transfected with pTRE.

Cells transfected with pTHr or the control plasmid pTRE were analyzed by immunofluorescence with monoclonal antibodies directed against the core, E2, and NS5A. A granular cytoplasmic staining was seen with antibodies against all three proteins (Fig. 3). A time-course experiment showed peak protein expression on day 2 and a significant decrease on day 4 after transfection (data not shown). The percentage of cells with fluorescence was $\approx$10%, despite the transfection efficiency of $\approx$50% with a GFP-containing plasmid (data not shown). No immunofluorescence was seen in the cells transfected with pTRE.

Western blot of cell lysates transfected with pTRE or pTHr showed the presence of core, E2, and NS5A in cells transfected with pTHr but not in cells transfected with pTRE (Fig. 4). As expected, viral protein was not detected in the presence of doxycycline (data not shown). Furthermore, little or no HCV protein was detected in pTHrGND-transfected cells, suggesting that viral replication is required for efficient protein production in this system (data not shown).

HCV Virion Production and Secretion. To assess the possibility of HCV particle production, culture medium of the pTHr- and pTHrGND-transfected cells was subjected to sucrose density gradient centrifugation. The fractions were analyzed for two HCV structural proteins, core and E2, and HCV RNA. These results are shown in Fig. 5*A*. In the culture medium from cells transfected with pTHr, a peak of HCV proteins and RNA coincided in fraction 5, which has the density of 1.16 g/ml. This density is consistent with the published density of free HCV virions (16). Viral particles were visualized by electron microscopy only in fraction 5 (Fig. 5*B*). These particles are heterogeneous in appearance and have at least two sizes ($\approx$50 and 100 nm in diameter) with the 50 nm being the major form. This heterogeneity has been described in ref. 17. Viral particles are double-shelled and appear to have spike-like projections from their surface. Shown in Fig. 5*A* are the results for pTHrGND-transfected cells. The HCV protein and RNA levels are at least 10-fold less than those of the pTHr-transfected cells.

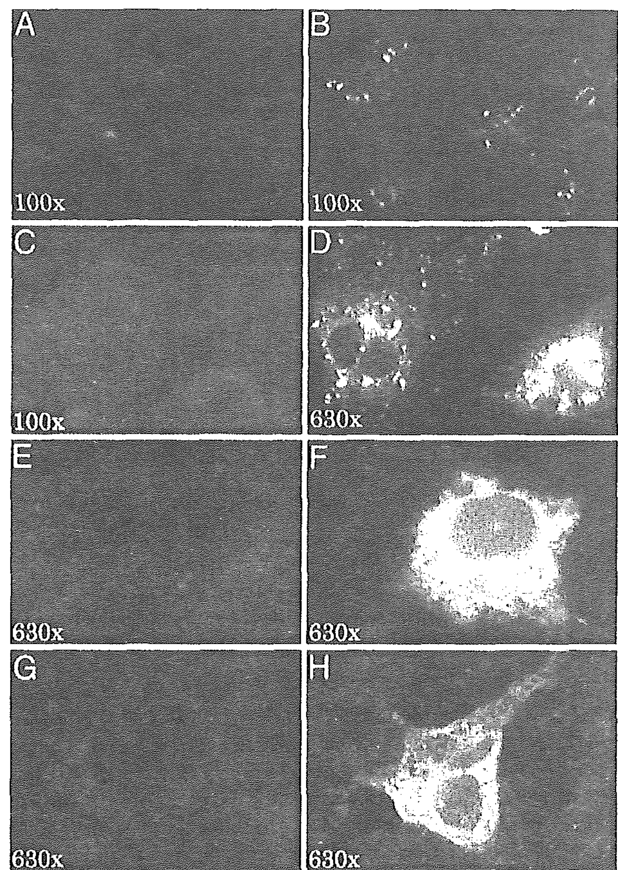

Fig. 3. Detection of HCV proteins by immunofluorescence. (*A*) Low-power view of cells transfected with pTHr and stained without primary antibody but with the secondary antibody. No fluorescence was seen. (*B*) Low-power view of cells transfected with pTHr and stained with anti-core. Multiple cells with fluorescence can be seen. (*C*) Low-power view of cells transfected with the control pTRE and stained with anti-core. There was no fluorescence. (*D*) High-power view of *B*. (*E* and *F*) High-power views of cells stained with anti-E2. Cells were transfected with pTRE (*E*) or pTHr (*F*). (*G* and *H*) High-power views of cells transfected with pTRE (*G*) and pTHr (*H*) and stained with anti-NS5A .

RACE. RACE was used to ensure the exact cleavage of the 5′ and 3′ ends of HCV by the ribozymes. *In vitro*-transcribed RNA from pHr and RNA from the culture medium of pTHr-transfected cells were analyzed by RACE. The 5′ end of the *in vitro*-transcribed RNA, as expected, had the same sequence as the cDNA construct (Fig. 6*A*). However the 3′ end of the *in vitro* transcript could not be amplified by RACE, possibly because of a less efficient cleavage by the 3′ ribozyme and subsequent difficulty in amplifying a heterogeneous population of the 3′ ends. Both the 5′ and 3′ ends of HCV RNA from the culture medium were successfully determined. Interestingly, a change in

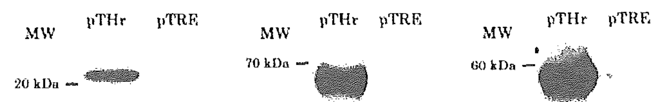

Fig. 4. Detection of HCV proteins by Western blot. In each blot, the first lane shows cells transfected with pTHr and the second lane shows cells transfected with pTRE. The molecular weights are shown on the left of the blots. (*Left*) Blot was probed with anti-core. (*Center*) Blot probed with anti-E2. (*Right*) Blot probed with anti-NS5A.

MICROBIOLOGY

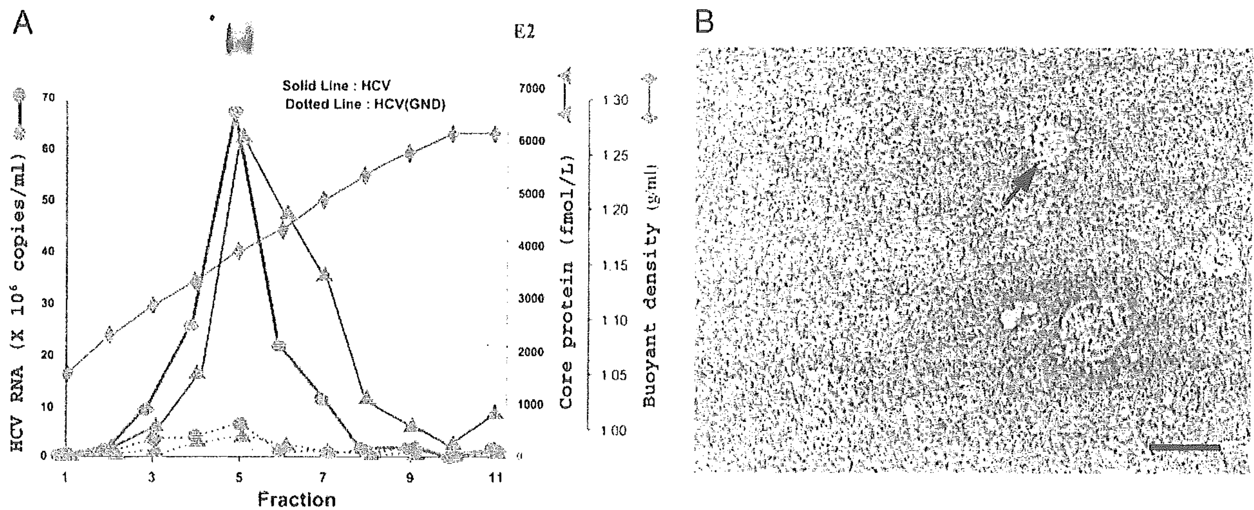

Fig. 5. Sucrose density gradient analysis of culture medium of HCV-transfected cells. (*A*) (*Lower*) Results of the sucrose gradient for pTHr (solid lines) and pTHrGND (dotted lines) transfections. The buoyant density of the sucrose is plotted with the levels of HCV RNA measured by TaqMan PCR and HCV core protein measured by core ELISA. (*Upper*) Western blot for the E2 protein in the fractions of the sucrose gradient of the pTHr transfection. Each lane corresponds to the fraction number below it on the *x* axis of the graph. Three hundred microliters of each fraction was spun at 100,000 × *g* for 90 min, and the pellet was resuspended in loading buffer and used for the Western blot. (*B*) Cryoelectron microscopy of fraction 5. (Bar, 100 nm.)

the most 5′ nucleotide from G to A was noted; this change has been frequently observed in HCV RNA replicons and circulating HCV RNA in infected humans (15). In the 3′ end, two nucleotide changes in the stem loop region were noted: U→A and A→U. These changes preserved the stem loop structure (Fig. 6*B*). Such changes have also been reported in HCV RNA from infected individuals (18). The RNA levels in the medium of the GND-transfected cells were not adequate to perform RACE.

