at the Department of Gastroenterology of Hokkaido University Graduate School of Medicine, Sapporo, Japan or any of seven hospitals making up the Hokkaido Gastrointestinal Cancer Study Group. Patients who met the following eligibility criteria could be enrolled: age 17–75 years; WHO performance status ≥2; estimated duration of survival ≥3 months; normal bone marrow function (white blood cell count [WBC] ≥4000/mm^3 and platelet count ≥100 000/mm^3); normal renal function (serum creatinine ≤1.5 mg/dL); and normal hepatic function (hepatic enzymes ≤3 times the upper limit of the institutional normal values [except in patients with liver metastases] and total bilirubin ≤1.5 mg/dL).

Written informed consent was obtained from all subjects. The present study was approved by the independent ethics committees of Hokkaido University and of each institution, as well as by the respective institutional review boards.

Pretreatment Evaluation and Monitoring

Prior to the start of treatment, a history was obtained and physical examination was performed. In addition, a complete blood count, hepatic and renal function tests, other biochemistry tests, ECG, chest x-ray examination, and CT scanning of the chest and abdomen were performed. During the study, blood tests were completed again at 5 weeks to detect drug toxicity. Toxicity was assessed according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 2.0.[14] After the completion of each course of therapy, a chest x-ray film and CT scans of the chest and abdomen were obtained to evaluate response. The response of assessable disease sites was evaluated according to the Response Evaluation Criteria in Solid Tumors.[15]

Treatment Plan

Irinotecan (Yakult Honsha Co., Ltd, Tokyo, Japan; and Daiichi Pharmaceutical Co., Ltd, Tokyo, Japan) was dissolved in 500 mL of saline or 5% glucose and was then administered by intravenous infusion over 90 minutes. Levoleucovorin was obtained from Wyeth Lederle Japan, Ltd (Tokyo, Japan). A dose of 250 mg/m^2 was mixed with 500 mL of saline and administered by intravenous infusion over 120 minutes immediately after the completion of irinotecan administration. Fluorouracil (Kyowa Hakko Kirin Co., Ltd, Tokyo, Japan) 600 mg/m^2 was administered intravenously as a bolus 1 hour after levoleucovorin administration. The modified-IFL combination evaluated in the current studies, unlike IFL therapies reported previously, included administration of irinotecan escalating to a dose of 150 mg/m^2 every 2 weeks (on days 1 and 15). This method was used because a dose of 150 mg/m^2 every 2 weeks is the standard regimen for irinotecan monotherapy in Japan in light of the fact that the drug often causes severe adverse reactions when administered once weekly, and it is difficult to administer irinotecan for four consecutive weeks. Conversely, a combination of fluorouracil and levoleucovorin can be administered satisfactorily on a weekly basis, and both of these drugs were accordingly administered on days 1, 8, 15 and 22 of the treatment cycle.

The doses of fluorouracil and levoleucovorin were fixed at 600 and 250 mg/m^2, respectively, while the irinotecan dose was escalated from 100 mg/m^2 in 25 mg/m^2 increments. At least three patients were entered at each dose level. If one of three patients developed dose-limiting toxicity (DLT) at a given dose level during treatment course 1, three additional patients were entered at that dose level. DLT was defined as any of the following NCI-CTC findings during treatment course 1: grade 3 non-haematological toxicity other than nausea, vomiting, anorexia, fatigue and hyponatraemia; grade 4 thrombocytopenia or grade 3 thrombocytopenia with haemorrhage; WBC <3000/mm^3; platelet count <10 000/mm^3 or non-haematological toxicity of grade 2 or higher on day 22, requiring treatment to be discontinued for ≥8 days. The MTD was defined as one dose level below the dose that resulted in DLTs in four of six patients during the first treatment course.

If dose level 1 was judged to be the MTD, a lower irinotecan dose of 80 mg/m^2 was scheduled to be assessed next. If the MTD was not reached at dose level 3, this dose would still be regarded as the recommended dose. This treatment course was repeated every 5 weeks with an allowance for

a delay in treatment if toxicity was observed. The next course was started only for patients whose organ biological parameters had been maintained at levels satisfying the eligibility criteria, except for the WBC (<3000/mm^3), and with no disease progression observed. Treatment was designed to be given on an outpatient basis, but the first course was administered in hospital, after which further courses were given in an ambulatory setting if adverse reactions were mild. To prevent diarrhoea due to irinotecan, 7.5 g of '*hangeshashintou*' (a herbal medicine) was administered after each meal.[16] In patients with constipation, a reduction in the dose or discontinuation of this medication was allowable. If diarrhoea occurred, loperamide was given.

