原虫性下痢症

a. アメーバ赤痢

赤痢アメーバ（*Entamoeba histolytica*）の感染により，下痢（粘血便），テネスムス，腹痛などの赤痢様症状が認められる．糞便内での栄養体や嚢子の存在を鏡検にて検出する．PCR法も診断に有用である．

b. ジアルジア症

ランブル鞭毛虫（*Giardia lamblia*）の経口感染により，1〜3週間の潜伏期を経て，嘔気・食欲不振・下痢・腹痛などが認められる．旅行者下痢症の原因となる．患者糞便内より，ランブル鞭毛虫抗原検出法および糞便内シスト検出蛍光抗体法を行い診断する．

c. クリプトスポリジウム症

Cryptospordium parvum の感染により，4〜5日の潜伏期を経て，水様性下痢・嘔気・嘔吐・発熱などが認められる．血便はみられない．患者糞便内よりオーシストを検出することにより診断する．PCR法による検査キットも市販されている．

治療の一般方針　B

治療方針の立て方

細菌性腸管感染症の急性期の治療は，下痢や発熱による脱水の是正に努める．また，腸内細菌叢の是正を図るためのプロバイオティクス（生菌製剤）の投与も考慮する．起因菌の排泄を抑制する止痢薬の投与は控える．

薬物療法

原因病原体が判明するまでの初期治療として，フルオロキノロン系薬（ノルフロキサシン300〜600 mg/日，分3）またはホスホマイシン（2 g/日，分3〜4）が3日間投与される．

a. 細菌性赤痢

成人にはフルオロキノロン系薬（レボフロキサシン水和物300 mg/日，分3），小児およびフルオロキノロン系薬が投与できない成人にはホスホマイシン（2 g/日，分3〜4）の5日間の投与が第1選択となる．耐性菌が増加しているので，感受性検査の結果により使用抗菌薬の変更を行う．

b. 腸チフス・パラチフス

クロラムフェニコール系薬は強い副作用があるため，フルオロキノロン系薬が第1選択薬である．投与量は通常の1.3倍として（レボフロキサシン400 mg/日，分4／トスフロキサシントシル酸塩水和物600 mg/日，分4），14日間投与する．フルオロキノロン系薬低感受性菌の場合，第3世代セフェム系薬（セフトリアキソンナトリウム水和物2 g/日，分1，静脈内投与/セフォタキシムナトリウム4 g/日，分2，静脈内投与）を併用する．

c. コレラ

脱水の治療のための補液をまず行う．菌の排泄期間を短縮するために，フルオロキノロン系薬またはホスホマイシンの通常量の経口投与を3日間行う．

d. EHEC感染症

感染早期における抗菌薬投与が有効であると考えられる．成人にはフルオロキノロン系薬（レボフロキサシン水和物300 mg/日，分3），小児にはホスホマイシン（2 g/日，分4）を3日間投与する．プロバイオティクスの投与も推奨されている．溶血性尿毒症症候群（HUS）の合併時には人工透析が有効である．

e. カンピロバクター腸炎

一般に自然治癒傾向の高い疾患であり，対症療法のみで軽快する例が多い．重篤に経過した場合や敗血症などを併発した場合にはマクロライド系（クラリスロマイシン400 mg/日，分2／ロキタマイシン600 mg/日，分3），ホスホマイシン（2 g，分3〜4）を3〜5日間投与する．

f. サルモネラ腸炎（非チフス性）

トスフロキサシントシル酸塩水和物（450 mg/日，分3）の7日間投与が推奨される．

g. 腸炎ビブリオ腸炎

自然治癒例が多い．脱水症状の改善には補液を行う．抗菌薬の投与は不要な場合が多い．

h. 病原性大腸菌感染症（EHEC除く）

自然治癒例が多い．補液，プロバイオティクスの投与などの補助療法を行う．重症例では成人にはフルオロキノロン系薬，小児・妊婦にはホスホマイシンの経口投与を行う．

i. 黄色ブドウ球菌性食中毒

食品中に蓄積されたエンテロトキシンによる疾病であるため，抗菌薬治療は無効である．脱水へ

の補液などの対症療法を行う．

j. ウェルシュ菌腸炎

自然治癒例が多いため，抗菌薬治療の必要はないと考えられる．

k. セレウス菌腸炎

アミノグリコシド系薬，マクロライド系薬が有効であるが，抗菌薬を使用しなくとも対症療法（脱水への処置）のみで軽快することが多い．

l. ウイルス性下痢症

ロタウイルス腸炎およびノロウイルス腸炎のいずれも自然治癒傾向がある．特異的抗ウイルス薬はないため，脱水への対策としての対症療法が行われる．

m. 原虫性下痢症

アメーバ赤痢に対してはメトロニダゾール（成人：1.0～1.5 g/日，分3～4，10日間）の経口投与が有効である．ジアルジア症にはメトロニダゾール（成人：0.75～1.0 g/日，分3～4，7～10日間）またはチニダゾール（2 g/日，分1，7～10日間）が有効である．クリプトスポリジウム症にアジスロマイシン水和物やロキシスロマイシンなどのマクロライド系薬が有効であったとの報告がある．

予　防　C

「付けない」「増やさない」「殺す」という食中毒予防のための3原則は，腸管感染症の予防にとっても有用である．食品に食中毒起因病原体を付けない，食材中で病原体を増殖させない，十分な加熱調理により病原体を死滅させることが重要である．加えて，宿主の生体防御能を高めるために，十分な睡眠・栄養をとることが大切である．

13. カンピロバクター感染症

1 病　名

カンピロバクター感染症（campylobacteriosis）。

2 概　要

カンピロバクター感染症には腹痛，下痢などの症状がみられるカンピロバクター腸炎と，敗血症や脳脊髄膜炎などを呈する全身性カンピロバクター症とがある。前者は主に *Campylobacter jejuni* subsp. *jejuni*（以下 *C. jejuni*）により，後者は主に *Campylobacter fetus* subsp. *fetus*（以下 *C. fetus*）によって引き起こされる。カンピロバクター腸炎は *Campylobacter* 属細菌によって汚染した食品の摂食により起こり，食中毒のピークは5～7月にある。全身性カンピロバクター症は妊婦や新生児に重篤な病態を引き起こす。カンピロバクター腸炎の後，脱髄性ニューロパチーであるギランバレー症候群（Guillan-Barré syndrome：GBS）が発症することがあり，両者の関連性が推定されている。治療としてマクロライド，ホスホマイシン，フルオロキノロンが有効である。

3 病原体

1）分類

Campylobacter 属細菌（curved rod の意味）の分類を表1に示す[1]。カタラーゼ陰性菌として *Campylobacter concisus*，*Campylobacter rectus*，*Campylobacter sputorum*

表1　主な *Campylobacter* 属菌の分類

Ⅰ．カタラーゼ陰性を示す菌群
Campylobacter concisus *Campylobacter curvus* *Campylobacter mucosalis* *Campylobacter rectus* *Campylobacter sputorum* subspecies *sputorum* *Campylobacter sputorum* subspecies *bubulus*
Ⅱ．カタラーゼ陽性を示す菌群
1．42 ℃ で発育可能な菌群（thermophilic *Campylobacter*） *Campylobacter coli* *Campylobacter hyointestinalis* *Campylobacter jejuni* subspecies *jejuni* *Campylobacter jejuni* subspecies *doylei*[*1] *Campylobacter lari* *Campylobacter upsaliensis* 2．42 ℃ で発育不能な菌群 *Campylobacter sputorum fetus*[*2] *Campylobacter sputorum fetus venerealis*

[*1]42 ℃ での発育は極めて弱い
[*2]ある菌株では 42 ℃ の発育が可能である

（筆者作成）

などがあり，カタラーゼ陽性菌は 42 ℃ で発育可能な *Campylobacter coli*, *C. jejuni*, *Campylobacter lari* などと発育不能な *C. fetus* とに分類される。

2）形態

グラム陰性で 0.2 ～ 0.5 × 0.5 ～ 5.0 μm 大の細長いらせん状形態を示す（図 1）。2 つの菌が並んでS字形や“かもめの翼”状（gull-wing shape）を示す場合もある。一端または両端に 1 本の鞭毛を持ち活発な運動性を示し，特徴的なコルク栓抜き様運動（cork-screw-like motion）を呈する。芽胞は作らない。長期間の培養による栄養分の枯渇などにより，本菌は球状形態（coccoid body）を示す。coccoid body は生きているが培養できない（viable but non-culturable）菌である[2,3]。

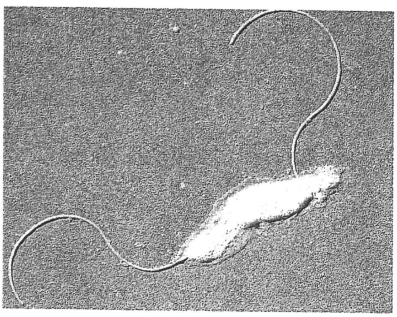

図 1　*C. jejuni* の電顕像
らせん状の菌体の両端に鞭毛が認められる。
（日本細菌学会教育用スライド集より出典，提供：横浜市立市民病院感染症部，相楽 裕子先生，病原菌の今日的意味 改訂3版（松本慶蔵 編）．医薬ジャーナル社，大阪，2003，p109）
（カラー図譜 32 頁）