Discussion

Since the discovery of HCV in 1989, working with HCV has proven to be difficult, mostly because of the lack of model systems (19). Each aspect of the life cycle has been difficult to reproduce *in vitro*. The infectious clone was developed after multiple attempts and had to be demonstrated in a chimpanzee (20, 21). Other small-animal models require complicated systems (22, 23). *In vitro*, virus obtained from infected individuals can replicate only in certain B cell lines and primary human hepatocytes but only at a low level (4, 5). Until the development of the replicon, most model systems have been difficult to work with (2, 24). Development of virus-like particles and pseudovirus have allowed study of viral entry into the cell but do not model other aspects of the viral life cycle (25–28). Therefore, a model system with viral replication, assembly, and release is urgently needed. Furthermore, genotype 1, the most prevalent form of HCV and the most difficult to treat, was chosen for this model.

By engineering two hammerhead ribozyme sequences, one at the 5′ end and the other at the 3′ end of an infectious HCV cDNA clone, we generated a DNA expression construct for the production of HCV virions. An important initial consideration was to ensure that the ribozymes are indeed functional. This functionality was demonstrated by *in vitro*-translation and RACE. Transfection of this HCV-ribozyme construct into Huh7 cells demonstrated the production of structural and nonstructural proteins by immunofluorescence and Western blot. Both positive- and negative-strand RNAs could be detected intracellularly. As expected, the positive strand is much more abundant than the negative strand.

The GND mutant was constructed as a control to determine the extent of replication in this model. Evidence for replication was derived from a number of results. The simplest evidence was the presence of negative-strand viral RNA in pTHr-transfected cells and the lack of negative strand in pTHrGND-transfected cells. A >10-fold difference in the relative amounts of the positive-strand viral RNA between the wild-type and GND constructs provided additional evidence. This observation can be explained by the lack of amplification as a result of defective replication. The positive strand seen with the GND mutant was generated from transcription of the cDNA plasmid. This difference in product was also evident in the culture medium. The amounts of viral RNA and core protein on the sucrose gradients were >10-fold higher in wild-type cells than in the GND mutant-transfected cells. The final and perhaps the most interesting evidence for replication is the RACE findings. The 5′ and 3′ nucleotide changes have been described in refs. 15 and 18. The G→A switch of the initial nucleotide of HCV is associated with replication *in vivo* and *in vitro* (15). A transposition from an A–T to a T–A base pairing has also been reported (18) and represents a base pair in the putative terminal stem loop of the 3′ end of

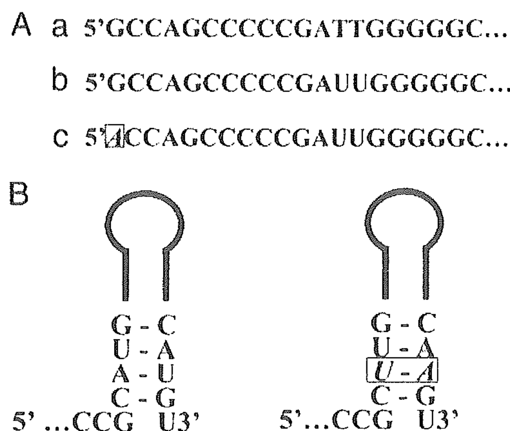

Fig. 6. Sequences of the 5′ and 3′ ends of HCV RNA. (*A*) The cDNA sequence for the 5′ end of the CG1B strain (*a*) and the RACE results for the 5′ ends of *in vitro*-transcribed RNA (*b*) and of the HCV RNA from the culture medium (*c*). (*B*) The cDNA sequences and the stem-loop structures of the 3′ ends of the CG1b strain (*Left*) and the HCV RNA from the medium (*Right*). Nucleotide changes are boxed.

HCV. These observations provide support for the replication of viral RNA in this system.

Evidence for assembly and release was derived in a number of ways. The presence of HCV RNA in the media with the exact 5′ and 3′ ends showed that the correctly processed RNA was secreted into the culture medium. The association of viral RNA and core and E2 protein in the same fraction on the sucrose gradient with a density of 1.16g/ml (the published density of free HCV virions) supported the interpretation that viral particles are assembled and secreted into the medium. The most compelling evidence is the visualization of particles resembling virions by electron microscopy, and these particles were visualized only in fraction 5, where viral RNA and proteins are present. It is interesting that the core protein extends into fractions 6 and 7 more than the viral RNA and E2 protein. This core reactivity might represent free core particles, although they were not seen on electron microscopy (29). The production and release of HCV particles is rather robust in this system, capable of achieving >10 million copies of HCV RNA per ml in the culture medium.

Although replicons using the full-length HCV genome have been developed, particles have not been described. In those replicons where sequence coding for the neomycin is included, difficulty in packaging a longer RNA molecule might be the problem. Alternatively the block could be the result of the inhibitory effects of the replicon adaptive mutations on virion assembly and release. Both possibilities are speculative. However, in the system presented here, there is no extraneous RNA and, although mutations can and do occur (see the RACE results), the source of the RNA (the cDNA) maintains a stable sequence without adaptive mutations. This difference might partially explain why particles are seen. It may also be of importance that there is a constant RNA production inside the cells being channeled directly into the appropriate cellular machinery for assembly.

This model system does not allow the study of viral entry and the earliest events in the HCV life cycle. In addition, whether these particles are infectious or not remains to be determined. The HCV sequence used is known to be infectious in chimpanzees. It should be noted that the sequence is genotype 1b. The results that would be obtained with other genotypes in this system is unknown. Despite these caveats, it represents a robust system to study the viral life cycle, specifically viral assembly and release. Very little is known about the assembly and release of HCV. This work might present an opportunity to better elucidate the biology of HCV as well as to develop therapeutic targets for the treatment of hepatitis C, in particular for genotype 1.

Note Added in Proof. During the preparation of this manuscript, two groups (T. Wakita, T. Takanobu, T. Date, and M. Miyamoto and T. Pietschmann, G. Koutsoudakis, S. Kallis, T. Kato, S. Foung, T. Wakita, and R. Bartenschlager) at the 11th International Symposium on HCV and Related Viruses (Heidelberg, Germany, Oct. 3–7, 2004) reported the production of infectious HCV in cell culture by transfecting a full-length HCV RNA genome.

We thank Z. Hong Zhou, Ph.D., for superb cryoelectron microscopy, Shintaro Yagi for providing the HCV core ELISA kit, and Anthony Davis for excellent technical assistance.

1. Liang, T. J., Rehermann, B., Seeff, L. B. & Hoofnagle, J. H. (2000) *Ann. Intern. Med.* **132,** 296–305.
2. Lohmann, V., Korner, F., Koch, J., Herian, U., Theilmann, L. & Bartenschlager, R. (1999) *Science* **285,** 110–113.
3. Ikeda, M., Yi, M., Li, K. & Lemon, S. M. (2002) *J. Virol.* **76,** 2997–3006.
4. Shimizu, Y. K., Iwamoto, A., Hijikata, M., Purcell, R. H. & Yoshikura, H. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 5477–5481.
5. Sung, V. M., Shimodaira, S., Doughty, A. L., Picchio, G. R., Can, H., Yen, T. S., Lindsay, K. L., Levine, A. M. & Lai, M. M. (2003) *J. Virol.* **77,** 2134–2146.
6. Raychaudhuri, G., Govindarajan, S., Shapiro, M., Purcell, R. H. & Emerson, S. U. (1998) *J. Virol.* **72,** 7467–7475.
7. Bukh, J., Pietschmann, T., Lohmann, V., Krieger, N., Faulk, K., Engle, R. E., Govindarajan, S., Shapiro, M., St Claire, M. & Bartenschlager, R. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 14416–14421.
8. Manns, M. P., McHutchison, J. G., Gordon, S. C., Rustgi, V. K., Shiffman, M., Reindollar, R., Goodman, Z. D., Koury, K., Ling, M. & Albrecht, J. K. (2001) *Lancet* **358,** 958–965.
9. Fried, M. W., Shiffman, M. L., Reddy, K. R., Smith, C., Marinos, G., Goncales, F. L., Jr., Haussinger, D., Diago, M., Carosi, G., Dhumeaux, D., *et al.* (2002) *N. Engl. J. Med.* **347,** 975–982.
10. Thomson, M., Nascimbeni, M., Gonzales, S., Murthy, K. K., Rehermann, B. & Liang, T. J. (2001) *Gastroenterology* **121,** 1226–1233.
11. Benedict, C. M., Pan, W., Loy, S. E. & Clawson, G. A. (1998) *Carcinogenesis* **19,** 1223–1230.
12. Dubuisson, J., Hsu, H. H., Cheung, R. C., Greenberg, H. B., Russell, D. G. & Rice, C. M. (1994) *J. Virol.* **68,** 6147–6160.
13. Triyatni, M., Saunier, B., Maruvada, P., Davis, A. R., Ulianich, L., Heller, T., Patel, A., Kohn, L. D. & Liang, T. J. (2002) *J. Virol.* **76,** 9335–9344.
14. Tanaka, E., Ohue, C., Aoyagi, K., Yamaguchi, K., Yagi, S., Kiyosawa, K. & Alter, H. J. (2000) *Hepatology* **32,** 388–393.
15. Cai, Z., Liang, T. J. & Luo, G. (2004) *J. Virol.* **78,** 3633–3643.
16. Kaito, M., Watanabe, S., Tsukiyama-Kohara, K., Yamaguchi, K., Kobayashi, Y., Konishi, M., Yokoi, M., Ishida, S., Suzuki, S. & Kohara, M. (1994) *J. Gen. Virol.* **75,** 1755–1760.
17. Andre, P., Komurian-Pradel, F., Deforges, S., Perret, M., Berland, J. L., Sodoyer, M., Pol, S., Brechot, C., Paranhos-Baccala, G. & Lotteau, V. (2002) *J. Virol.* **76,** 6919–6928.
18. Kolykhalov, A. A., Feinstone, S. M. & Rice, C. M. (1996) *J. Virol.* **70,** 3363–3371.
19. Choo, Q. L., Kuo, G., Weiner, A. J., Overby, L. R., Bradley, D. W. & Houghton, M. (1989) *Science* **244,** 359–362.
20. Kolykhalov, A. A., Agapov, E. V., Blight, K. J., Mihalik, K., Feinstone, S. M. & Rice, C. M. (1997) *Science* **277,** 570–574.
21. Yanagi, M., Purcell, R. H., Emerson, S. U. & Bukh, J. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 8738–8743.
22. Mercer, D. F., Schiller, D. E., Elliott, J. F., Douglas, D. N., Hao, C., Rinfret, A., Addison, W. R., Fischer, K. P., Churchill, T. A., Lakey, J. R., *et al.* (2001) *Nat. Med.* **7,** 927–933.
23. Labonte, P., Morin, N., Bowlin, T. & Mounir, S. (2002) *J. Med. Virol.* **66,** 312–319.
24. Blight, K. J., Kolykhalov, A. A. & Rice, C. M. (2000) *Science* **290,** 1972–1974.
25. Baumert, T. F., Ito, S., Wong, D. T. & Liang, T. J. (1998) *J. Virol.* **72,** 3827–3836.
26. Nam, J. H., Bukh, J., Purcell, R. H. & Emerson, S. U. (2001) *J. Virol. Methods* **97,** 113–123.
27. Bartosch, B., Bukh, J., Meunier, J. C., Granier, C., Engle, R. E., Blackwelder, W. C., Emerson, S. U., Cosset, F. L. & Purcell, R. H. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 14199–14204.
28. Logvinoff, C., Major, M. E., Oldach, D., Heyward, S., Talal, A., Balfe, P., Feinstone, S. M., Alter, H., Rice, C. M. & McKeating, J. A. (2004) *Proc. Natl. Acad. Sci. USA* **101,** 10149–10154.
29. Maillard, P., Krawczynski, K., Nitkiewicz, J., Bronnert, C., Sidorkiewicz, M., Gounon, P., Dubuisson, J., Faure, G., Crainic, R. & Budkowska, A. (2001) *J. Virol.* **75,** 8240–8250.