Pharmacokinetic Study

The blood concentrations of fluorouracil, levoleucovorin, irinotecan and SN-38 (the major active metabolite of irinotecan) were measured in three patients during the first course of therapy. Blood samples for the pharmacokinetic study were collected before starting infusion of irinotecan; at 30, 60 and 90 (completion of administration) minutes after the initiation of infusion; and 15 minutes, 30 minutes and 1, 2, 4, 6, 8, 12, 24, 48 and 72 hours after completion of administration.

Results

Between February 2001 and December 2003, nine patients were enrolled in the phase I study and an additional 13 patients were enrolled in the phase II study. Of these 22 patients, 14 were male and 8 were female. Patient characteristics are listed in table I. The median age was 55 years (range 17–75 years) and the performance status was 0 or 1 in most of the patients. The mean number of treatment cycles was 4, and 93% of the patients completed at least two courses.

In the phase I study, three patients were assigned to dose level 1 and six patients received dose level 2. All nonhaematological toxicities were mild, except for grade 2 diarrhoea and nausea/vomiting in one patient at dose level 2 (table II). On the other hand, grade 3 neutropenia occurred in one patient at dose level 1 and in three patients at dose level 2. One of the three patients with neutropenia at dose level 2 also had a temperature ≥38°C. Therefore, grade 3 neutropenia was regarded as the DLT. Grade 4 neutropenia also occurred in one patient at dose level 2. Two patients showed partial remission (PR) at dose level 1 and five patients achieved PR at dose level 2, for a response rate of 77.8%. Because the DLT occurred in four patients at dose level 2, this was considered to be the MTD and the recommended dose of irinotecan for the phase II study was set at 100 mg/m^2, which was dose level 1.

The phase II study included an additional 13 patients (n = 22), all receiving the irinotecan dose level of 100 mg/m^2 to assess efficacy and safety. In this study, the response rate was also high (63.6%; table III). The response rate was 75.0% for undifferentiated cancer and 61.1% for differentiated cancer. Four patients developed grade 4 neutropenia, but this resolved after withdrawal of therapy or dose reduction. The median time to progression was 197 days (95% CI 111, 283) and the MST was 414 days (95% CI 116, 712). The 1-year survival rate was 55.5%. When survival was stratified by the site of metastasis, the MST was 705 days (95% CI 489, 921) for patients without liver metastasis versus 188 days (95% CI 14, 362) for those with liver metastasis, and the

Table I. Baseline characteristics of the study population (n = 22)

Parameter	n (%)[a]
Sex	
Male	14 (64)
Female	8 (36)
Age (y)	
Median (range)	55 (17–75)
Pathology	
Differentiated	18 (82)
Undifferentiated	4 (18)
Location	
Caecum-ascending colon	5 (23)
Transverse-descending colon	3 (14)
Sigmoid	6 (27)
Rectum	8 (36)

a Except where stated otherwise.

Table II. Number of patients experiencing different types of toxicity by irinotecan dose level

Dose level/adverse event	Toxicity grade			
	1	2	3	4
1: irinotecan 100 mg/m² (n = 3)				
Neutropenia	0	0	1	0
Leukopenia	0	1	0	0
Anaemia	1	2	0	0
Diarrhoea	0	0	0	0
Nausea/vomiting	1	0	0	0
2: irinotecan 125 mg/m² (n = 6)				
Neutropenia	1	0	3	1
Leukopenia	0	2	3	0
Anaemia	2	3	1	0
Diarrhoea	3	1	0	0
Nausea/vomiting	0	1	0	0
3: irinotecan 150 mg/m² (n = 0)				
Neutropenia	0	0	0	0
Leukopenia	0	0	0	0
Anaemia	0	0	0	0
Diarrhoea	0	0	0	0
Nausea/vomiting	0	0	0	0

prognosis was significantly better in the former group ($p = 0.0079$).