3）性状

Campylobacter 属細菌は，増殖に 3 ～ 15%酸素を必要とする微好気性細菌（microaerophilic bacterium）である。オキシダーゼ陽性，ウレアーゼ陰性，リパーゼ陰性である。炭水化物非分解性で，アミノ酸または TCA 回路中の中間代謝物の異化によりエネルギーを獲得する。表 2 に主な *Campylobacter* 属細菌の性状を示す[1]。

C. jejuni NCTC11168 株は，1,641,481 bp 長のゲノムを有し，1,654 の蛋白と 54 の RNA 分子をコードしている[5]。ゲノム上繰り返し配列が多数みられるため，滑り誤対合（slipped-strand misparing）により抗原変異が起こりやすく，宿主の免疫能から回避することが可能である。プラスミドやバクテリオファージなどの遺伝因子が存在する[6]。テトラサイクリン，カナマイシン，クロラムフェニコール耐性はプラスミド上に存在する。

C. jejuni は比較的低温で生残しやすい性状を有し，25 ℃ で 3 日間しか生残しないが，10 ℃ 以下では 20 日間以上生残する。*C. coli* は馬尿酸加水分解陰性であり，*C. jejuni* と区別される。カンピロバクター腸炎の原因の 5 %程度を占める。*C. lari* はカタラーゼ陽性で，ナリジクス酸，セファロチンに耐性を示す。急性胃腸炎を引き起こす。*C. fetus* はウシやヒツジの流産を引き起こす菌であるが，ヒトにも感染する。免疫不全者や易感染性宿主（特に心臓弁膜症患者）に感染し，敗血

表 2　*Campylobacter* 属菌の性状

菌種	生化学性状			感受性*1		発育	
	オキシダーゼ	カタラーゼ	馬尿酸分解	ナリジクス酸	セファロチン	25 ℃	42 ℃
C. jejuni subsp. *jejuni*	+	+	+	S	R	−	+
C. coli	+	+	−	S	R	−	+
C. lari	+	+	−	R	R	−	+
C. fetus subsp. *fetus*	+	+	−	R	S	+	−
C. hyointestinalis	+	+	−	R	S	+*2	+

*1 R：抵抗性　S：感受性
*2 菌株により異なる

（筆者作成）

症，髄膜炎，卵管炎，胎児感染，流産などの全身性感染症が起こり，妊婦や新生児に重篤な病態を誘導する。小児などではまれにカンピロバクター腸炎に続発して，敗血症や髄膜炎が併発することがある。*C. hyointestinalis* はブタの腸内より検出される菌である。胃腸炎患者より分離されるが，ヒトに対する病原性は不明である。

4）血清型別およびゲノタイピング

C. jejuni や *C. coli* などを対象とした耐熱性抗原（Penner 型）および易熱性抗原（Lior 型）による血清型別が行われる。Penner 型[7]は 100 ℃，1 ～ 2 時間加熱による菌体抽出抗原（リポ多糖〔LPS〕）の違いに基づくものであり，*C. jejuni* は 50 型以上，*C. coli* は 17 型以上に型別される。Lior 型[8]は，ホルマリン死菌の鞭毛抗原の違いに基づくものであり，*C. jejuni*，*C. coli*，*C. lari* を含め 90 型以上に分けられる。

フラジェリン遺伝子型，パルスフィールド電気泳動パターン，リボタイピング，RAPD 法などの遺伝的手法を用いた菌株のゲノタイピングが行われ，原因菌の特定，感染経路の推定などの疫学的解析に有用である[9]。

5）病原因子

鞭毛蛋白遺伝子 *flaA* が不活化された場合，鞭毛は短くなり運動性も低下するが，同遺伝子 *flaB* の不活化は鞭毛の形態，運動性に影響を与えない[10, 11]。鞭毛は付着や細胞内侵入にも重要であり，鞭毛を欠く菌では宿主細胞への侵入性が低下する。*C. jejuni* は PEB1 や CadF などの付着因子を有する。PEB1 はグラム陰性菌の ABC トランスポート系蛋白のホモログであり，CadF はフィブロネクチオン結合性蛋白である。外膜蛋白や LPS も本菌の腸管上皮細胞への付着因子として作用する[12, 13]。*C. jejuni* はミクロフィラメントやミクロチュブルスを介して宿主細胞に侵入することが報告されており[14, 15]，本菌の細胞内侵入は腸炎患者や感染サルの腸管組織に認められている。腸管上皮細胞への本菌の侵入性は菌株により異なる。本菌は腸管上皮細胞の管腔側の他，基底膜（basolateral membrane）を介して（特に隣接細胞への二次感染時）細胞内に侵入するものと考えられている。

なお，*C. jejuni* は，細菌毒素として細胞致死性伸張化毒素（cytolethal distending toxin：CDT）遺伝子（*cdtA*，*cdtB*，*cdtC* から構成される）を有する[16]。CdtA，B，C は三量体を形成し，このうち CdtB が毒素活性である DNase 活性を有する。CDT の作用により，宿主細胞は伸張化し，細胞周期が G2/M 期にアレストされ，やがて細胞死が起こる。CDT は outer membrane visicle を介して菌体外へ分泌されている可能性が報告されている[17]。また，本毒素は宿主細胞からの IL-8 産生を誘導させる。本菌の鞭毛機能が CDT の分泌と IL-8 産生誘導を調節していることが報告されている[18]。熱ショック蛋白遺伝子 *htrA* の不活化変異株は細胞侵入性の点で親株より劣り，熱ショック蛋白の侵入性への関与が想定される[19]。

本菌感染後，宿主組織で産生されるプロスタグランディン E_2（PGE_2）や，ロイコトリエン B_4（LTB_4）などの炎症性メディエーターも本菌の病原性発現に関与する[20]。*C. jejuni* は Ⅲ 型分泌装置の遺伝子を保有しないが，ある種の菌株では Ⅳ 型分泌装置遺伝子を含む 37 kb のプラスミド pVir を保有することが明らかにされた。プラスミド上の Vir 遺伝子を欠失させた変異株では細胞内侵入ができない。約 10％の菌株が本プラスミドを有すると報告されている[21]。シリンジ状の Ⅳ 型分泌装置を宿主細胞に刺し，*C. jejuni* が産生するエフェクター蛋白（CiaB：*Campylobacter* invasion antigen B など）を宿主細胞内に移入させるものと想定される。

4 生態，疫学

Campylobacter 属細菌は，ウシ，ブタ，ウマ，ヒツジ，イヌ，ネコ，ハムスター，ニワトリ，カモメなどの動物の，主として腸管（その他生殖器，口腔内など）に生息する。ヒト腸管や口腔内からも *Campylobacter* 属細菌が検出される（病原性の強い *C. jejuni*，*C. fetus* などは通常検出されない）。本菌に汚染された生肉，生乳，飲料水，食品（特に鶏肉）を介してヒトへの経口感染する。鶏肉からの *Campylobacter* 属細菌の検出率は，わが国では 58.8％（912 試料/1,551 試料）を示す[22]。世界的にも鶏肉からの *Campylobacter* 属細菌の検出率は高く，平均 50％以上であることが報告されている[22]。

表3　病因物質別食中毒発生状況（2007 ～ 2009 年，厚生労働省統計より）

病因物質	平成 19 年（2007）			平成 20 年（2008）			平成 21 年（2009）		
	事件数	患者数	死者数	事件数	患者数	死者数	事件数	患者数	死者数
総数	1,289	33,477	7	1,369	24,303	4	1,048	20,249	7
細菌	732	12,964	−	778	10,331		536	6,700	−
サルモネラ属菌	126	3,603	−	99	2,551		67	1,518	−
ブドウ球菌	70	1,181	−	58	1,424	−	41	690	−
ボツリヌス菌	1	1	−			−			−
腸炎ビブリオ	42	1,278	−	17	168	−	14	280	−
腸管出血性大腸菌（VT 産生）	25	928	−	17	115	−	26	181	−
その他の病原大腸菌	11	648	−	12	501	−	10	160	−
ウエルシュ菌	27	2,772	−	34	2,088		20	1,566	−
セレウス菌	8	124	−	21	230	1	13	99	−
エルシニア・エンテロコリチカ	−	−	−	−	−	−	−	−	−
カンピロバクター・ジェジュニ / コリ	416	2,396	−	509	3,071	−	345	2,206	−
ナグビブリオ	1	1	−	1	5	−			−
コレラ菌	−	−	−	3	37	−	−	−	−
赤痢菌	−	−	−	3	131	−	−	−	−
チフス菌	−	−	−	−	−	−	−	−	−
パラチフスA菌	−	−	−	−	−	−	−	−	−
その他の細菌	5	32	−	4	10	−			−
ウイルス	348	18,750	−	304	11,630	−	290	10,953	−
ノロウイルス	344	18,520	−	303	11,618	−	288	10,874	−
その他のウイルス	4	230	−	1	12	−	2	79	−
化学物質	10	93	−	27	619	−	13	552	−
自然毒	113	355	7	152	387	3	92	290	7
植物性自然毒	74	266	4	91	283		53	195	4
動物性自然毒	39	89	3	61	104	3	39	95	3
その他	8	20	−	17	47	−	17	19	−
病因物質の不明のもの	78	1,295	−	91	1,289	−	100	1,735	−