GERDの薬物治療（H_2ブロッカー，消化器運動機能改善薬の使用法）

稲森 正彦・阿部 泰伸・中島　淳

- nocturnal gastric acid breakthough はPPI投与中に起こる夜間の酸分泌であり，そのコントロールにはH_2RAの眠前投与が効果的である．
- H_2RAの効果発現は速く，症状出現に応じた治療（on demand therapy）に適した薬剤である可能性がある．
- 酸分泌抑制剤を補助する目的で，消化管機能改善薬の効果が期待されている．

胃食道逆流症（以下GERD）の治療において，プロトンポンプ阻害薬（以下PPI）の投与を第1選択とすることは諸外国ならびにわが国において広く認められている．しかしH_2受容体拮抗薬（以下H_2RA）の投与により，最適な酸分泌抑制効果が得られる状態があることが知られており，また消化器運動改善薬の併用により症状の軽快をみることを実地診療の場においてしばしば経験する．今回，GERD診療におけるH_2RAおよび消化器運動機能改善薬の位置付けにつき，私見を交え解説したい．

H2受容体拮抗薬

1．nocturnal gastric acid breakthough関連

nocturnal gastric acid breakthrough（以下NAB）は1998年，Peghiniら[1]により報告された概念で，PPI投与中にもかかわらず夜間に胃内のpHが下降し，pH 4以下が1時間以上続く状態と定義される．NABの起こるメカニズムは解明されていないが，特に*Helicobacter pylori*陰性例において発生しやすく，PPI投与に加えたH_2RAの就寝前投与の有用性が認められている．臨床的には，夜間頻回に食道への酸逆流が起こる，重症の逆流性食道炎の治療の際問題になっていると推察される．NABに対するH_2RAの有用性に関する報告の抜粋を示す（表1）[2~5]．H_2RAの連続投与では酸分泌抑制効果が減弱する耐性の問題が指摘されているが，NAB抑制を目的とした，PPIに加えた就寝前H_2RAの継続投与の臨床的有効性を示唆する報告もあり，今後さらなる

表1　nocturnal gastric acid breakthrough（NAB）の有用性に関する報告（抜粋）

Author	Journal	Year	Subjects	Summary
Peghini PLら[2]	Gastroenterology	1998	Healthy volunteers	PPI投与に加えた就寝前H_2RA投与によりNABが抑制された．
Katsube Tら[3]	Aliment Pharmacol Ther	2000	Healthy volunteers	*H. pylori*感染がないことがNABの発生と関係する．NABはPPI投与に加えた就寝前H_2RA投与で抑制される．
Xue Sら[4]	Aliment Pharmacol Ther	2001	GERD patients	PPI投与に加えた就寝前H_2RA投与によりNABは抑制され，夜間の胃および食道のpHが上昇する．
Adachi Kら[5]	J Clin Gastroenterol	2004	GERD patients	PPI投与に加えた就寝前H_2RAの継続投与は臨床的に有用である可能性を示唆．

いなもり まさひこ，あべ やすのぶ，なかじま あつし：横浜市立大学大学院医学研究科分子消化管内科学　〒236-0004　横浜市金沢区福浦3-9

0025-7699/05/¥500/論文/JCLS

表2 H_2RA の立ち上がりの速さに関する報告(抜粋)

Author	Journal	Year	Subjects	Summary
Hurlimann S ら[6]	Aliment Pharmacol Ther	1994	Healthy volunteers	投与初日は PPI より H_2RA の酸分泌抑制効果が高いが，その後減弱する．
Chassany O ら[7]	Curr Ther Res	1996	ICU patients	静注初期には PPI より H_2RA の酸分泌抑制効果が高い．
Arnestad JS ら[8]	Aliment Pharmacol Ther	1997	Healthy volunteers	空腹時単回投与後 11 時間の計測で PPI より H_2RA の酸分泌抑制効果が高い．
Hedenstrom H ら[9]	Aliment Pharmacol Ther	1997	Healthy volunteers	空腹時単回投与後 4 時間の計測で PPI より H_2RA の酸分泌抑制効果が高い．
Khoury RM ら[10]	Aliment Pharmacol Ther	1999	Healthy volunteers	食後の単回投与後 6 時間の計測で PPI より H_2RA の酸分泌抑制効果が高い．
Abe Y ら[11]	J Gastroenterol	2004	Healthy volunteers	空腹時単回静注投与後 4 時間の計測で PPI より H_2RA の酸分泌抑制効果が高い．

表3 消化管運動改善薬

一般名	商品名	主な作用機序
Metoclopramide	プリンペラン®，他	ドーパミン D_2 受容体遮断作用，セロトニン 5-HT_3 受容体遮断作用および 5-HT_4 受容体刺激作用．制吐作用および消化管運動促進作用．
Domperidone	ナウゼリン®，他	ドーパミン D_2 受容体遮断作用，消化管運動促進作用．
Itopride hydrochloride	ガナトン®	ドーパミン D_2 受容体拮抗作用とアセチルコリンエステラーゼ阻害作用．消化管運動賦活作用．
Mosapride citrate	ガスモチン®	セロトニン 5-HT4 受容体アゴニスト．消化管運動促進作用．
Trimebutine maleate	セレキノン®	オピオイド μ 受容体刺激薬．消化管運動正常化作用．

評価が必要である．また近年では PPI の分割投与も NAB 抑制に有効である可能性が明らかになっている．

2．H_2RA の立ち上がりの速さ

あまり知られていないことであるが，投与初期において H_2RA の胃酸分泌抑制効果は PPI より速やかであり，特に空腹時にこの差は顕著である．H_2RA の立ち上がりの速さに関する報告の抜粋を示す(表2)[6~11]．

この H_2RA の特徴を活かしうる状況の一つとして，胸やけが断続的にしか出現しない比較的軽症の逆流症患者に対して，症状のあるときにだけ短期間の治療をする(on demand therapy)という考えがある．われわれは 2000 年 4 月より胃食道逆流症専門外来(胸やけ外来)を開設しているが，GERD の診断後約 7 割の患者は PPI の投与で満足されたが，2 割弱の患者は H_2RA の投薬を希望され，そのうちの半数は on demand で服用されていることを報告した[12]．従来胸やけに関しては医療サイド，患者サイドともに病気としての認識が薄かったと考えられるが，GERD の概念の普及とともに軽症例での医療機関受診も増えることが予想され，on demand therapy は今後の検討課題の一つではないかと考えられている．

消化管運動改善薬

消化管運動改善薬は下部食道内圧(lower esophageal sphincter：LES)の上昇作用，食道胃運動亢進作用などを目的に使用されている．現在よく用いられる薬剤としては表3のようなものがあり，おのおの特徴があるが，逆流性食道炎，胃食道逆流症の適応は必ずしも得られていない．

これらの薬剤の単独投与では，残念ながら GERD 治療において十分な効果を示すとは言い難い．しかし，酸分泌抑制薬と併用することで真価を発揮する可能性があり，今後の検討が期待さ