No drug interactions were detected in three patients evaluated in the pharmacokinetic study. The mean maximum peak concentrations of irinotecan (1.786 μg/mL) and its major metabolite SN-38 (22.54 ng/mL) were similar to those obtained with irinotecan monotherapy (1.33 μg/mL and 20.6 ng/mL, respectively) in an earlier study by Saltz et al.[17]

Discussion

In 2000, the US FDA approved the use of combined therapy with irinotecan, fluorouracil and levoleucovorin. However, an alert was subsequently issued after a high early death rate was reported in the IFL groups of two trials (N9741[18] and CALGB [Cancer And Leukemia Group B] 89803[19]). As careful review of these trials revealed that most of the deaths were caused by gastrointestinal toxicity or thrombosis, the FDA issued an alert about IFL therapy.[8,20] The results of the N9741 trial, however, demonstrated the significant efficacy of FOLFOX4 (oxaliplatin/leucovorin/fluorouracil) therapy.[21] When FOLFIRI therapy and FOLFOX6 (another oxaliplatin/leucovorin/fluorouracil combination) therapy were compared in a Groupe Coopérateur Multidisciplinaire en Oncologie (GERCOR) trial, toxicity was found to be acceptable for both regimens (although the nature of the toxicity differed between regimens) and there was no difference in mortality up to 60 days.[22] Based on these results, and an indirect comparison with FOLFOX therapy, FOLFIRI therapy with continuous infusion of fluorouracil was considered to be a better treatment than IFL therapy with bolus administration of fluorouracil, and FOLFIRI and FOLFOX in this way became recognized as the standard treatments. Goldberg et al.[23] reviewed the dosages used in IFL therapy and conducted a trial of reduced-IFL therapy (R-IFL) after the high early death rate in the IFL group in the N9741 trial became controversial. They reported that the early death rate (death within 60 days) was 2.7% in the R-IFL group and 2.0% in the FOLFOX4 group. Goto et al.[24] also reported that the modified Saltz regimen (in which fluorouracil is administered as a bolus) is safe and efficacious for Japanese patients.

The present modified-IFL regimen was designed to reduce the toxicity of the original IFL therapy developed by Saltz et al.[9] without any decrease in efficacy, and was also intended to be suitable for administration in an ambulatory setting. It was decided that irinotecan should be given on days 1 and 15 of each course. Although fluorouracil is generally administered as a continuous infusion, the phase III study conducted by Kohne et al.[25] showed that bolus administration is not inferior to continuous infusion

Table III. Overall response in the study population (n = 22)

Response type	No. reported
Complete response	0
Partial response	14
Stable disease	6
Progressive disease	2
Non-evaluable	0
Response rate (%)	63.6 (95% CI 43.5, 83.7)

because there was no significant difference in survival. With reference to the Roswell Park Memorial Institute (RPMI) method,[4] it was decided to administer four doses of fluorouracil/levoleucovorin (on days 1, 8, 15 and 22), and to repeat this treatment every 5 weeks after a 1-week withdrawal period. Using this modified-IFL therapy, diarrhoea occurred in 18% of patients, but grade 3 or 4 diarrhoea was not observed. The incidence of nonhaematological adverse reactions was also reduced, because administration of irinotecan was limited to days 1 and 15. Grade 4 neutropenia occurred in five patients (23%) during the combined phase I/phase II studies, but treatment could be continued after reducing the irinotecan dose according to the protocol, suggesting that this therapy can be used on an outpatient basis.

In patients administered modified-IFL therapy, irinotecan is likely to be the major cause of adverse reactions. Abnormal irinotecan metabolism has been reported in people with reduced uridine diphosphate glucuronosyl transferase 1 (UGT1A1) activity, resulting in more severe adverse reactions.[26] UGT1A1 homozygosity was found among patients with grade 4 neutropenia in the phase I study, but a dosage reduction allowed patients to be treated safely. This finding suggests that it may be useful to conduct a study in which the irinotecan dose is modified after identification of genetic variations in UGT1A1 activity. In terms of efficacy, the modified-IFL regimen achieved a high response rate of 63.6%, while the MST and median time to progression were 414 and 197 days, respectively, results that were comparable with the response to the Saltz regimen.[9]

Conclusions

Modification of the IFL regimen by administering irinotecan every 2 weeks is considered to have reduced the occurrence of adverse reactions in this study. Since the fluorouracil/levoleucovorin dosage for RPMI therapy was adopted without modification, the modified-IFL regimen also achieved a high response rate. However, enrolment of previously untreated patients may also have led to a higher response rate. Our modified-IFL regimen can be expected to achieve a stronger anti-tumour effect than FOLFOX because fluorouracil is administered as a bolus. Hurwitz et al.[27] reported that IFL is highly compatible with bevacizumab, suggesting that our modified-IFL regimen may also be used in combination with molecular-target drugs. The main finding of the study, i.e. that the modified-IFL regimen will be an effective treatment option for some patients and/or some situations of advanced colorectal cancer, is applicable globally and not only to Japan.