事件数はカンピロバクター・ジェジュニ / コリが最も多く，次いでノロウイルスであった。患者数ではノロウイルスによる食中毒が断然多く，1 万人を超える。次いで多いのはカンピロバクター・ジェジュニ / コリ（2008 ～ 2009 年）およびサルモネラ属細菌（2007 年）であった。

事件あたりの患者数では，カンピロバクター・ジェジュニ / コリで少数（5.8 ～ 6.4 人）だが，ウエルシュ菌（61.4 ～ 102.7 人）やノロウイルス（37.8 ～ 53.8 人）では多数となる特徴がある。

（医薬食品局食品安全部監視安全課「平成 19 年食中毒統計」，「平成 20 年食中毒統計」，「平成 21 年食中毒統計」より）

検出率が高率である国としてオーストラリア（100%），アルゼンチン（92.9%），ニュージーランド（89.1%），トリニダード・トバゴ（84.6%）などがある。

一方，検出率が低率である国として，エストニア（8.1%），ベルギー（17.0%），旧ソ連（19.1%），スイス（25.1%）などがある。また，ペットを介したヒトへの感染も起こる。

わが国の食中毒事件の 34.3%（1,270 事件/3,706 事件：2007 ～ 2009 年）が，*Campylobacter* 属細菌（80 ～ 90%は *C. jejuni*）の感染による（表 3）。事件数（300 ～ 500 事件 / 年）では，2007 年以降 *Campylobacter* による食中毒が首位を占めている。しかし，患者数ではノロウイルスによる 10,874 人（2009 年）が最も多く，カンピロバクターによる 2,206 人（2009 年）は 2 位となっている。カンピロバクター腸炎では 1 件あたりの患者数は 5.8 ～ 6.4 人 / 事件であり，ウェルシュ菌（61.4 ～

102.7人/事件），ノロウイルス（37.8～53.8人/事件），サルモネラ（12.5～17.7人/事件）に比べ少ないのが特徴となる。散発性下痢症患者（特に小児）から高頻度に検出される。食中毒の発生は5～7月がピークとなり，サルモネラ属細菌による食中毒発生ピークより約2カ月先行する。本属細菌は比較的低温で生残しやすく，乾燥に弱いなどの性状を有するためと考えられている。*C. jejuni*は25℃で3日間しか生残しないが，10℃以下では20日間以上生残する。

全身性カンピロバクター感染症は，ウマ，ウシ，ヒツジなどの腸管に生息する*C. fetus*が，これらの糞便を介してヒトに感染する。また，性行為を介した感染も想定されている。

GBS患者の10～30%で，血清学的に*C. jejuni*感染が推測され，そのうちの25～88%で糞便より本菌の分離が陽性であった。分離菌株では，Penner 19型が最も多く（50%以上），次いで2型，4型が多い。また，Lior 7型菌の検出率も高い。カンピロバクター感染者の約0.1%の割合でGBSが発症するものと考えられている[23, 24]。

5 臨床

1）症状

1．カンピロバクター腸炎

原因菌は*C. jejuni*，*C. coli*，*C. lari*などであるが，80～90%は*C. jejuni*感染による。*C. jejuni*は感染性が強く，ヒトを対象とした研究において500～800 cfuの本菌の投与が感染症状を引き起こした[25]。本菌に汚染した食肉（特に鶏肉），生乳，飲料水を摂取後1～7日（平均3日）で，下痢，腹痛，発熱，全身倦怠感などの症状が認められる。時に嘔吐や血便などもみられる。下痢は1日4～12回にもおよび，水様便，泥状便で，膿，粘液，血液が混じることがある。本症では腹痛が強く長く続くという特徴がある。

2．全身性カンピロバクター症

*C. fetus*はウシやヒツジの流産を引き起こす菌であるが，ヒトにも感染する。免疫不全者や易感染性宿主（特に心臓弁膜症患者）に感染し，敗血症，髄膜炎，卵管炎，胎児感染，流産などの全身性感染症が起こり，妊婦や新生児に重篤な病態を誘導する。小児などではまれにカンピロバクター腸炎に続発して，敗血症や髄膜炎が併発することがある。感染源は不明な場合が多い。

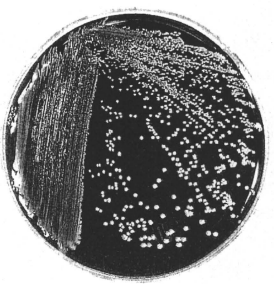

図2　*C. jejuni*のコロニー
血液寒天培地にて42℃，2日間微好気培養を行った。
（筆者提供）

3．ギランバレー症候群（GBS）

*C. jejuni*腸炎の後，1～3週に炎症性脱髄性ニューロパチーであるGBSが合併することがある。症状として筋力低下，顔面神経麻痺などの運動麻痺や異常知覚，不整脈，多汗などの自律神経障害などが認められる。

*C. jejuni*のLPSに神経細胞に存在する糖脂質GM_1，ガングリオシドと類似した構造があり（分子相同性〔molecular mimicry〕），本菌感染後抗GM_1抗体が産生され，これが自己免疫的に神経細胞に作用する発症メカニズムが想定されている[26, 27]。外眼筋麻痺，小脳性失調，深部腱反射消失を3徴とするフィッシャー症候群（Fischer's syndrome）と*C. jejuni*感染との関連も想定されている。

2）診断

患者材料（糞便，血液，膿，脳脊髄液）や原因食品を直接塗沫してグラム染色を行い，菌の存在を確認する[28]。同材料をスキロー（Skirrow）培地（ポリミキシンB，トリメトプリム，バンコマイシン含有），またはCCDA培地（charcoal，セフォペラゾン，デオキシコール酸含有）に接種する[29]。微好気ガス（O_2 5%，CO_2 10%，N_2 85%）下に42℃，2日間微好気培養を行う。直径1～2 mmの湿潤でやや褐色を帯びたコロ

ニーが形成される（図2）。

C. jejuni, *C. coli* はナリジクス酸感受性，セファロチン耐性を示すが，*C. lari* はナリジクス酸耐性，セファロチン感受性である。*C. jejuni* と *C. coli* とは，馬尿酸分解能により同定可能である（*C. jejuni* 陽性，*C. coli* 陰性）。馬尿酸加水分解試験を簡易・迅速化した判定キット（RIDZyme HIP-M）が市販されており，少量の菌を用いて迅速に（1時間余り），*C. jejuni* と *C. coli* の判別が可能となる[30]。*C. fetus* を考慮する場合は培養温度を37℃とする。らせん状のグラム陰性菌でオキシダーゼ陽性を確認する。

培養検査によらない迅速診断法として，DNAプローブ法やPCR法などの遺伝子学的診断法も有用である。PCR法は感度の高い検査法であるため，下痢便などのように被験材料中に存在する起因菌数が多い場合のみならず，原因食のように含有起因菌数が少ない場合にも起因菌の検出が可能である。近年，フラジェリン遺伝子型，パルスフィールド電気泳動パターン，リボタイピング，RAPD法などの遺伝的手法を用いた菌株のゲノタイピングが行われており，原因菌の特定，感染経路の推定などの疫学的解析に有用である。耐熱性抗原（LPS），および易熱性抗原（鞭毛抗原）に基づく血清型別の判定は，疫学調査に有用である。また，患者血清中の補体結合（CF）抗体価を測定することも可能だが，本症の経過が急性であることや分離培養が可能であることより，本症に血清学的診断はそれほど重要ではない。

3）治療

1．カンピロバクター腸炎

一般に自然治癒傾向の高い疾患であり，対症療法のみで軽快する例が多い。重篤に経過した場合や敗血症などを併発した場合にはエリスロマイシン，クラリスロマイシン，アジスロマイシンなどのマクロライド，ホスホマイシン，フルオロキノロンの投与を行う。近年，これらの抗菌薬に対する耐性株が増加している。*C. jejuni* のマクロライドおよびフルオロキノロンに対する耐性率は，それぞれ10%および19～47%と高率である[31]。抗菌薬の決定は薬剤感受性試験に基づき行うべきである。

2．全身性カンピロバクター症

抗菌薬を投与することが要求される。*C. fetus* にも *C. jejuni* や *C. coli* と同様，エリスロマイシン，ホスホマイシン，フルオロキノロンなどが有効である。その他第3世代セフェム，アンピシリン，アミノグリコシドなども用いられる。*C. fetus* はバシトラシン，ノボビオシンには耐性である。

3．ギランバレー症候群（GBS）

カンピロバクター感染との関与が疑われているが，その治療はGBSの神経症状が対象となる。

4）予防

C. jejuni，*C. coli*，*C. fetus* などの *Campylobacter* 属細菌は低温を好む傾向がみられるため，これらの菌に汚染された食材は長期間冷蔵保存せずに十分な加熱調理を行う。生理食塩水中での *C. jejuni* の生存期間は，10℃以下で20日以上，15℃で15日，20℃で7日，25℃で3日であることが報告される[32]。感染者の糞便から本菌は2～3週間にわたり検出されるが，手指や食品を介して健常人へ感染する可能性は極めて低い。患者の糞便や血液は滅菌処理し，これらに汚染されたと思われる衣類，物品は消毒を行う。本属細菌は，通常の消毒剤（0.1～0.2% 塩化ベンザルコニウム，0.05～0.1% グルコン酸クロルヘキシジン）に感受性である。

（神谷　茂）

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Reduced serum vascular endothelial growth factor receptor-2 (sVEGFR-2) and sVEGFR-1 levels in gastric cancer patients