れている。

その他GERDの病因の一つである一過性下部食道括約筋弛緩を抑制するGABA-B受容体アゴニスト(Baclofen)の効果も期待されている。

また5-HT_4受容体アゴニストであるCisaprideには比較的多くのエビデンスがあり，GERD患者に広く用いられてきたが，QT延長などの副作用により残念ながら使用できなくなっている。

おわりに GERD診療におけるH_2RA，消化管運動機能改善薬につき概説した。GERD治療においてはPPIを主軸とした薬物療法がGold standardであることは間違いないが，今後個々の患者の求めるものに即し，薬剤の特徴を活かした投与法の検討も必要である。

文献

1) Peghini PL, et al : Nocturnal recovery of gastric acid secretion with twice-daily dosing of proton pump inhibitors. Am J Gastroenterol 93 : 763-767, 1998
2) Peghini PL, Katz PO, Castell DO : Ranitidine controls nocturnal gastric acid breakthrough on omeprazole : a controlled study in normal subjects. Gastroenterology 115 : 1335-1339, 1998
3) Katsube T, et al : *Helicobacter pylori* infection influences nocturnal gastric acid breakthrough. Aliment Pharmacol Ther 14 : 1049-1056, 2000
4) Xue S, et al : Bedtime H_2 blockers improve nocturnal gastric acid control in GERD patients on proton pump inhibitors. Aliment Pharmacol Ther 15 : 1351-1356, 2001
5) Adachi K, et al : Administration of H_2 receptor antagonist with proton pump inhibitor is effective for long-term control of refractory reflux esophagitis. J Clin Gastroenterol 38 : 297-298, 2004
6) Hurlimann S, et al : Comparison of acid inhibition by either oral high-dose ranitidine or omeprazole. Aliment Pharmacol Ther 8 : 193-201, 1994
7) Chassany O, et al : The comparative effects of single intravenous doses of *cimetidine*, *ranitidine*, famotidine, and omeprazole on intragastric pH. Curr Ther Res 57 : 159-169, 1996
8) Arnestad JS, Kleveland PM, Waldum HL : In single doses ranitidine effervescent is more effective than lansoprazole in decreasing gastric acidity. Aliment Pharmacol Ther 11 : 355-358, 1997
9) Hedenstrom H, et al : Intragastric pH after oral administration of single doses of ranitidine effervescent tablets, omeprazole capsules and famotidine fast-dissolving tablets to fasting healthy volunteers. Aliment Pharmacol Ther 11 : 1137-1141, 1997
10) Khoury RM, Katz PO, Castell DO : Post-prandial ranitidine is superior to post-prandial omeprazole in control of gastric acidity in healthy volunteers. Aliment Pharmacol Ther 13 : 1211-1214, 1999
11) Abe Y, et al : The comparative effects of single intravenous doses of omeprazole and famotidine on intragastric pH. J Gastroenterol 39 : 21-25, 2004
12) 稲森正彦・他：胃食道逆流症専門外来(胸やけ外来)における臨床的検討．日内会誌92 Suppl : 214, 2003

MEDICAL BOOK INFORMATION ―――― 医学書院

<日本医師会生涯教育シリーズ>

腹部エコーのABC 第2版

編集・発行　日本医師会
監修　竹原靖明
編集　竹原靖明・秋本　伸・木村邦夫・跡見　裕
●B5　頁460　2004年
定価5,775円(本体5,500円+税5%)
[ISBN4-260-17517-3]

日本医師会生涯教育シリーズ。最もわかりやすい腹部エコー入門書として大ベストセラーになった初版を，読者の熱い要望にこたえて大幅改訂。豊富な症例と簡潔な解説はそのままに，頻度の高い重要な疾患を改めて厳選し，写真・記述ともに時代に合ったクオリティに刷新した。初心者のみならず超音波診断に携わる医療者すべてに必要十分な1冊。

J Gastroenterol 2005; 40:213–216

Journal of
Gastroenterology

© Springer-Verlag 2005

Letters to the editor

Evaluation of the effects of anti-secretory drugs on intragastric acidity: is combined therapy with a proton pump inhibitor and an H_2 receptor antagonist effective as initial treatment?

To the Editor: The ideal medication for acid-related gastric diseases should have a rapid onset of action to decrease intragastric pH. Proton pump inhibitors are currently the most potent inhibitors of gastric acid secretion when used regularly.[1] On the other hand, previous reports have described that H_2 receptor antagonists increase intragastric pH more rapidly than proton pump inhibitors,[2] and we[3] have also observed that famotidine acts significantly faster and provides stronger inhibition of intragastric acid secretion than omeprazole. However, few studies have examined the effects of the intravenous dosing of a proton pump inhibitor and an H_2 receptor antagonist combined as an initial treatment.

Six healthy male volunteers (mean age, 28 years; range, 22–34 years) participated in this study. All subjects were negative for anti- *Helicobacter pylori* immunoglobulin G antibodies (SRL, Tokyo, Japan). Their isoenzyme CYP2C19 profiles were genotyped; three subjects were homozygous extensive metabolizers, one was a heterozygous extensive metabolizer, and two subjects were poor metabolizers (SRL, Tokyo, Japan).

All subjects received, at various times, 20 mg omeprazole, 20 mg famotidine, and 20 mg omeprazole plus 20 mg famotidine, intravenously. The drugs were administered separately, with a washout period of at least 7 days between each study. The subjects fasted overnight before treatment and for 4 h after receiving the drug. An antimony pH electrode was inserted transnasally and placed in the body of the stomach. The gastric pH was measured, and the data were analyzed using established software (Chemical Instrument, Tokyo, Japan). Statistical evaluation was carried out using the Wilcoxon signed-ranks test. The level of significance was $P < 0.05$. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Yokohama City University School of Medicine.

Omeprazole plus famotidine maintained a gastric pH of more than 3 significantly longer than either famotidine alone or omeprazole alone during the first hour of the study period (mean, 70.7% versus 27.7% and 19.8%, respectively; $P < 0.05$), and the combination also maintained a gastric pH of more than 3 significantly longer than omeprazole alone during the third hour (mean, 100% versus 19.0%; $P < 0.05$) and the fourth hour (mean, 89.8% versus 11.3%; $P < 0.05$; Fig. 1).

During the 4-h study period, omeprazole plus famotidine sustained pH values of more than 2, 3, 3.5, and 4 longer than omeprazole alone and famotidine alone (mean, 93.8% versus 41.3% and 81.5% respectively; $P < 0.05$; 90.0% versus 27.0% and 76.3% respectively; $P < 0.05$; 89.5% versus 24.0% and 74.5% respectively; $P < 0.05$; and 88.0% versus 20.8% and 72.0% respectively; $P < 0.05$). The combination also sustained pH values of more than 5, 6, and 7 longer than omeprazole alone (mean, 82.5% versus 12.5%; $P < 0.05$; 74.3% versus 7.8%; $P < 0.05$; and 57.3% versus 3.3%; $P < 0.05$; Fig. 2).

Values for holding times at pH more than 3 for omeprazole plus famotidine, famotidine alone, and omeprazole alone during the 4-h study period were 88.5%, 73.8%, and 24.8% in CYP2C19 extensive metabolizers versus 93.0%, 81.5%, and 31.5% in CYP2C19 poor metabolizers (mean values). Our data showed that significant differences were not observed between the extensive metabolizers and poor metabolizers.

Our results support previous study findings which have indicated that the antisecretory activity of omeprazole increases progressively after repeated oral and intravenous administrations, with a steady state being achieved after about 5 days.[4] Daily doses of omeprazole at 40 mg, given as intravenous injections were not sufficient to maintain an intragastric pH of more than 4 during the first day of treatment.[5] However, after 5–7 days of treatment, oral omeprazole was more effective than famotidine for normal subjects.[6] Our study was done in fasted volunteers for a long period; therefore, this experimental condition is not suitable for a proton pump inhibitor. However, combined therapy with a proton pump inhibitor and an H_2 receptor antagonist can compensate for the disadvantage of the proton pump inhibitor during the initial treatment of acid-related diseases.

In conclusion, the administration of omeprazole plus famotidine increased intragastric pH more rapidly than either omeprazole alone or famotidine alone in healthy volunteers. The clinical meaning of this result is not clear; however, there is a possibility that combined therapy with a proton pump inhibitor and an H_2 receptor antagonist is suitable for initial therapy, when given intravenously.