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機能性ディスペプシアおよび食欲不振に対する漢方治療

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要旨：機能性ディスペプシア（FD）治療に用いられる代表的な方剤である六君子湯は，食欲不振に対する効果を手がかりとして，そのユニークな作用機序の解明が進んでいる．六君子湯の作用機序としては，従来，胃排出促進および胃適応弛緩の改善が知られていたが，摂食促進ホルモンであるグレリンの分泌を亢進させることがごく最近明らかとなった．シスプラチンや選択的セロトニントランスポーター阻害薬（SSRI）投与は，消化管粘膜や脳内のセロトニンを増加させ，セロトニン2Bあるいは2C受容体を介してグレリン分泌を抑制することがその副作用の発現に関わっているが，六君子湯はセロトニン2Bあるいは2C受容体に拮抗してグレリン分泌を改善する．六君子湯のグレリン分泌促進作用は，FDに対する効果に関わっている可能性も示唆されている．また，六君子湯は高齢動物の視床下部でレプチンの作用と拮抗することにより食欲を改善させる機序も有しており，今後さらに多くの作用点が見出される可能性が高い．

索引用語：機能性ディスペプシア，食欲不振，六君子湯，シスプラチン，グレリン

はじめに

機能性ディスペプシア（functional dyspepsia；FD）に対する漢方薬の臨床効果に関しては，質の高いエビデンスはきわめて少なく特に英文の文献はほとんど存在しない[1)2)]．しかしながら，FDの治療に関しては，西洋薬に関するエビデンスも決して満足すべきものではない[3)4)]漢方治療は証に基づいて行うのが基本とされてきたが，最近では西洋医学と区別なく使用して有効であったとする報告が増加しており，通常の治療に反応しない症例において漢方が有効である可能性も指摘されている．FD治療に用いられる代表的な方剤である六君子湯については，食欲不振に対する効果を手がかりとして，その作用機序の解明が進んでいる．本稿では，六君子湯のもつユニークな作用機序の解明とその臨床応用の現状について概括する．

Ⅰ FDおよび食欲不振治療における漢方の役割

FDの治療目標は自覚症状の改善および消失，quality of lifeの向上である．FD患者の病態に応じて治療法を選択するのが理想的であるが，FD患者の病態把握が困難であるため，FDに対しては経験的治療が主体となっている．FDの病態としては胃排出の低下，適応性弛緩の障害，内臓知覚過敏が中心と考えられ，心理学的要因や最近では酸の関与も指摘されている[3)4)]．FDの治療では，複雑な病態に対応するため，酸分泌抑制，胃

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Role of Kampo medicine in the treatment of dyspepsia and anorexia
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排出促進，適応弛緩の改善，内臓知覚過敏の改善などの作用を期待して，さまざまな薬物や治療法が試みられている．しかしながら，これらの薬剤で，必ずしも十分な治療効果が得られているとはいい難く，漢方の効果に期待が寄せられている．

FDに処方される一般的な漢方薬は10種類程度あり，原則として東洋医学的観点から処方が決定されている．その使い分けに関しては明確な基準を見出すことは難しいものの，ある程度のコンセンサスが得られている[1)5)]．食後愁訴症候群では，六君子湯を第一選択とし，特徴的な自覚症状，他覚所見があればそれに応じて半夏瀉心湯，茯苓飲，人参湯が使い分けられる．一方，心窩部痛症候群では安中散を第一選択とし，特徴的な自覚症状，他覚所見があればそれに応じて半夏瀉心湯，茯苓飲，人参湯，黄連湯，柴胡剤（大柴胡湯，柴胡桂枝湯，四逆散）が使い分けられている．これらが無効な時，抗不安作用，抗うつ作用を期待できる香蘇散，平胃散が考慮される．なお，漢方薬がFD患者に使用される場合は，食欲不振も重要な症状の1つと考えて対処されることが多い．