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The relationship between gastric cancer and serum vascular endothelial growth factor receptor-1 (sVEGFR-1) and sVEGFR-2, which are soluble form receptor proteins of vascular endothelial growth factor (VEGF), has not been extensively studied. VEGF, sVEGFR-1 and sVEGFR-2 were measured in the sera obtained before surgical operation from 164 gastric cancer patients and from 164 healthy controls matched for age and gender. Compared with controls, the cases showed elevated VEGF ($P < 0.01$) and reduced sVEGFR-1 ($P = 0.07$) and sVEGFR-2 ($P = 0.02$). The difference in VEGF levels was small among men and when the outcome was early cancer. The difference in sVEGFR-1 levels was significant or borderline significant only in men and when the outcome was diffuse type cancer. The difference in sVEGFR-2 levels was significant only in men and when the outcome was advanced or diffuse type cancer. The sensitivities and specificities of VEGF, sVEGFR-1 and sVEGFR-2 were all approximately 60%. For diffuse type cancer, sVEGFR-2 showed a sensitivity of 62.4% and a specificity of 63.4%, which was similar to serum pepsinogen. In conclusion, elevated VEGF and reduced sVEGFR-1 and sVEGFR-2 in serum are characteristic of gastric cancer patients, and the value of serum sVEGFR-2 in the diagnosis of diffuse type gastric cancer should be further evaluated. (*Cancer Sci*, doi: 10.1111/j.1349-7006.2011.01860.x, 2011)

Although the incidence and mortality of gastric cancer have been declining among the younger generation in Japan,[1] it remains as the second highest cause of cancer death.[2] Recently, it has been proposed that serum *Helicobacter pylori* antibody and pepsinogen values should be used for risk assessment of gastric cancer in adults,[3,4] and gastric cancer prevention programs are expected to become more cost-effective, if the programs do not target at subjects without *H. pylori* infection or abnormal serum pepsinogen values, who have very low risks of gastric cancer.[4,5] However, the sensitivity of serum pepsinogen is poor for diffuse type gastric cancer.[6] Vascular endothelial growth factor (VEGF) is a factor promoting vascularization, and it plays a role in both physical and malignant conditions.[7] Staining with VEGF antibodies has revealed the presence of VEGF in some malignant tissues.[8] Previous studies have shown that serum VEGF concentration is high in several cancers, such as breast and colon cancers.[9,10] In gastric cancer patients, elevated VEGF predicts a poor prognosis[8,11] and is often accompanied by other malignant factors such as TGFβ-1.[12]

The biological effects of VEGF are mediated by two receptor tyrosine kinases, VEGFR-1 and VEGFR-2, which are almost exclusively expressed within endothelial cells. In addition to VEGFR-1 and VEGFR-2, a soluble form of VEGF-R1 (sVEGFR-1), a naturally occurring and alternatively spliced variant, functions as a high-affinity receptor of VEGF. Compared to VEGFR-1, VEGFR-2 is more widely distributed and expressed in all vessel-derived endothelial cells. The VEGF/VEGFR-2 signaling pathway plays a crucial role in tumor angiogenesis. Although the exact role of VEGFR-1 remains controversial, the available evidence have shown that VEGFR-1 functions to limit VEGF/VEGFR-2 mediated angiogenesis with intact receptor acting as a decoy and soluble form creating inert receptors by dimerization with VEGFR-2 or sequestering free ligand.[7] Soluble form of VEGFR-2 (sVEGFR-2) as well as sVEGFR-1 can be detected in serum. Circulating VEGF is known to be higher in gastric cancer patients than in healthy subjects.[13] However, the data on circulating sVEGFR-1 and sVEGFR-2 levels are limited.

In this study, serum VEGF, sVEGFR-1 and sVEGFR-2 levels were compared between gastric cancer patients and matched healthy controls. Secondary analyses examined different subtypes of gastric cancer defined by progression stage and histopathological type. To evaluate the diagnostic accuracy of the VEGF, sVEGFR-1 and sVEGFR-2 levels, the optimal cutoff values, sensitivities and specificities for gastric cancer were calculated.

Materials and Methods

Subjects in this study were originally enrolled in our previous study; the details of the subject recruitment and data collection are provided elsewhere.[14,15] Briefly, sera were collected from 787 gastric cancer patients who were younger than 70 years of age and were admitted to the surgical division of nine hospitals in the Tokyo Metropolitan Area between June 1993 and July 1995. Phlebotomy of each patient was performed before cancer treatment (surgical operation or chemotherapy). The sera were also collected from 1007 apparently healthy subjects who were admitted for health screening programs between June 1993 and November 1994. Informed consent was obtained from all subjects. The diagnosis of cancer was confirmed, and other information, including histological types and progression stages, was collected from histopathological reports for resected or biopsy specimens.

In three of the nine hospitals, prognosis information for gastric cancer patients was available. Of the 571 patients from these three hospitals, 198 cases were randomly selected so that young patients were included, and the proportion of men and women were similar in each 10-year age group.

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Table 1. Characteristics of subjects

	Control	Case	*P*-value
Number of subjects	164	164	
Age (years)	53.9 ± 10.0*	54.0 ± 9.9*	Matched factor
Male/Female	78/86	78/86	Matched factor
Smoking dose (number of cigarettes per day multiplied by smoking years)			
No smoking history	84 (51.2%)	81 (49.4%)	$P = 0.43$
1–399	27 (16.5%)	23 (14.0%)	
400–799	22 (13.4%)	25 (15.2%)	
800+	21 (12.8%)	30 (18.3%)	
Unknown	10 (6.1%)	5 (3.0%)	
Drinking dose (amount of alcohol consumed [g] per week multiplied by drinking years)			
No drinking history	47 (28.7%)	54 (32.9%)	$P < 0.01$
Occasional/1–134.9	38 (23.2%)	28 (17.1%)	
135–1349.9	35 (21.3%)	27 (16.5%)	
1350+	19 (11.6%)	41 (25.0%)	
Unknown	25 (15.2%)	14 (8.5%)	
Helicobacter pylori seropositive†	105 (64.0%)	159 (97.0%)	$P < 0.01$

*Mean ± standard deviation. †Measured using J-HM-Cap (Kyowa Medex Co. Ltd., Tokyo).

A healthy control was matched to each case based on age (within 2 years) and gender. In 34 pairs, the serum sample from either the case or the control had already been used up, so data on 164 matched pairs were available for this study. Based on the pathological information, cases were classified into early (depth of invasion was within submucosa) and advanced (depth of invasion includes propria muscle) gastric cancer or into intestinal and diffuse type cancers.

VEGF, VEGFR-1 and VEGFR-2 were measured in the sera with the commercial ELISA kit Quantikine from R&D systems (Minneapolis, MN, USA) for human VEGF, sVEGF R1 and sVEGF R2 by a researcher who was blind to the case status associated with the samples. Serum VEGF, sVEGFR-1 and sVEGFR-2 levels were compared between cases and controls by paired *t*-tests. To evaluate the diagnostic accuracy of the factors, the optimal cutoff values and the sensitivity and specificity were calculated for all, early, advanced, intestinal and diffuse type cancers. In these calculations, the controls were restricted to those who were paired to cases belonging to the specific classification.

Results

Table 1 shows the characteristics of the subjects. Although no remarkable difference in smoking was observed between cases and controls, the cases drank more alcohol and had a higher prevalence of *H. pylori*. VEGF and sVEGFR-2 levels were measured for all subjects, but the sVEGFR-1 level was measured only in 147 pairs because of insufficient sera.

The VEGF level was higher in cases than in controls (Tables 2,3), but the difference was weak among men and when the outcome was early cancer. Compared with controls, the cases tended to have lower sVEGFR-1 levels, and the difference was significant among male subjects and borderline significant when the outcome was diffuse type cancer. The sVEGFR-2 level was lower in cases of advanced or diffuse-type cancer than in their matched controls.

Table 4 shows the optimal cutoff values for VEGF, sVEGFR-1 and sVEGFR-2. Of the three factors, VEGF showed the best sensitivity and specificity, 63.5% and 65.1%, respectively, for intestinal-type cancer when the cut-off value was 415 pg/mL. sVEGFR-2 gave the best diagnostic accuracies for all advanced and diffuse type cancers where cut-off values (sensitivities and specificities) were 8520 pg/mL (61.0% and 60.4%), 8314 pg/mL (66.3% and 61.6%) and 8520 pg/mL (62.4% and 63.4%), respectively. For early gastric cancer, sVEGFR-1 gave the best sensitivity and specificity, 60.0% and 58.6%, respectively, when the cut-off value was 46.0 pg/mL.

Table 2. VEGF, sVEGFR-1 and sVEGFR-2 levels in cases and controls

	VEGF (pg/mL) Mean ± SD	sVEGFR-1 (pg/mL) Mean ± SD	sVEGFR-2 (pg/mL) Mean ± SD
Total			
Number of pairs	164	147	164
Controls	479.1 ± 350.8	56.96 ± 34.34	8853 ± 1888
Cases	640.6 ± 516.8	48.47 ± 32.45	8397 ± 2014
P-value*	$P < 0.01$	$P = 0.07$	$P = 0.02$
Male			
Number of pairs	78	71	78
Controls	511.6 ± 372.4	56.10 ± 36.26	9304 ± 1822
Cases	649.3 ± 517.6	42.58 ± 30.46	8343 ± 2186
P-value*	$P = 0.06$	$P = 0.02$	$P = 0.04$
Female			
Number of pairs	86	76	86
Controls	479.6 ± 329.4	55.82 ± 32.69	8443 ± 1864
Cases	632.8 ± 519.0	53.97 ± 33.47	8173 ± 1828
P-value*	$P < 0.01$	$P = 0.74$	$P = 0.28$

*Results from paired *t*-tests. VEGF, vascular endothelial growth factor; sVEGFR-1/2, serum vascular endothelial growth factor receptor-1/2.