Masahiko Inamori, Jun-ichi Togawa, Gaku Chiguchi,
Yasunobu Abe, Taisuke Kikuchi, Kenichi Muramatsu,
Harunobu Kawamura, Noritoshi Kobayashi,
Hiroyuki Kirikoshi, Takeshi Shimamura, Takashi Sakaguchi,
Tomoo Takamura, Norio Ueno, and Atsushi Nakajima
The Third Department of Internal Medicine, Yokohama City
University School of Medicine, 3-9 Fuku-ura, Kanazawa-ku,
Yokohama 236-0004, Japan

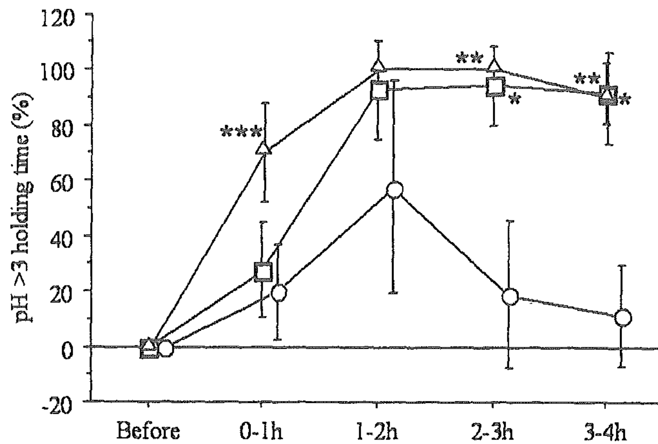

Fig. 1. Famotidine plus omeprazole (*triangles*) sustained a pH > 3 during the first hour of the study period, compared to omeprazole alone (*circles*) and famotidine alone (*squares*). (***$P < 0.05$ by the Wilcoxon signed-ranks test). Famotidine plus omeprazole sustained a pH > 3 during the third and fourth hours of the study period, compared to omeprazole alone. (**$P < 0.05$ by the Wilcoxon signed-ranks test). Famotidine alone sustained a pH > 3 during the third and fourth hours of the study period, compared to omeprazole alone. (*$P < 0.05$ by Wilcoxon signed-ranks test). *Triangles*, *circles*, and *squares*, mean values; *vertical lines*, SD; *horizontal line*, ±SD

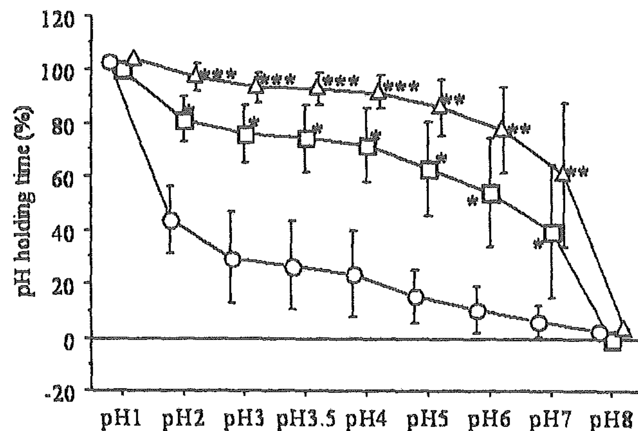

Fig. 2. During the first 4h of the study period, famotidine plus omeprazole (*triangles*) sustained pH values of more than 2, 3, 3.5, and 4 for longer than both omeprazole alone (*circles*) and fomotidine alone (*squares*) (***$P < 0.05$ by the Wilcoxon signed-ranks test). During the first 4h of the study period, famotidine plus omeprazole sustained pH values of more than 5, 6, and 7 for longer than both omeprazole alone and *fomotidire alone*. (**$P < 0.05$ by the Wilcoxon signed-ranks test). During the first 4h of the study period, famotidine alone sustained pH values of more than 2, 3, 3.5, 4, 5, 6, and 7 for longer than omeprazole alone (*$P < 0.05$ by the Wilcoxon signed-ranks test). *Triangles*, *circles*, and *squares*, mean values; *vertical lines*, SD; *horizontal line*, ±SD

References

1. Burget DW, Chiverton SG, Hunt RH. Is there an optimal degree of acid suppression for healing of duodenal ulcers? A model of the relationship between ulcer healing and acid suppression. Gastroenterology 1990;99: 345–51.
2. Khoury RM, Katz PO, Castell DO. Post-prandial ranitidine is superior to post-prandial omeprazole in control of gastric acidity in healthy volunteers. Aliment Pharmacol Ther 1999;13:1211–4.
3. Abe Y, Inamori M, Togawa JI, Kikuchi T, Muramatsu K, Chiguchi G, et al. The comparative effects of single intravenous doses of omeprazole and famotidine on intragastric pH. J Gastroenterol 2004; 39:21–5.
4. Jansen JB, Lundborg P, Baak LC, Greve J, Ohman M, Stover C, et al. Effect of single and repeated intravenous doses of omeprazole on pentagastrin stimulated gastric acid secretion and pharmacokinetics in man. Gut 1988;29:75–80.
5. Cederberg C, Thomson AB, Mahachai V, Westin JA, Kirdeikis P, Fisher D, et al. Effect of intravenous and oral omeprazole on 24-h intragastric acidity in duodenal ulcer patients. Gastroenterology 1992;103:913–8.
6. Ohara S, Hongo M, Asaki S, Sato A, Shibuya D, Sato H, et al. The effects of famotidine and omeprazole on 24-h intragastric pH of normal subjects (in Japanese with English abstract). Nippon Shokakibyo Gakkai Zasshi (Jpn J Gastroenterol) 1988;85:1353–9.

Received: November 20, 2003 / Accepted: April 5, 2004
Reprint requests to: M. Inamori
DOI 10.1007/s00535-004-1521-8

Villous tumor of the rectum that started secreting watery fluid after colectomy

To the Editor: An 80-year-old man who had a large villous tumor (over 10 cm in maximum diameter) in the lower rectum was admitted to our hospital because of severe left lower abdominal pain. On May 22, 2001, an emergency operation was performed, with a diagnosis of localized peritonitis due to perforation of the intestine. Exploration of the abdomen revealed a perforated sigmoid colon, which contained many firm stools. We performed left hemicolectomy, transverse colostomy, and construction of a mucus fistula. Because the general condition of the patient was very poor, we had to minimize the operation time. Therefore, the large villous tumor in the rectum was not resected. The surgical specimen and microscopic findings showed the features of a typical stercoral ulcer.

After the operation, the patient had a watery discharge of 1000–2000 ml/day from the mucus fistula and anus. Laboratory data for the anal discharge fluid revealed the following values: sodium, 103 mEq/l; potassium, 49 mEq/l; and chloride, 121 mEq/l. Culture of the discharge fluid revealed no evidence of infectious enterocolitis. The villous tumor was assumed to have started secreting the watery discharge, although the mechanism was unclear.

On June 19, 2001, a Hartmann operation was performed to control the secretion from the villous tumor. The gross appearance of the resected specimen showed a 15 × 12-cm villous tumor, with a wide base, attached to the lower rectal mucosa (Fig. 1). Microscopic examination revealed cancer in the villous adenoma, and part of the villous adenoma showed rich mucin production (Fig. 2).

Villous tumor of the colon, which causes secretory diarrhea, may lead to depletion syndrome, which is characterized by hyponatremia, hypokalemia, severe dehydration, and metabolic acidosis, and death in severe cases. Generally, any acute diarrheal illness in which intestinal fluid loss exceeds 10 ml/kg per day is classified as secretory diarrhea. Causes of secretory diarrhea are classified as infectious, such as infection with *Vibrio cholerae* and *Escherichia coli*, and noninfectious, such as hormones secreted by tumors, including villous tumors.[1] Cases of noninfectious secretory diarrhea are numerically uncommon. According to a previ-

J Gastroenterol 2005; 40:453–458
DOI 10.1007/s00535-005-1569-0

Journal of
Gastroenterology
© Springer-Verlag 2005

Early effects of lafutidine or rabeprazole on intragastric acidity: which drug is more suitable for on-demand use?

Masahiko Inamori, Jun-Ichi Togawa, Tomoyuki Iwasaki, Yutaka Ozawa, Taisuke Kikuchi, Kenichi Muramatsu, Gaku Chiguchi, Shuhei Matsumoto, Harunobu Kawamura, Yasunobu Abe, Hiroyuki Kirikoshi, Noritoshi Kobayashi, Takeshi Shimamura, Kensuke Kubota, Takashi Sakaguchi, Satoru Saito, Norio Ueno, and Atsushi Nakajima

Gastroenterology Division, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan

Editorial on page 549

Background. Medication for the relief of heartburn should have the rapid onset of action required for on-demand use. We studied the inhibition of gastric acid secretion by lafutidine and rabeprazole, given in single doses to fasting and postprandial subjects. ***Methods.*** A total of 22 healthy male, *Helicobacter pylori*-negative volunteers participated in this randomized, two-way crossover study. They were randomly assigned to receive a single oral dose of 10mg lafutidine or 20mg rabeprazole after fasting overnight (12 subjects, fasting study) or after eating a test meal (noodles, 364kcal; protein, 10.1g; fat, 16g; carbohydrates, 44.9g; NaCl, 1.1g; 10 subjects, postprandial study). Intragastric pH was monitored continuously for 6h after treatment. The other drug was given after a washout period of at least 7 days, and intragastric pH was similarly monitored. ***Results.*** In the fasting study, lafutidine sustained pH at >3 and >4 during the second, third, fourth, fifth, and sixth hours of the study for significantly longer than rabeprazole. During the first 6h after treatment, lafutidine sustained pH at more than 2, 3, 3.5, 4, 5, 6, and 7 longer than rabeprazole. In the postprandial study, lafutidine sustained pH >3 and >4 for longer periods than rabeprazole during the third, fourth, fifth, and sixth hours of the study. During the first 6h after treatment, lafutidine sustained pH at more than 2, 3, 3.5, 4, 5, 6, and 7 longer than rabeprazole. ***Conclusions.*** Lafutidine 10mg produces a prompter rise in intragastric pH than rabeprazole 20mg in fasting and postprandial *Helicobacter pylori*-negative male subjects.