II　エビデンスからみたFDの漢方治療の現状

残念ながら，FDに対する漢方薬の臨床効果に関する質の高いエビデンスはきわめて少なく，特に英文の文献はほとんど存在しない．そのなかでは，FDに対して第一選択として用いられることの多い六君子湯についての研究が最も多く報告されている．最近のSuzukiらの総説[2)]によれば，六君子湯に関する英文文献は，臨床研究9件，動物実験8件の合計17件抽出されている．ただし，臨床研究9件のなかでランダム化比較試験（RCT）を行っているものはわずか1件のみであり，残りは観察研究であった．一方，和文文献を含めたEBMに関しては，日本東洋医学会のEBM特別委員会により漢方治療エビデンスレポート2009として構造化抄録とともに同学会ホームページに公開されている[6)]．それによるとFD（当時の病名は胃炎もしくは上腹部不定愁訴）に対する漢方治療のRCTは，英文1件，和文8件の合計9件報告されている．このうち半夏瀉心湯の1件を除くと残りはすべて六君子湯に関する報告であった[1)6)]．

プラセボの使用により厳密に二重盲検化した検討は行われていないが，これらのRCTのなかで最も質が高いと思われる研究は原澤らの報告[7)]である．これは，運動不全型のFDに対する多施設共同臨床試験を行い，六君子湯の上腹部症状改善効果を示したもので，この試験で注目すべき点として，前治療として他の運動改善薬で効果を得られなかった症例に対しても，約50%の有効性が示されたこと，胃もたれ感とともに食欲不振の改善効果が高かったこと，臨床効果が投与3日目というきわめて早期から認められた点である．このことは，六君子湯が他の運動改善薬とは異なるユニークな作用メカニズムを有することを示唆している．

III　FDおよび食欲不振に対する六君子湯の作用機序

六君子湯のFDおよび食欲不振に対する作用機序としてまず最初に考えられるのは，胃排出能の改善である．Tatsutaら[8)]，原澤ら[7)]は，アセトアミノフェン法を用いてFD患者の胃排出を検討し，六君子湯投与により胃排出の促進がみられることを示した．Tominagaらは，六君子湯による胃排出促進作用に5-HT3受容体が関与する可能性を示唆しており[9)]，Kidoらは，六君子湯に含まれるhesperidinおよびL-arginineが，胃排出改善作用の活性本体であることを示している[10)]．

胃適応性弛緩に対する六君子湯の作用も検討されている．Hayakawaらは，モルモットを用いた実験的胃適応性弛緩障害モデルを用い，六君子湯が一酸化窒素の産生を介して胃適応性弛緩を改善することを示した[11)]．さらに，この作用はメトクロプラミド，トリメブチンおよびシサプリドでは認められず，六君子湯特有の作用と考えられた．

IV　グレリン分泌に対する六君子湯の作用

最近，われわれは，シスプラチンをラットに投与するという食欲不振モデルを用い，六君子湯の食欲不振に対する効果が，グレリン分泌の促進を介していることを初めて明らかにした[12)]．シスプラチンはラット消化管粘膜のセロトニンを放出