Table 3. VEGF, sVEGFR-1 and sVEGFR-2 levels in cases and controls with respect to progression stage (early or advanced) and histopathological type (intestinal or diffuse)

	VEGF (pg/mL) Mean ± SD	sVEGFR-1 (pg/mL) Mean ± SD	sVEGFR-2 (pg/mL) Mean ± SD
Early gastric cancer (depth of tumor invasion is within submucosa)			
Number of pairs	78	70	78
Controls	485.4 ± 344.0	52.68 ± 30.79	8853 ± 1770
Cases	607.4 ± 422.8	46.43 ± 28.81	8811 ± 2075
P-value*	$P = 0.06$	$P = 0.24$	$P = 0.88$
Advanced gastric cancer (depth of invasion includes propria muscle)			
Number of pairs	86	77	86
Controls	473.3 ± 358.7	58.93 ± 37.23	8852 ± 2001
Cases	670.8 ± 590.2	50.32 ± 35.53	8021 ± 1891
P-value*	$P < 0.01$	$P = 0.16$	$P < 0.01$
Intestinal type gastric cancer			
Number of pairs	63	57	63
Controls	474.5 ± 391.3	50.64 ± 32.38	8501 ± 1764
Cases	658.6 ± 541.3	47.28 ± 30.96	8556 ± 2208
P-value*	$P = 0.03$	$P = 0.58$	$P = 0.87$
Diffuse type gastric cancer			
Number of pairs	101	90	101
Controls	481.9 ± 325.0	59.32 ± 35.29	9072 ± 1938
Cases	629.4 ± 503.4	49.22 ± 33.51	8297 ± 1887
P-value*	$P = 0.02$	$P = 0.06$	$P < 0.01$

*Results of paired *t*-tests. VEGF, vascular endothelial growth factor; sVEGFR-1/2, serum vascular endothelial growth factor receptor-1/2.

Discussion

VEGF was elevated in gastric cancer patients compared to controls. The presence of VEGF in a gastric cancer lesion[11] and a

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Table 4. Optimal cutoff values (sensitivity, specificity) of the factors for all, early, advanced, intestinal type and diffuse type cancers

Factor	All	Early	Advanced	Intestinal	Diffuse
VEGF (pg/mL)*	420 (60.4%, 57.9%)	428 (61.5%, 56.4%)	420 (58.1%, 60.5%)	415 (63.5%, 65.1%)†	428 (56.4%, 54.5%)
sVEGFR-1 (pg/mL)‡	45.6 (57.8%, 57.1%)	46.0 (60.0%, 58.6%)†	45.5 (57.1%, 55.8%)	40.7 (50.9%, 57.9%)	45.8 (58.9%, 60.0%)
sVEGFR-2 (pg/mL)‡	8520 (61.0%, 60.4%)†	8959 (55.1%, 52.6%)	8314 (66.3%, 61.6%)†	8400 (58.7%, 57.1%)	8520 (62.4%, 63.4%)†

*Positive is defined as the marker level being greater than or equal to the value given. †The best sensitivity and specificity of the three markers. ‡Positive is defined as the marker level being less than the value given. VEGF, vascular endothelial growth factor; sVEGFR-1/2, serum vascular endothelial growth factor receptor-1/2.

high level of circulating VEGF indicate a poor prognosis.[16–19] The result of the current study on VEGF is consistent with the results of previous studies.[13,20,21] The VEGF may have originated from gastric cancer cells[8] and the production and secretion may be increased with the progression of the cancer, but whether the cancer is intestinal or diffuse may have little influence on the VEGF level. It has been reported that *H. pylori* infection elevates serum VEGF level,[22,23] which can be a reason for the elevated VEGF level in gastric cancer patients. However, no association was observed between *H. pylori* serology and VEGF, sVEGFR-1 or sVEGFR-2 level in controls of this study.

Compared to the controls, the sVEGFR-1 and sVEGFR-2 levels were reduced in gastric cancer patients. One possibility is that the antibody used for ELISA recognizes the same or a near region as the ligand binds. Elevated VEGF levels may bind to these receptors and thereby reduce the sVEGFR-1 and sVEGFR-2 levels. Vascularization may promote cancer progression, and soluble VEGFR-1 and VEGFR-2 may act as decoys and disturb the binding of VEGF to VEGFR-2 on the surface of target cells. Reduced VEGFR-1 and VEGFR-2 levels and an elevated VEGF level stimulate the progression of gastric cancer and thus may be characteristic in gastric cancer patients.

Compared with VEGF, limited studies have examined circulating sVEGFR-1 level for gastric cancer and their role remains elusive. Colorectal cancer patients showed lower serum sVEGFR-1 level than controls did,[24] which is consistent with this study. Studies on pancreatic and biliary tract cancers gave similar results with this study on serum VEGF levels, but they showed higher sVEGFR-1 levels in patients than in controls.[25,26] Several studies to date have investigated relationships between sVEGFR-1 levels and prognosis as for several sites of cancers, which is inconsistent.[27] On sVEGFR-2 level, studies have been more limited. Further studies are warranted to clarify the role of sVEGFR-1 and sVEGFR-2 and their interactions with VEGF in the development of gastric cancer. The difference in the levels of the three factors between gastric cancer patients and controls was affected by gender, progression stage and histopathological type. The difference in the sVEGFR-1 level was not as clear as that for sVEFGR-2, which may be due to the obscure role of VEGFR-1 in vascularization. The differences in the VEGF and sVEGFR-2 levels were more striking for advanced cancer than early cancer, which can be explained by greater VEGF secretion from advanced gastric cancer tissues and by the binding of circulating sVEGFR-2. The difference in VEGF levels between matched cases and controls was smaller among men than women, while the differences in sVEGFR-1 and sVEGFR-2 levels between matched cases and controls were greater in men than in women. The underlying reason for the gender difference is unknown, but hormonal differences could exert some effect.

The difference in VEGF between matched cases and controls was similar between the intestinal and diffuse type cancers, whereas the difference in sVEGFR-1 and sVEGFR-2 levels was greater in the diffuse type cancer than in intestinal cancer. An explanation for the reduced sVEGFR-2 level in diffuse type gastric cancer is that advanced cancer was more frequent in diffuse type cancer than in the intestinal type. Actually, 54% of early and 69% of advanced cancers were diffuse types ($P = 0.06$). TGF-β1 is upregulated in patients with diffuse type gastric cancer[28,29] and TGF-β1 downregulates the expression of VEGFR-2 in endothelial cells.[30] These facts may be associated with the reduced sVEGFR-2 level in diffuse type gastric cancer. Serum pepsinogen II showed a sensitivity of 83.3% and a specificity of 76.9% for gastric cancer among those younger than 40 years of age,[31] although the sensitivity and specificity were weaker in those over 40 years. The sensitivities and specificities for the optimal cut-off values of VEGF, sVEGFR-1 and sVEGFR-2 were all approximately 60%, which is a somewhat unsatisfactory level. However, there were two interesting findings. One was that sVEGFR-2 showed a relatively good diagnostic accuracy compared with VEGF, although with VEGF, there was a smaller *P*-value than sVEGFR-2 in the paired *t*-test between cases and controls. The other finding was that sVEGFR-2 gave a similar diagnostic accuracy for diffuse type gastric cancer than serum pepsinogen. Serum pepsinogen, which is a good marker for gastric cancer and its risk,[32] does not show a good diagnostic accuracy for the diffuse type cancer.[6] When the diagnostic accuracy of serum pepsinogen for diffuse type cancer was calculated, and a positive result was defined as a pepsinogen I concentration not more than 70 ng/mL and the pepsinogen I to II ratio not more than 3.0,[33] the sensitivity and specificity were 65.3% and 59.4%, respectively. Compared with these values, the values for sVEGFR-2 of 62.4% and 63.4% were similar. Serum sVEGFR-2 can be used in the diagnosis of gastric cancer as the diagnostic accuracy of serum pepsinogen is not excellent. However, cautions are needed when using it to detect gastric cancer, because serum sVEGFR-2 is not specific for gastric cancer. The value of serum sVEGFR-2 in the diagnosis of gastric cancer, especially of the diffuse type, should be evaluated in further studies.

Because this study was not prospective, the differences in the serum levels of the markers may have been due to gastric cancer, and thus, the evaluation of the markers as risk indicators was impossible. We took this into consideration when interpreting the results. Another weakness of our study was that the measurement of the marker levels in the sera was performed after several years of frozen preservation. The preservation condition was not different between the cases and controls, and the measurement was performed by a researcher who was blinded to the case status for each serum sample. Thus, neither bias nor a difference in the preservation condition was expected to distort the results.