Key words: intragastric acidity, lafutidine, rabeprazole, on-demand therapy, heartburn

Received: June 3, 2004 / Accepted: January 19, 2005
Reprint requests to: M. Inamori

Introduction

The prevalence of gastroesophageal reflux disease (GERD) in Japan is estimated to be lower than that in Western countries. However, recent studies indicate that the number of Japanese patients with reflux esophagitis is increasing, because of changes in dietary habits, the increase in the elderly population, improved techniques for endoscopic examination, and more widespread use of diagnostic procedures for GERD.[1]

Heartburn is a common problem in Japan. Although proton pump inhibitors given daily potently inhibit gastric acid secretion, whether these drugs are effective as on-demand therapy for specific symptoms remains largely uninvestigated.

Medication for on-demand treatment should have a rapid onset of action. Many types of drugs can be obtained over the counter for the treatment of heartburn, including antacids, H_2-receptor antagonists,[2] and proton pump inhibitors. Proton pump inhibitors have been established to be potent inhibitors of gastric acid secretion when used regularly;[3] however, the effect of intermittent single doses for on-demand treatment remains unclear.

Lafutidine is a newly synthesized H_2-receptor antagonist. Previous studies have shown that lafutidine more promptly and potently inhibits gastric acid secretion than other H_2-receptor antagonists,[4,5] especially during the daytime.[6] In subjects without *Helicobacter pylori* infection, lafutidine markedly elevates the daytime as well as the nocturnal intragastric pH. In contrast, other H_2-receptor antagonists markedly elevate only the nocturnal intragastric pH.

Lafutidine (10mg twice daily) and the proton pump inhibitor rabeprazole (20mg once daily) are both approved for the treatment of peptic ulcers in Japan. Rabeprazole 20-mg tablets rapidly suppress gastric acid secretion, as compared with other proton pump inhibitiors.[7–9] Because patients with GERD often have

symptoms during the daytime, we compared the early effect on intragastric pH of lafutidine 10 mg with that of rabeprazole 20 mg, the maximum dose levels approved in Japan, each given as a single dose, to fasting and postprandial subjects.

Methods

Subjects

The subjects were 12 healthy male volunteers in a fasting state and 10 healthy male volunteers in a postprandial state. None of the subjects were receiving acid-suppressive medications, such as antacids, H_2-receptor antagonists, or proton pump inhibitors. All subjects were negative for anti-*H. pylori* immunoglobulin G antibodies (SRL, Tokyo, Japan).

Fasting study

Twelve subjects participated in this randomized, two-way crossover study. They were randomly assigned to receive 10 mg lafutidine (Protecadin; Taiho Pharmaceutical, Tokyo, Japan) or 20 mg rabeprazole (Pariet; Eisai, Tokyo, Japan). The other drug was then given after a washout period of at least 7 days.

To monitor gastric pH, a pH electrode was inserted transnasally into the body of the stomach, with the subjects under local anesthesia. Gastric pH was measured at 10-s intervals by means of a portable pH meter attached to an antimony pH electrode (Chemical Instrument, Tokyo, Japan). The pH electrode was calibrated before each recording, using standard buffers of pH 4.01 and 6.86. The pH data were analyzed with the use of established software (Chemical Instrument). The times during which the intragastric pH remained above 1, 2, 3, 3.5, 4, 5, 6, 7, and 8 were also measured over the course of 6 h after treatment.

Postprandial study

Ten subjects participated in this randomized, two-way crossover study. The subjects were randomly assigned to receive a single oral dose of 10 mg lafutidine (Protecadin; Taiho Pharmaceutical) or 20 mg rabeprazole (Pariet; Eisai) after a test meal (noodles, 364 kcal; protein, 10.1 g; fat, 16 g; carbohydrates, 44.9 g, NaCl 1.1 g). The other drug was given after a washout period of at least 7 days.

Statistical analysis

Statistical evaluation was carried out with the use of the Wilcoxon signed-rank test. The level of significance was $P < 0.05$. Statistical analyses were performed with Stat View software (SAS Institute, Cary, NC, USA).

Ethics

The study was conducted in accordance with the Declaration of Helsinki. He study protocol was approved by the Ethics Committee of Yokohama City University School of Medicine.

Results

Twelve male subjects (mean age, 28.3 years; range, 22–35 years) completed the fasting study, and 10 male subjects (mean age, 28.5 years; range, 21–36 years) completed the postprandial study. No adverse events occurred during either study.

Fasting study

Holding time (%) of pH > 3

Lafutidine 10 mg maintained gastric pH at >3 significantly longer than rabeprazole 20 mg during the second (1–2 h; mean, 74.9% versus 18.8%; $P < 0.01$), third (2–3 h, 95.8% versus 33.3%; $P < 0.01$), fourth (3–4 h, 93.8% versus 67.4%; $P < 0.05$), fifth (4–5 h, 95.3% versus 67.6%; $P < 0.01$), and sixth (5–6 h, 93.0% versus 5.24%, $P < 0.01$) study periods (Fig. 1). The holding time (%) of pH > 3 did not differ significantly between the drugs during the first (0–1 h) study period.

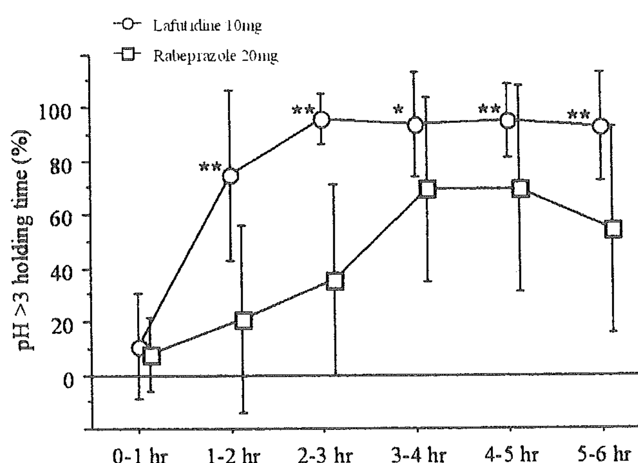

Fig. 1. In the fasting study, lafutidine sustained a pH of >3 for significantly longer than rabeprazole during the second, third, fourth, fifth, and sixth study periods. *Circles, and squares*, mean values; *vertical lines*, SDs. $^{**}P < 0.01$ and $^{*}P < 0.05$ by the Wilcoxon signed-rank test

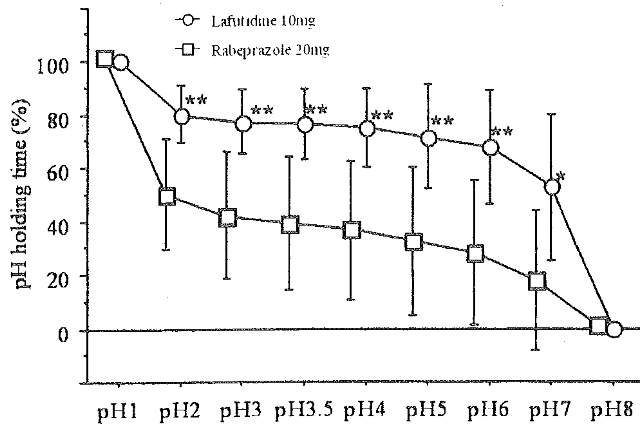

Fig. 2. In the fasting study, lafutidine sustained pH at above 2, 3, 3.5, 4, 5, 6, and 7 for significantly longer than rabeprazole during the first 6h of the study. *Circles, and squares*, mean values; *vertical lines*, SDs. $^{**}P < 0.01$ and $^{*}P < 0.05$ by the Wilcoxon signed-rank test

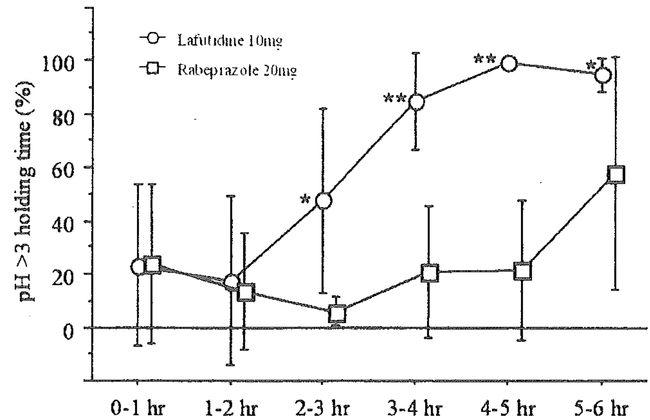

Fig. 3. In the postprandial study, lafutidine sustained pH at >3 for significantly longer than rabeprazole during the third, fourth, fifth, and sixth study periods. *Circles, and squares*, mean values; *vertical lines*, SDs. $^{**}P < 0.01$ and $^{*}P < 0.05$ by the Wilcoxon signed-rank test

Holding time (%) of pH > 4

Lafutidine 10mg maintained gastric pH at >4 significantly longer than rabeprazole 20mg during the second (1–2h; mean, 70.7% versus 15.6%; $P < 0.01$), third (2–3h, 93.4% versus 26.8%; $P < 0.01$), fourth (3–4h, 91.6% versus 58.9%; $P < 0.05$), fifth (4–5h, 93.8% versus 63.2%; $P < 0.01$), and sixth (5–6h, 91.8 versus 45.4%; $P < 0.01$) study periods. There was no significant difference between the drugs during the first (0–1h) study period.