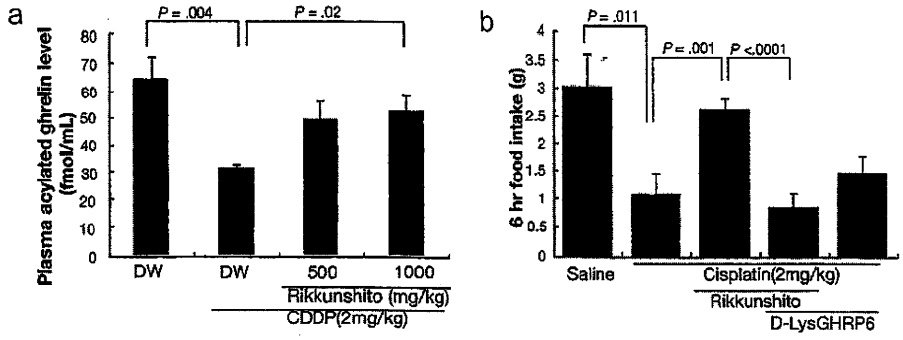

Figure 1. シスプラチン投与ラットにおける六君子湯の効果 a：血漿アシルグレリン，b：摂食量．文献12より改変引用．

し，血漿グレリン分泌を減少させて食欲を低下させたが，驚いたことに，この食欲低下に関与するセロトニン受容体は，予想された5-HT3受容体ではなく，これまで機能が不明であった胃粘膜の5-HT2B受容体および中枢神経に存在し食欲の制御に関わっている5-HT2C受容体であった．六君子湯の構成生薬の1つである陳皮，人参および生姜に含まれるflavonoid，coumarinおよびphenol類が，セロトニン2Bおよび2C受容体に拮抗してグレリン分泌を促進し，食欲を回復させることが明らかとなった[12](Figure 1)．なお，このグレリン分泌促進作用は，従来の消化管運動改善薬であるドンペリドン，メトクロプラミドには認められず，六君子湯特有の作用と考えられた．最近，同様のモデルを用い，シスプラチンが5-HT2C受容体を介して視床下部のグレリン分泌と食欲を低下させること，六君子湯は5-HT2C受容体拮抗作用を介して視床下部のグレリン分泌を改善させることが示されている[13]．

さらに，抗うつ薬として広く使用されている選択的セロトニントランスポーター阻害薬（SSRI）が，5-HT2C受容体を介してグレリン分泌を低下させ食欲不振を引きおこすこと，六君子湯はSSRIによるグレリン分泌，食欲を改善することが，ラットを用いた実験で示されている[14]．このことは，SSRIの使用開始直後にしばしば認められる消化器症状の原因として，5-HT2C受容体刺激によるグレリン分泌の低下が関与することを強く示唆するものである．実際，臨床的検討においても，六君子湯の併用がfluvoxamineの副作用を軽減させることが明らかにされている[15]．さらに，最近の健康ボランティアによる検討でも，六君子湯の2週間投与により，血漿アシルグレリンが有意に増加することが報告されている[16](Figure 2)．

グレリンは視床下部弓状核のAgRP/NPYニューロンに働いて食欲を亢進させるとともに，迷走神経末端のグレリン受容体に作用して迷走神経求心路を刺激し，迷走神経遠心路経由で胃運動を促進することが知られている[17]．六君子湯は，グレリンの増加を介して食欲を亢進させるとともに，胃運動を促進することによってFDをはじめとする上部消化管の機能異常を改善する可能性が考えられる．また，これらの研究によって，使用目標にも示されている食欲不振に対する六君子湯の効果が科学的に実証されたと考えられる[18](Figure 3)．

V 六君子湯は加齢による食欲不振を異なる機序で改善させる

六君子湯をはじめとする漢方薬の特質として，多成分系であるがゆえに，生体に対する作用点が複数存在することがあげられる．六君子湯の食欲不振改善作用の機序として，グレリン分泌促進作用以外にも複数の作用点が存在することが予想されるが，実際，われわれが行った老齢マウスを用いた検討では，グレリン分泌とは異なった機序で食欲を亢進させることが明らかになっている[19]．

高齢者における食欲不振の原因としては，社会的孤独，うつや身体疾患などの併存疾患，味覚・

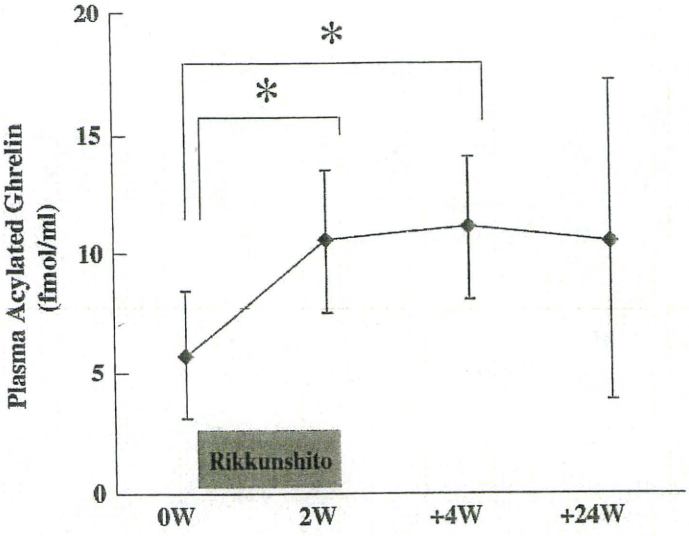

Figure 2. 健康ボランティアに六君子湯を2週間投与した時の血漿アシルグレリン値．文献16より改変引用．＊；P＜0.01.