In conclusion, serum VEGF was elevated and sVEGFR-1 and sVEGFR-2 levels were reduced in gastric cancer patients. The difference in the levels of the three factors between gastric cancer patients and controls was affected by gender, progression stage and histopathological type. sVEGFR-2 showed a sensitivity and specificity for predicting diffuse type cancer that was similar to that of serum pepsinogen.

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Disclosure Statement

The authors have nothing to declare as financial disclosures.

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ORIGINAL ARTICLE

In vitro and in vivo effects of the Mongolian drug Amu-ru 7 on *Helicobacter pylori* growth and viability

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ABSTRACT

Amu-ru 7, a Mongolian folk medicine, is used to treat digestive diseases such as gastritis and gastric and duodenal ulcers. We examined the effect of Amu-ru 7 on the growth and viability of *Helicobacter pylori* in vivo and in vitro. By the agar dilution method, the MIC of Amu-ru 7 for *H. pylori* strains was shown to be 100–200 μg/mL with a MIC_{90} of 200 μg/mL. Two hundred micrograms per milliliter of Amu-ru 7 exhibited potent bactericidal activity against *H. pylori* in the stationary phase of growth 6 hr after treatment. Amu-ru 7 inhibited the growth of both AMPC-resistant and CAM-resistant strains, and also had a combined effect with AMPC on AMPC-resistant strain 403. The Amu-ru 7 inhibited biofilm formation by *H. pylori* and induced morphological changes, such as bleb-like formation and shortening of the cell. Although colonization of the stomach of the Mongolian gerbil by *H. pylori* was not cured by treatment with Amu-ru 7, both the mean number of *H. pylori* colonized and the colonization rate were decreased in Amu-ru 7 treated gerbils. These results suggest the effectiveness Amu-ru 7 as an adjunct therapy for eradication therapies consisting of a PPI combined with antibiotics.

Key words Amu-ru 7, bactericidal effect, *Helicobacter pylori*, Mongolian medicine.

Helicobacter pylori is a major cause of chronic gastritis and peptic ulcers and is also a risk factor for gastric adenocarcinoma and MALT lymphoma (1, 2). In 1994, a working group of the WHO International Agency for Research on Cancer classified *H. pylori* as a Group 1 carcinogen in humans. The prevalence of *H. pylori* infection worldwide is approximately 50% and in developing countries is 80–90% (3), suggesting that environmental factors are important for transmission of *H. pylori*. *H. pylori* infection is curable with regimens of multiple antimicrobial agents in combination with a PPI, but recently antimicrobial resistance has become the leading cause of treatment failure (4).

A new combination of folk medicines, Amu-ru 7 is composed of the extracts of *Rhei rhizoma*, *Hedychium spicatum*, *Radix aucklandiae*, *Terminalia chebula*, Cape Jasmine fruit, *Piper longum*, and Calcite; and is used for the treatment of patients with gastritis and peptic ulcer diseases. Although stomach cancer incidence rates have been decreasing slowly over recent decades in China, it was estimated that there were 400 000 new cases diagnosed and 300 000 deaths from this malignancy (5). Therefore, this disease remains an important public health burden throughout the world, especially in developing counties such as China. Furthermore, in Inner Mongolia the

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List of Abbreviations: AMPC, amoxicillin; BHS, Brucella broth supplemented with 7% horse serum; CAM, clarithromycin; CFU, colony-forming units; FCS, fetal calf serum; HBSS, Hanks' balanced salt solution; MALT, mucosa associated lymphoid tissue; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; PPI, proton pump inhibitor.

pathogenesis of *H. pylori* has received much attention. The effects of Amu-ru 7 on gastric ulcers induced by ethanol, acetic acid, or cold-water restraint stress have been reported. In the present study, Amu-ru 7 was examined for its effect on the colonization of *H. pylori* in the gastric mucosa of the Mongolian gerbil and inhibition of the growth of *H. pylori*.

MATERIALS AND METHODS

Test compound

Amu-ru 7 was synthesized in the Department of Medicine of the Inner Mongolian National University (Tongliao, China). *Rhei rhizoma, Hedychium spicatum, Radix aucklandiae, Terminalia chebula*, Cape Jasmine fruit, and *Piper longum* were extracted in hot-water or alcohol separately and dried. Amu-ru 7 contains *Rhei rhizoma, Hedychium spicatum, Radix aucklandiae, Terminalia chebula*, Cape Jasmine fruit, *Piper longum* and calcium at concentrations of 30%, 20%, 16%, 10%, 8%, 8%, and 8%, respectively.

Bacterial strains

Twenty *H. pylori* strains—TK 1003, TK 1008, TK 1021, TK 1022, TK 1046, TK 1047, TK 1101, TK 1102, TK 1103, TK 1108, TK 1117, TK 1126, TK 1301, TK 1304, TK 1405, TK 1407, KR 2002, KR 2003, TK 1402, and TK 1029—isolated from gastric biopsy specimens of patients attending the Tokai University Hospital (TK) and Kyorin University Hospital (KR) were used in this study (6). Two standard strains of *H. pylori*—ATCC 43503 and NCTC 11638—were used and three AMPC-resistant *H. pylori* strains—403, 116, and 175—were kindly provided by Dr Myung-Woong Chang (Kosin Medical College and Gospel Hospital, Department of Microbiology, Korea). The MIC of AMPC for strains 403, 116, and 175 were 2.0, 8.0, and 4.0 μg/mL, respectively (breakpoint of AMPC, 0.03 μg/mL). Cultures of these strains were stored at −80°C in Brucella broth containing 15% glycerol. The MIC of CAM for the *H. pylori* strain TK 1047 was 1.5 μg/mL by E-test; therefore, this strain was used as the CAM-resistant strain.

Reagent

Brucella broth and Bacto agar were purchased from Becton Dickinson (Detroit, MI, USA). Horse serum was purchased from Sigma-Aldrich Japan (Tokyo, Japan).

Determination of the MIC of Amu-ru 7 for *H. pylori*

The MICs of Amu-ru 7 were determined by the agar dilution method using Brucella broth supplemented with 7% horse serum and 1.5% agar (BHS agar). The 25 *H. pylori* strains were preincubated in Brucella broth with 7% horse serum for 18 hr under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2), and then inoculated (1 μL of sample) with a multipoint inoculator, MIT-F; Sakuma, Tokyo, Japan) on BHS agar plates containing various concentrations of Amu-ru 7 (100, 200, 400, 800, and 1000 μg/mL). BHS agar without Amu-ru 7 was used as the positive control. All plates were incubated for 3 days at 37°C under microaerobic conditions. The MIC was determined as the lowest concentration at which the compound inhibited visible bacterial growth.

Inhibitory effect of Amu-ru 7 on antibiotic resistant *H. pylori* strains

AMPC resistant *H. pylori* strains 403, 116, and 175 were inoculated on BHS agar plates supplemented with each of the aforementioned concentrations of AMPC and Amu-ru 7. The CAM-resistant *H. pylori* strain TK 1047 was inoculated on a BHS agar plate supplemented with each of the previously indicated concentrations of CAM and Amu-ru 7. Cultures of all strains were inoculated using standard loop (1/500 mL, Medical Wire and Equipment, Wiltshire, UK). BHS agar plates were used as a positive control. After inoculation, the plates were incubated under microaerophilic conditions for 72 hr.

Biofilm formation, quantification and inhibition in vitro

H. pylori strain TK 1402, which forms the thickest biofilm among the 12 *H. pylori* strains tested, was used for biofilm formation studies. Sterilized glass cover slips (approximately 22 × 22 mm, 0.12 to 0.17 mm thick; Matsunami Glass, Tokyo, Japan) were placed into 12-well microtiter plates. Each well was filled with 2 mL BHS to allow adherence of *H. pylori* at the air–liquid interface. The formation of biofilms was initiated by inoculating 10 μL of precultured cell suspension (approximately 5 × 10^5 cells in Brucella broth) into each well as described by Yonezawa *et al.* (7). The cultures were incubated under microaerobic conditions at 37°C for 2 days with shaking (125 rpm). After incubation, the cover slips were washed with phosphate-buffered saline (PBS). After washing, those slips were transferred into new 12-well microtiter plates. Each well was filled with 2 mL BHS and1/16, 1/8, 1/4, 1/2, 1, 10, and 25 MIC of the test compound. The cultures were incubated under microaerobic conditions at 37°C for 24 hr with shaking (125 rpm). After incubation, the cover slips were washed with PBS. The samples were then air dried and stained with 0.1% crystal violet for 30 sec. After staining, the cover slips were rinsed with distilled PBS to remove excess dye and then air dried for 30 min. All dye

associated with the biofilms was dissolved in 1 mL of ethanol and 200 μL of the ethanol solution was used to measure the absorbance at 595 nm with a microplate reader (Bio-Rad Tokyo, Japan) to determine the amount of biofilm formation.

Evaluation of bactericidal activity of Amu-ru 7

The bactericidal activity of Amu-ru 7 against *H. pylori* TK 1402 was assessed at each of the concentrations (200, 400, and 800 μg/mL). The bacterial suspension (20 μL) was inoculated into 2 mL of BHS containing twofold serial dilutions of the compounds. The culture was serially diluted 10-fold with BHS, and 50 μL of the diluted sample was plated on a BHS agar plate. The plate was incubated at 37°C under microaerobic conditions for 3 days; colonies of *H. pylori* were then counted.