Holding time (%) of various pH levels during the 6-h study period

During the 6-h study period, lafutidine 10mg provided longer durations of pH above 2, 3, 3.5, 4, 5, 6, and 7 than did rabeprazole 20mg (mean, 80.1% versus 48.7%; $P < 0.01$; 77.3% versus 40.7%; $P < 0.01$; 76.2% versus 37.8%; $P < 0.01$; 74.9% versus 35.1%; $P < 0.01$; 71.4 versus 31.2%; $P < 0.01$; 67.5% versus 26.6%; $P < 0.01$; and 52.6% versus 16.5%; $P < 0.05$; respectively) (Fig. 2).

Postprandial study

Holding time (%) of pH > 3

Lafutidine 10mg maintained gastric pH at >3 significantly longer than rabeprazole 20mg during the third (2–3h; mean, 47.7% versus 2.8%; $P < 0.05$), fourth (3–4h; 84.9% versus 17.8%; $P < 0.01$), fifth (4–5h; 99.7% versus 18.9%; $P < 0.01$), and sixth (5–6h, 95.2% versus 55.3%; $P < 0.05$) study periods (Fig. 3). No significant differences were found at the first (0–1h) or second (1–2h) study periods between the two drugs.

Holding time (%) of pH > 4

Lafutidine 10mg maintained gastric pH at >4 significantly longer than did rabeprazole 20mg during the third (2–3h) (mean, 36.0% versus 2.4%; $P < 0.05$), fourth (3–4h, 79.7% versus 15.4%; $P < 0.01$), fifth (4–5h, 98.7% versus 14.6%; $P < 0.01$), and sixth (5–6h, 92.5% versus 48.0%; $P < 0.05$) study periods. There were no significant differences between the drugs during the first (0–1h) or second (1–2h) study periods.

Holding time (%) of various pH levels during the 6-h study period

During the 6-h study period, lafutidine 10mg provided longer durations of pH above 2, 3, 3.5, 4, 5, 6, and 7 than did rabeprazole 20mg (mean, 70.3% versus 27.5%; $P < 0.01$; 61.2% versus 21.0%; $P < 0.01$; 58.2% versus 19.4%; $P < 0.01$; 56.0% versus 17.4%; $P < 0.01$; 49.8% versus 12.6%; $P < 0.01$; 42.7% versus 6.7%; $P < 0.01$; and 34.8% versus 3.3%, $P < 0.01$, respectively) (Fig. 4).

Discussion

This study examined changes in intragastric pH after a single dose of lafutidine (10mg) or rabeprazole (20mg) during the first 6h after the dose in *H. pylori*-negative subjects. During this early period, lafutidine had a faster onset of action and more strongly inhibited intragastric acid secretion than did rabeprazole, in both the fasting and postprandial subjects. These results support the findings of previous studies by Khoury et al.,[10] Arnestad et al.,[11] Hedenstrom et al.,[12] Hurlimann et al.,[13] Chassany et al.,[14] and Abe et al.,[15] showing that H_2

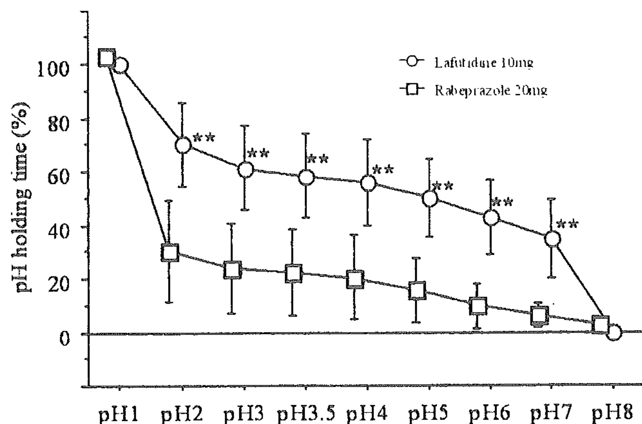

Fig. 4. In the postprandial study, lafutidine sustained pH at above 2, 3, 3.5, 4, 5, 6, and 7 for significantly longer than rabeprazole during the first 6h of the study. *Circles, and squares*, mean values; *vertical lines*, SDs. $^{**}P < 0.01$ by the Wilcoxon signed-rank test

receptor antagonists increase intragastric pH more rapidly than proton pump inhibits.

We believe that data obtained from *H. pylori*-negative subjects will be of greater value for the evaluation of H_2-receptor antagonists and proton pump inhibitors than those obtained from *H. pylori*-positive subjects. Currently, antisecretory drugs are used to treat most patients with peptic ulcer, who usually have concurrent *H. pylori* infection, as well as being used for the treatment of GERD, in which *H. pylori* infection is not involved; indeed, there may be a negative association between GERD and *H. pylori* infection.[16,17]

We selected rabeprazole for study, among various currently available proton pump inhibitors, for several reasons. First, rabeprazole is metabolized mainly via a nonenzymatic pathway, in contrast to other proton pump inhibitors, which are metabolized by the CYP2C19 and CYP3A4 genotypes of cytochrome P450.[18] The plasma drug concentrations of these other proton pump inhibitors differ considerably between CYP2C19-extensive metabolizers and -poor metabolizers, influencing the degree of inhibition of acid secretion.[19,20] Second, previous studies have reported that rabeprazole more promptly inhibits acid secretion than various other proton pump inhibitors in vivo,[7,8] consistent with the results of our previous pH monitoring report.[9] In the present study, however, lafutidine inhibited intragastric acid secretion even more promptly than rabeprazole (which was previously shown to act more promptly than various other proton pump inhibitors).

Our study showed that an H_2-receptor antagonist more strongly inhibited intragastric acid secretion than a proton pump inhibitor 0 to 6h after the dose was taken. This finding was unexpected, because proton pump inhibitors have antisecretory activity and promote ulcer healing more effectively than H_2 receptor antagonists.[21] However, prior studies have shown that the antisecretory activity of proton pump inhibitors increases progressively after repeated oral or intravenous administration, with a steady state achieved after about 5 days.[22,23] Another study has demonstrated that a daily intravenous injection of proton pump inhibitor did not maintain intragastric pH at >4 during the first day of treatment.[24] Nonetheless, after 5 to 7 days of treatment, an oral proton pump inhibitor was more effective than an H_2-receptor antagonist for suppressing the intragastric pH in normal subjects, patients with duodenal ulcer, and patients with GERD.[25–27]

Our results, showing that an H_2 receptor antagonist increased intragastric pH more rapidly than a proton pump inhibitor, are also supported by the findings of an autoradiography study. Nakamura et al.[28] found that H_2-receptor antagonists accumulated uniformly on parietal cells, whereas proton pump inhibitors were bound only to young activated parietal cells. Other proton pumps were quickly activated, resulting in a slower onset of antisecretory action with proton pump inhibitors than with H_2-receptor antagonists in the early period after the drug was commenced.

On-demand therapy for patients with GERD is considered to be safe and cost-effective after they receive initial treatment with proton pump inhibitors.[29,30] Drugs for on-demand therapy, which target heartburn, should have a rapid onset of action. Many patients have heartburn after meals,[31–33] and H_2-receptor antagonists are unsuitable for the treatment of postprandial heartburn, because the state of the stomach after meals blunts their effectiveness.[34] However, our results suggested that lafutidine increased intragastric pH more rapidly than rabeprazole not only in fasting subjects but also in postprandial subjects. This rapid onset of action in postprandial subjects was attributed to the fact that lafutidine is a unique H_2-receptor antagonist that affects levels of calcitonin gene-related peptide (CGRP).[35–39] Lafutidine activates capsaicin-sensitive afferent neurons and stimulates the release of CGRP from afferent neurons, in addition to having strong binding affinity for H_2-receptors. Lafutidine has been shown to significantly increase plasma somatostatin levels 20 to 120min after a dose has been taken and CGRP levels 40 to 120min after a dose has been taken, as compared with a placebo group.[36] In that study,[36] the physiological release of plasma secretin was reduced and did not alter gastrin levels. CGRP released from afferent neurons in the gastric mucosa stimulates D cells in the antral and fundic glands and increases somatostatin secretion from D cells. Somatostatin inhibits gastric acid secretion, acting directly on somatostatin receptors on parietal cells

and indirectly by decreasing gastrin secretion from antral G cells.

Although many factors are implicated in the development of GERD, acid reflux to the esophagus is considered to be the major cause of this disease. Treatment with a proton pump inhibitor to provide potent, long-term suppression of gastric acid is essential for disease management. On the other hand, the transient heartburn associated with mild GERD is attributed mainly to temporary, short-term gastric acid reflux. Therefore, rapid acid suppression is one of the most important factors for the resolution of these symptoms. Because lafutidine promptly suppressed gastric acid secretion, it is considered to be a useful drug for the on-demand treatment of mild GERD.

The ideal medication for the treatment of heartburn should have the rapid onset of action needed for on-demand treatment, as well as a sufficient duration of action to assure that symptoms are controlled. On the basis of our results, we conclude that lafutidine 10mg produces a prompter rise in intragastric pH than rabeprazole 20mg in *H. pylori*-negative subjects. The clinical implications of our results remain unclear; however, our findings suggest that 10-mg lafutidine tablets are suitable for on-demand treatment in patients with mild GERD.