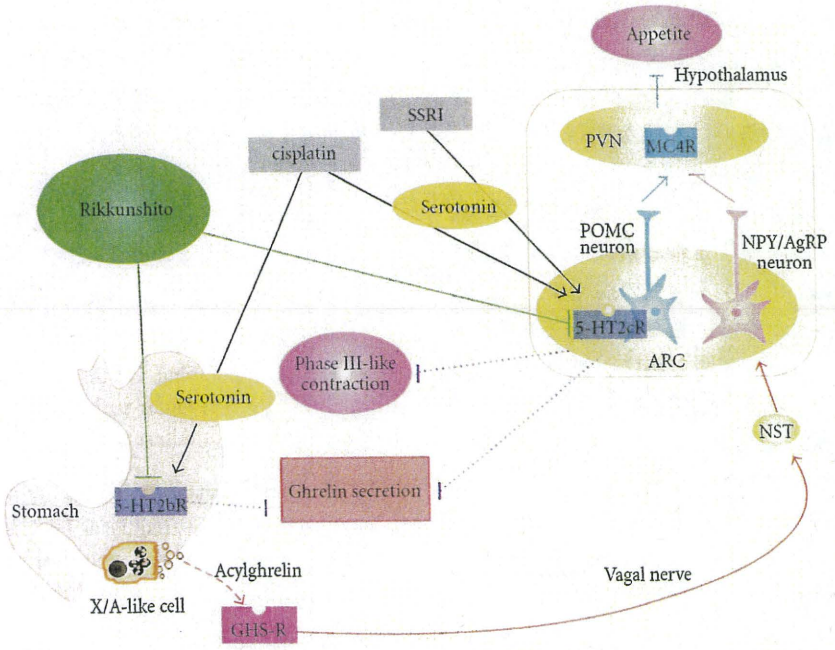

Figure 3. 六君子湯の食欲に対する作用機序．文献18より引用.

嗅覚の低下，薬剤など多数の要因が知られている[20)21)]．液性因子の異常としては，コレシストキニン，ペプチドYY，グルカゴン様ペプチド1，インスリン，レプチンなどの摂食抑制ホルモンの高値が指摘されている．一方，加齢によるグレリン動態に関してはわずかなデータしかない．空腹時グレリン濃度については，若年と高齢で差があるとする報告と差がないとする報告があり，結論

が出ていない[20][21]．食後には，若年者，高齢者ともにグレリン濃度が低下し4時間後に前値まで回復する．しかしながら，高齢者においてはグレリン濃度の上昇にともなう空腹感が生じないことが明らかにされており，このようなグレリン感受性の低下には，高濃度のレプチンとインスリンが関与していると考えられている[22]．レプチンによるグレリン感受性低下のメカニズムとしては，レプチンが弓状核NPY/AgRPニューロンにおいてホスファチジルイノシトール3キナーゼ（PI3K）およびホスホジエステラーゼ3（PDE3）を活性化する機構が想定されていた[23][24]．最近われわれは，老化マウスにおいてPI3K-PDE3系が亢進していること，六君子湯は血漿グレリンには影響を与えず，視床下部PDE3の阻害により老化マウスの食欲を回復させることを明らかにしている[19]．

おわりに

FDあるいは食欲不振は，病態が複雑でしかも機序が複数である可能性が高い．もしそうであれば，これまで開発が続けられてきた，単一の作用点を標的とした薬剤では，FDや食欲不振に対する効果が不十分であるのは当然かもしれない．通常の治療に抵抗するFDや食欲不振に対して漢方薬が顕著な効果を発揮することがあるが，その場合はおそらく漢方薬に含まれる複数の活性成分の作用点がその患者が有する複数の病態をうまくカバーできた時と思われる．ただ残念なことに，漢方薬の作用点はほとんど明らかにされていない上に，個々の患者の病態を的確に捉えることができていないことから，作用点と病態がうまくマッチしないと臨床効果が全く認められないことにもつながると考えられる．東洋医学的には，“証”に従って処方する“随証治療”を必須と考えているのはそのような理由と思われる．

FDに対し，漢方薬をファーストチョイスとするには，現時点ではエビデンス，特に質の高い英文の研究報告が不足しているが，通常の治療に反応しないFDや食欲不振に対しては，セカンドもしくはサードチョイスとして試みても良い治療法と思われる．漢方薬では，通常副作用がほとんどないことも有利な点である．今後漢方薬の複数の作用点が明らかにされ，個々のFD患者の病態を的確に捉えることができれば，漢方薬をファーストチョイスとして使用することも可能になるかもしれない．

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Similar changes of hypothalamic feeding-regulating peptides mRNAs and plasma leptin levels in PTHrP-, LIF-secreting tumors-induced cachectic rats and adjuvant arthritic rats