Electron microscope observation

For scanning electron microscope analysis, *H. pylori* TK 1402 was cultured in Brucella broth containing 5% FBS for 18 hr and incubated with Amu-ru 7 (0, 200 and 400 μg/mL). After 3 hr incubation, *H. pylori* TK 1402 was fixed in 2.5% glutaraldehyde. Specimens were examined using scanning electron microscope (JEOL LSM-5600LV, Tokyo, Japan) as described previously (8, 9).

Animals

Four- or five-week-old male Mongolian gerbils (20 gerbils in total; specific-pathogen free) were purchased from Kyudou (Fukuoka, Japan) and bred under specific-pathogen free conditions (room temperature, 23 ± 2°C; relative humidity, 40 to 60%; 12-hr light–dark cycle) in the animal facility of Kyorin University. A standard diet (CE-2; Clea Japan, Tokyo, Japan) and sterilized tap water were provided ad libitum in microisolator units as described by Nakagawa *et al.* (10). The experiments were approved by the Experimental Animal Ethics Committee at the Kyorin University School of Medicine.

In vivo experiments

Strain TK 1402 was isolated from duodenal and gastric ulcer patients, and can effectively colonize Mongolian gerbil (8, 10) and mice (11, 12). TK 1402 was grown on BHS agar at 37°C for 2 days under microaerobic environment. After incubation, bacteria were harvested in HBSS (Sigma-Aldrich Japan, Tokyo, Japan) and 1 mL of bacterial solution containing 1–2 × 10^9 CFU was inoculated intragastrically on two consecutive days. The gerbils were fasted for 16 hr from before the first inoculation to after completion of the second inoculation.

Either 50 MIC (200 mg/kg) or 200 MIC (800 mg/kg) of Amu-ru 7 in 1 mL of sterile water was administered to each of the gerbils (5 gerbils/group) orally once each day for 16 days, and sterile tap water was administered to each gerbil in the control group (5 gerbils/group). The gerbils were sacrificed 8 weeks after infection. The stomach was opened along the greater curvature and the contents were emptied, then the stomach was divided into two parts. One half of the stomach, including the forestomach-to-pylorus was scraped off with a spatula, collected into 500 μL HBSS and homogenized to determine the number of microorganisms in the mucus layer: 50 μL of gastric sample in HBSS was inoculated on *H. pylori*-selective medium (Nissui, Tokyo, Japan) and incubated for 5 days under microaerophilic conditions at 37°C. Purple colonies were counted manually and the number of viable *H. pylori* cells was expressed as the number of CFU per gram of stomach. Brucella agar medium supplemented with 7% HS was inoculated with a single colony for identification of the bacteria. The isolated strain was shown to be positive for urease, catalase and oxidase with a Gram–negative helical form and was identified as *H. pylori*.

Statistic analysis

Statistic analysis was examined by the software StatView (HuLinks, Tokyo, Japan). Also, statistically significant differences in bacterial numbers in the stomach between the *H. pylori*-infected and the non-infected gerbils were examined using Student's *t*-test.

RESULTS

Effect of Amu-ru 7 on the growth of *H. pylori*

The MICs of Amu-ru 7 for 25 strains of *H. pylori* are shown in Table 1. Growth of all *H. pylori* strains was inhibited by Amu-ru 7, and the MIC_{90} of Amu-ru 7 was 200 μg/mL. Table 2 shows the combined effect of Amu-ru 7 with AMPC to AMPC-resistant *H. pylori* strains. *H. pylori* strain 403 was resistant to 4.0 μg/mL of AMPC; however, AMPC (4.0 μg/mL) plus Amu-ru 7 inhibited its growth. The effect of Amu-ru 7 on CAM-resistant strain TK 1047 was also examined. CAM-resistant strain TK 1047 was sensitive to Amu-ru 7, but the combination of Amu-ru 7 with CAM was not synergistic on the strain (data not shown).

The bactericidal activity of Amu-ru 7 is shown in Figure 1. No visible bacterial colonies were detected after incubation of *H. pylori* with 800 μg/mL of Amu-ru 7 for 1 hr. Following incubation of *H. pylori* with 400 and 200 μg/mL of Amu-ru 7, no *H. pylori* was detected at 2 and 6 hr, respectively (Fig. 1).

Table 1. Minimum inhibitory concentrations of *Helicobacter pylori* strains to Amu-ru 7

	Concentration of Amu-ru 7 (μg/mL)					
H. pylori strain	0	100	200	400	800	1000
TK 1003	2+	W	–	–	–	–
TK 1008	2+	W	–	–	–	–
TK 1021	2+	W	–	–	–	–
TK 1022	2+	W	–	–	–	–
TK 1029	2+	W	–	–	–	–
TK 1046	2+	W	–	–	–	–
TK 1047	2+	W	–	–	–	–
TK 1101	2+	W	–	–	–	–
TK 1102	2+	W	–	–	–	–
TK 1103	2+	W	–	–	–	–
TK 1108	2+	W	–	–	–	–
TK 1117	2+	W	–	–	–	–
TK 1126	2+	W	–	–	–	–
TK 1301	2+	W	–	–	–	–
TK 1304	2+	W	–	–	–	–
TK 1402	2+	W	–	–	–	–
TK 1405	2+	W	–	–	–	–
TK 1407	2+	W	–	–	–	–
KR 2002	2+	W	–	–	–	–
KR 2003	2+	W	–	–	–	–
ATCC 43503	2+	W	–	–	–	–
NCTC 11638	2+	–	–	–	–	–
403¶	2+	W	+	–	–	–
116¶	2+	W	+	–	–	–
175¶	2+	W	+	–	–	–

¶, AMPC-resistant strain. 2+, good growth, W, weak growth; –, no growth.

Table 2. Inhibitory effect of AMPC and Amu-ru 7 on AMPC-resistant strains

		Helicobacter pylori strain								
		403			116			175		
Amu-ru 7 (μg/mL)		0	100	200	0	100	200	0	100	200
AMPC (μg/mL)	0	+	+	+	+	+	+	+	+	+
	0.25	+	+	+	+	+	+	+	+	+
	0.5	+	+	+	+	+	+	+	+	+
	1.0	+	+	+	+	+	+	+	+	+
	2.0	+	+	+	+	+	+	–	–	–
	4.0	+	–	–	–	–	–	–	–	–
	8.0	–	–	–	–	–	–	–	–	–
	16	–	–	–	–	–	–	–	–	–

+, good growth; –, no growth.

Effect of Amu-ru 7 on biofilm formation by *H. pylori*

We examined the effect of Amu-ru 7 on biofilm formation by strain TK 1402 (Fig. 2). Amu-ru 7 (2000 and 5000 μg/mL, 10 MIC and 25 MIC) did not disperse the *H. pylori* biofilm that had been formed over two days of incubation (Fig. 2a). In contrast, the initial addition of Amu-ru 7 (12.5, 25, 50, 100, and 200 μg/mL) significantly inhibited biofilm formation in a dose-dependent manner for 3 days (Fig. 2b).

Morphological changes of *H. pylori* treated with Amu-ru 7

The effect of Amu-ru 7 on the morphology of *H. pylori* strain TK 1402 was examined by scanning electron micrography (Fig. 3). Bleb-like structures and shortening of the cells were observed in *H. pylori* treated with 200 μg/mL and 400 μg/mL of Amu-ru 7 for 3 hr. Such changes were not detected in untreated *H. pylori*. There was no difference in the number of coccoid cells between Amu-ru 7-treated and untreated *H. pylori*.

Effect of Amu-ru 7 on colonization of *H. pylori* in the Mongolian gerbil

When 200 mg/kg of Amu-ru 7 was administered orally to the Mongolian gerbil after *H. pylori* infection, the mean titer ($10^{4.63\pm0.90}$ CFU/g tissue) of *H. pylori* in gastric mucosa of treated animals was lower than that ($10^{5.35\pm1.02}$ CFU/g tissue) of control gerbils, although differences did not reach statistical significance ($P = 0.129$) (Table 3). Following inoculation with a higher dose of Amu-ru 7 (800 mg/kg), the *H. pylori* colonization rate (4/5 gerbils) was lower than that in the controls (5/5 gerbils), but there was no significant difference ($P = 0.209$) in the mean number of *H. pylori* colonized between the Amu-ru 7-treated and control gerbils.

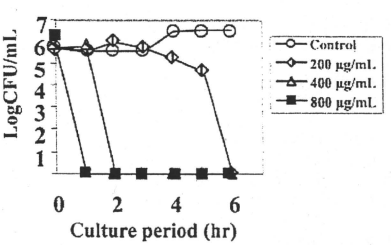

Fig. 1. Bactericidal effect of Amu-ru 7 on *Helicobacter pylori* strain TK 1402. *H. pylori* TK 1402 was incubated in Brucella broth containing (○) 0 μg/mL, (◆) 200 μg/mL, (△) 400 μg/mL, or (■) 800 μg/mL of Amu-ru 7.