References

1. Furukawa N, Iwakiri R, Koyama T, Okamoto K, Yoshida T, Kashiwagi Y, et al. Proportion of reflux esophagitis in 6010 Japanese adults: prospective evaluation by endoscopy. J Gastroenterol 1999;34:441–4.
2. Hatlebakk JG, Berstad A. Pharmacokinetic optimisation in the treatment of gastro-oesophageal reflux disease. Clin Pharmacokinet 1996;31:386–406.
3. Burget DW, Chiverton SG, Hunt RH. Is there an optimal degree of acid suppression for healing of duodenal ulcers? A model of the relationship between ulcer healing and acid suppression. Gastroenterology 1990;99:345–51.
4. Fujisawa T, Adachi K, Komazawa Y, Mihara T, Azumi T, Katsube T, et al. *Helicobacter pylori* infection prevents the occurrence of the tolerance phenomenon of histamine H2 receptor antagonists. Aliment Pharmacol Ther 2004;20:559–65.
5. Haruki S, Matsumoto H, Kodama K, Takebe M, Takayanagi H, Tei S. Pharmacokinetic studies of lafutidine (FGR-8813) in man. Jpn Pharmacol Ther 1995;23:3049–59.
6. Saitoh T, Fukushima Y, Otsuka H, Hirakawa J, Ishikawa M, Takahashi N, et al. Lafutidine, a novel histamine H2 receptor antagonist, inhibits daytime acid secretion in both *H. pylori* infected and non-infected human subjects (abstract). J Gastroenterol Hepatol 2002;17(Suppl):828.
7. Shirai N, Furuta T, Moriyama Y, Okochi H, Kobayashi K, Takashima M, et al. Effects of CYP2C19 genotypic differences in the metabolism of omeprazole and rabeprazole on intragastric pH. Aliment Pharmacol Ther 2001;15:1929–37.
8. Saitoh T, Fukushima Y, Otsuka H, Hirakawa J, Mori H, Asano T, et al. Effects of rabeprazole, lansoprazole and omeprazole on intragastric pH in CYP2C19 extensive metabolizers. Aliment Pharmacol Ther 2002;16:1811–7.
9. Inamori M, Togawa J, Takahashi K, Yoneda M, Fujisawa N, Iwasaki T, et al. Comparison of the effect on intragastric pH of a single dose of omeprazole or rabeprazole: which is suitable for on-demand therapy? J Gastroenterol Hepatol 2003;18:1034–8.
10. Khoury RM, Katz PO, Castell DO. Post-prandial ranitidine is superior to post-prandial omeprazole in control of gastric acidity in healthy volunteers. Aliment Pharmacol Ther 1999;13:1211–4.
11. Arnestad JS, Kleveland PM, Waldum HL. In single doses ranitidine effervescent is more effective than lansoprazole in decreasing gastric acidity. Aliment Pharmacol Ther 1997;11:355–8.
12. Hedenstrom H, Alm C, Kraft M, Grahnen A. Intragastric pH after oral administration of single doses of ranitidine effervescent tablets, omeprazole capsules and famotidine fast-dissolving tablets to fasting healthy volunteers. Aliment Pharmacol Ther 1997;11:1137–41.
13. Hurlimann S, Abbuhl B, Inauen W, Halter F. Comparison of acid inhibition by either oral high-dose ranitidine or omeprazole. Aliment Pharmacol Ther 1994;8:193–201.
14. Chassany O, Bergmann JF, Simoneau G, Elouaer-Blanc L, Segrestaa JM, Caulin C. The Comparative effects of single intravenous doses of cimetidine, ranitidine, famotidine, and omeprazole on intragastric pH. Curr Ther Res 1996;57:159–69.
15. Abe Y, Inamori M, Togawa J, Kikuchi T, Muramatsu K, Chiguchi G, et al. The comparative effects of single intravenous doses of omeprazole and famotidine on intragastric pH. J Gastroenterol 2004;39:21–5.
16. Newton M, Bryan R, Burnham WR, Kamm MA. Evaluation of *Helicobacter pylori* in reflux oesophagitis and Barrett's oesophagus. Gut 1997;40:9–13.
17. Haruma K, Hamada H, Mihara M, Kamada T, Yoshihara M, Sumii K, et al. Negative association between *Helicobacter pylori* infection and reflux esophagitis in older patients: case-control study in Japan. Helicobacter 2000;5:24–9.
18. Ishizaki T, Horai Y. Cytochrome P450 and the metabolism of proton pump inhibitors: emphasis on rabeprazole (review article). Aliment Pharmacol Ther 1999;13(Suppl 3):27–36.
19. Andersson T. Pharmacokinetics, metabolism and interactions of acid pump inhibitors. Focus on omeprazole, lansoprazole and pantoprazole. Clin Pharmacokinet 1996;31:9–28.
20. Furuta T, Ohashi K, Kosuge K, Zhao XJ, Takashima M, Kimura M, et al. CYP2C19 genotype status and effect of omeprazole on intragastric pH in humans. Clin Pharmacol Ther 1999;65:552–61.
21. Hunt RH, Malfertheiner P, Yeomans ND, Hawkey CJ, Howden CW. Critical issues in the pathophysiology and management of peptic ulcer disease. Eur J Gastroenterol Hepatol 1995;7:685–99.
22. Howden CW, Forrest JA, Reid JL. Effects of single and repeated doses of omeprazole on gastric acid and pepsin secretion in man. Gut 1984;25:707–10.
23. Jansen JB, Lundborg P, Baak LC, Greve J, Ohman M, Stover C, et al. Effect of single and repeated intravenous doses of omeprazole on pentagastrin stimulated gastric acid secretion and pharmacokinetics in man. Gut 1988;29:75–80.
24. Cederberg C, Thomson AB, Mahachai V, Westin JA, Kirdeikis P, Fisher D, et al. Effect of intravenous and oral omeprazole on 24-hour intragastric acidity in duodenal ulcer patients. Gastroenterology 1992;103:913–8.
25. Ohara S, Hongo M, Asaki S, Sato A, Shibuya D, Sato H, et al. The effects of famotidine and omeprazole on 24-hour intragastric pH of normal subjects. (in Japanese with English abstract). Nippon Shokakibyo Gakkai Zasshi (Jpn J Gastroenterol) 1988;85:1353–9.
26. Ducrotte P, Guillemot F, Elouaer-Blanc L, Hirschauer C, Thorel JM, Petit A, et al. Comparison of omeprazole and famotidine on esophageal pH in patients with moderate to severe esophagitis: a cross-over study. Am J Gastroenterol 1994;89:717–21.

27. Corinaldesi R, DeGiorgio R, Stanghellini V. Comparison of the antisecretory effects of omeprazole and famotidine on peptone-meal-stimulated gastric acid secretion in patients with duodenal ulcer. Curr Ther Res 1991;49:560–5.
28. Nakamura M, Oda M, Akiba Y, Inoue J, Ito T, Tsuchiya M, et al. Autoradiographic demonstration of lansoprazole uptake sites in rat antrum and colon. J Clin Gastroenterol 1995;20(Suppl 2):S8–13.
29. Bardhan KD. Intermittent and on-demand use of proton pump inhibitors in the management of symptomatic gastroesophageal reflux disease. Am J Gastroenterol 2003;98(Suppl 3):S40–8.
30. Ofman JJ. The economic and quality-of-life impact of symptomatic gastroesophageal reflux disease. Am J Gastroenterol 2003; 98(Suppl 3):S8–14.
31. Allen ML, Mellow MH, Robinson MG, Orr WC. The effect of raw onions on acid reflux and reflux symptoms. Am J Gastroenterol 1990;85:377–80.
32. Vantrappen G, Janssens J. Recent studies of the pathophysiology and diagnosis of esophageal symptoms. Scand J Gastroenterol Suppl 1990;175:34–41.
33. Lanza FL, Smith V, Page-Castell JA, Castell DO. Effectiveness of foaming antacid in relieving induced heartburn. South Med J 1986;79:327–30.
34. Colin-Jones DG. The role and limitations of H2-receptor antagonists in the treatment of gastro-oesophageal reflux disease. Aliment Pharmacol Ther 1995;9(Suppl 1):9–14.
35. Sato H, Kawashima K, Yuki M, Kazumori H, Rumi MA, Ortega-Cava CF, et al. Lafutidine, a novel histamine H2-receptor antagonist, increases serum calcitonin gene-related peptide in rats after water immersion-restraint stress. J Lab Clin Med 2003;141:102–5.
36. Itoh H, Naito T, Takeyama M. Lafutidine changes levels of somatostatin, calcitonin gene-related peptide, and secretin in human plasma. Biol Pharm Bull 2002;25:379–82.
37. Nishihara K, Nozawa Y, Nakano M, Ajioka H, Matsuura N. Sensitizing effects of lafutidine on CGRP-containing afferent nerves in the rat stomach. Br J Pharmacol 2002;135:1487–94.
38. Onodera S, Shibata M, Tanaka M, Inaba N, Yamaura T, Ohnishi H. Gastroprotective activity of FRG-8813, a novel histamine H2-receptor antagonist, in rats. Jpn J Pharmacol 1995;68:161–73.
39. Kawashima K, Ishihara S, Karim Rumi MA, Moriyama N, Kazumori H, Suetsugu H, et al. Localization of calcitonin gene-related peptide receptors in rat gastric mucosa. Peptides 2002;23:955–66.