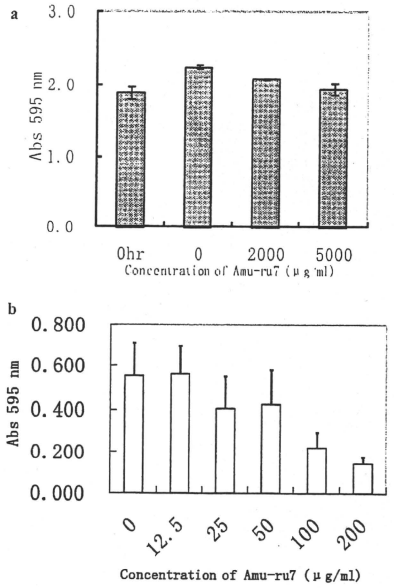

Fig. 2. Inhibitory effect of Amu-ru 7 on biofilm formation by *Helicobacter pylori*. Amu-ru 7 was added (a) after biofilm formation, or (b) from the start of the culture. Error bars are shown as the standard deviations using triplicate experiments.

In order to check the toxicity of Amu-ru 7, the Mongolian gerbils without infection were administered Amu-ru 7 orally for the same number of days. We did not find any visible damage in the stomachs or microscopic damage in the gastric samples stained with Hematoxylin–Eosin. This result indicates that Amu-ru 7 has no cytotoxicity to gastric epithelial cells.

DISCUSSION

H. pylori is one of the causative agents of various gastroduodenal diseases, and eradication of *H. pylori* is an important clinical goal; however, the rate of drug resistance of *H. pylori* to CAM, a first-line antibiotic used for eradication therapy, has been recently increasing (13). In Japan, the rates of isolation of CAM-resistant *H. pylori* strains were 7.0%, 18.9%, and 21.3% in 1999–2000, 2002–2003, and 2003–2004 surveys by the Japanese Society of Chemotherapy, respectively (14). In Beijing, China, it was reported that the isolation rate of CAM-resistant strains was 13.5% and is increasing (15); therefore, a secondary or tertiary regimen for the eradication of *H. pylori* is needed.

In the present study, the mean number of *H. pylori* in the Amu-ru 7 (200 mg/kg) treated gerbils was lower than that of the controls and the colonization rate in the Amu-ru 7 (800 mg/kg) treated gerbils was lower than that of the controls.

There have been many reports on the effects of Kampo (16), a traditional Chinese medicine (17, 18), on pathogenic bacteria including *H. pylori*; however, only a few reports on the effect of traditional Mongolian medicines on pathogens exist (19). As Amu-ru 7, which contains seven different kinds of Mongolian medicine, is used for the treatment of gastric diseases including gastritis and peptic ulcer diseases, the effect of this drug on *H. pylori* needs to be clarified. This is the first report on both in vitro and in vivo effects of Amu-ru 7 on *H. pylori*. We demonstrated that Amu-ru 7 has a bactericidal effect on *H. pylori* at a concentration of 200 μg/mL. Emodin, which is detected in *Rhei rhizoma*, was reported to inhibit the growth and DNA damage of *H. pylori* (20). It was also reported that *Piper longum* in the long pepper inhibited the growth of *H. pylori* by more than 99% after incubation for 60 min, but not the adhesion of *H. pylori* to gastric tissue (21). Malekzadeh *et al.* (22) reported that *Terminalia chebula* Retz in black myrobalan showed an anti-bacterial effect with a MIC of 125 μg/mL and MBC of 150 μg/mL for *H. pylori* strains. Similarly, gallic acid and ethyl gallate in *Terminalia chebula* were reported to have a bactericidal effect on pathogenic bacteria, including methicillin-resistant *Staphylococcus aureus* (23). In the present study, Amu-ru 7, which contains the above four medicinal components, exhibited a bactericidal effect on *H. pylori* strains, including CAM- and AMPC-resistant strains.

There have been other reports on the effects of these components. *Terminalia chebula* and *Rhei rhizoma* were reported to protect the gastric mucus membrane and decrease inflammatory changes in patients with gastritis (24). Although we did not examine the bactericidal effect of each component in Amu-ru 7 on *H. pylori*, the effect of six components in Amu-ru 7 on urease activity was examined (the effect of calcium was not examined due to its insolubility in water). Inhibition rates of *Rhei rhizome* (0.026 μg/mL), *Piper longum* (0.046 μg/mL), *Hedychium spicatum* (0.029 μg/mL), Cape Jasmine fruit (0.026 μg/mL), *Terminalia chebula* (0.034 μg/mL), and *Radix aucklandiae* (0.034 μg/mL) on urease activity were 54%, 29%, 4%, 3%, 1%, and 0%, respectively, suggesting that *Rhei rhizome* is the most effective antibacterial component on *H. pylori*.

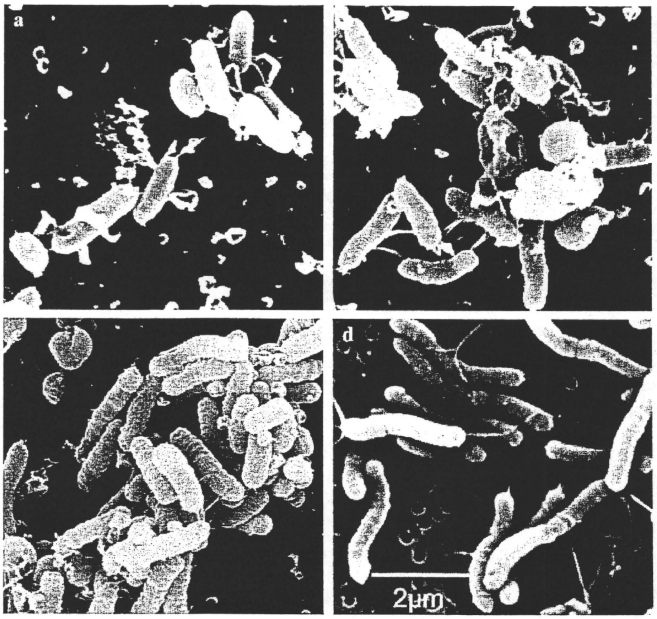

Fig. 3. Electron microscope analysis of *Helicobacter pylori* treated with Amu-ru 7. *H. pylori* was treated with (a,b) 400 μg/mL, (c) 200 μg/mL, or (d) 0 μg/mL of Amu-ru 7 for 3 hr at 37°C.

In this study, AMPC resistant strains 403, 116, and 175 were used for the evaluation of the growth inhibitory activity of Amu-ru 7; 200 μg/mL of Amu-ru 7 inhibited the growth of these AMPC-resistant strains. In addition, a combination of Amu-ru 7 (100 μg/mL) and AMPC (4.0 μg/mL) inhibited the growth of the AMPC-resistant strain 403, although only AMPC (4.0 or 2.0 μg/mL) inhibited the growth of both strains 116 and 175.

CAM is one of the most useful antibiotics against *H. pylori*. It is an acid-stable macrolide with a broad spectrum of antibacterial activity, well-absorbed with a wide tissue distribution and with mild side effects (25). In this

Table 3. Isolation of *Helicobacter pylori* from the stomach of infected Mongolian gerbils

		Infection status†	No. of *H. pylori* isolated					Mean No. of *H. pylori* isolated (Log CFU/g of tissue)‡
Exp. 1	Control	5/5	4.66	5.87	3.89	6.23	6.08	5.35 ± 1.02
	Amu-ru 7 (200 mg/kg)	5/5	5.75	5.16	3.49	4.05	4.77	4.63 ± 0.90
Exp. 2	Control	5/5	3.51	3.26	2.72	4.35	4.52	3.67 ± 0.75
	Amu-ru 7 (800 mg/kg)	4/5	4.35	4.37	2.85	ND	3.96	3.63 ± 0.84

†The number of *H. pylori*-positive gerbils/number of gerbils tested. ‡Mean ± SE, $n = 5$. ND, not detected.

study, the combination of Amu-ru 7 with CAM did not exhibit any synergistic effects on CAM-resistant *H. pylori* strain TK 1047. However, as the CAM-resistant *H. pylori* strain was shown to be sensitive to Amu-ru 7, this drug could play a useful role in the treatment of patients with *H. pylori* infection, particularly for patients infected with CAM-resistant *H. pylori*.

Strain TK 1402 forms the thickest biofilm among the 12 *H. pylori* strains tested, as previously reported (7), and this strain could effectively colonize the stomach of the Mongolian gerbil (8, 10). Biofilm formation is important for the survival of *H. pylori* in the gastric mucosa. Chellini *et al.* (26) have shown that biopsy samples from *H. pylori*-positive patients had coccoid bacteria arranged in a microbial biofilm. Bacterial biofilm is more resistant to killing by phagocytes and antibiotics, presumably due to the differentiation and survival of bacteria with a slow growth rate (27, 28). In the present study, it was shown that Amu-ru 7 inhibited biofilm formation by *H. pylori* when it was added from the beginning of the culture, mainly due to its bactericidal effect against *H. pylori*.

Electron microscope analysis of the Amu-ru 7 treated *H. pylori* indicated that Amu-ru 7 (200 μg/mL and 400 μg/mL) induced bleb-like formations on the cell surface and a shortening of the cell. It is likely that these morphological changes induced by Amu-ru 7 are the main causes for the bactericidal effect of this drug on the organisms.

In the present study, we demonstrated that the Mongolian medicinal drug Amu-ru 7 exhibited a bactericidal effect on *H. pylori* strains, and showed that it has a bactericidal effect with a MIC_{90} of 200 μg/mL and induced morphological changes of bleb-like formation and cell shortening. Although the effectiveness of Amu-ru 7 on *H. pylori* was demonstrated in vitro, this drug also needs to be assessed in vivo in combination with a PPI and/or antibiotics and in further clinical trials.

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