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Parathyroid hormone-related protein (PTHrP) is a causative factor of humoral hypercalcemia in malignancy. However, it is difficult to explain the mechanism of anorexia/cachexia with PTHrP secretion in detail. Previously, we demonstrated that the expressions of orexigenic peptides increased and anorexigenic peptides decreased under cachectic conditions in rats carrying tumors secreting PTHrP. In this study, we investigated whether such changes in the expression of hypothalamic feeding-regulating peptides can be solely attributed to PTHrP or are a general response under cachectic conditions. Cachectic syndromes were induced in rats by: (*i*) inoculation of human lung cancer LC-6 cells that secreted PTHrP, (*ii*) inoculation of human melanoma SEKI cells that secrete not PTHrP but LIF1, (*iii*) injection of heat-killed *Mycobacterium* leading to arthritis (AA) and (*iv*) oral administration of a high dose of $1\alpha,25(OH)_2D_3$ that resulted in hypercalcemia. The LC-6-bearing rats and AA rats were treated with or without anti-PTHrP antibody and indomethacin, respectively, and the expression of the hypothalamic feeding-regulating peptide mRNAs were examined by *in situ* hybridization histochemistry. The orexigenic peptide mRNAs, such as neuropeptide Y and agouti-related protein, were significantly increased, and that of anorexigenic peptide mRNAs, such as proopiomelanocortin, cocaine- and amphetamine-regulated transcript and corticotropin-releasing hormone were significantly decreased when they developed cachectic syndromes and AA. A high dose of $1\alpha,25(OH)_2D_3$ caused hypercalcemia and body weight loss but did not affect the expression of hypothalamic feeding-regulating peptide mRNAs. The expressions of the hypothalamic feeding-regulating peptides change commonly in different chronic cachectic models without relating to serum calcium levels.

Cachexia is characterized by weight loss involving massive depletion of adipose tissue and lean body mass. Nutritional supplementation cannot replenish the loss of lean body mass.[1,2] The severity of cachexia in disease states such as cancer, end-stage renal disease, rheumatoid arthritis (RA) and acquired immunodeficiency syndrome may be the primary determining factor in both the quality of life and eventual mortality.[3,4] Hypercalcemia is also a frequent paraneoplastic syndrome and represents an important factor affecting the morbidity and mortality of cancer patients.[5] The main cause of humoral hypercalcemia in malignancy (HHM) is the tumor production of parathyroid hormone-related protein (PTHrP) that stimulates osteoclastic resorption and renal reabsorption of calcium.[6]

The homeostasis of food intake and body weight is controlled by complex mechanisms. The hypothalamus receives and integrates the neural and humoral signals that inform energy status from peripheral tissues.[7] Appetite and feeding behaviors are primarily controlled by feeding centers in the lateral hypothalamic area (LHA), the satiety center in the ventromedial hypothalamic nucleus, the arcuate nucleus (Arc) and the paraventricular nucleus (PVN) in the hypothalamus.[8,9] Among the hypothalamic feeding-regulating peptides, neuropeptide Y (NPY) and agouti-related protein (AgRP) in the Arc are potent orexigenic neuropeptides. Under physiological conditions, the orexigenic hormone ghrelin increases energy intake by increasing NPY and AgRP neurons.[10] Plasma ghrelin levels in anorexia nervosa patients

Key words: cachexia, PTHrP, calcium, feeding-regulating peptides, hypothalamus
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are high and return to control levels after weight gain by renutrition.[11] On the other hand, anorexigenic hormone leptin, which derives mainly from fat tissue, decreases the activity of NPY and AgRP neurons and suppresses energy intake.[12] AgRP is an endogenous antagonist of the anorexigenic neuropeptide α-melanocyte-stimulating hormone (αMSH), which is derived from the proopiomelanocortin (POMC). AgRP promotes food intake *via* the inhibition of αMSH-stimulated signaling and antagonist of Type 4 central melanocortin receptors (MC4R).[13] Early studies in obese humans showed that leptin mRNA concentrations in adipose tissue and serum leptin concentrations correlated positively and closely with fat mass.[14] The leptin receptor is located in the hypothalamus as well as in some peripheral tissues. Injury to the hypothalamus can cause obesity, partly by destroying neurons that express the leptin receptor. In addition to NPY and AgRP, orexins in the LHA are also thought to participate in feeding regulation. Bolus injection of orexins to the rat lateral ventricle stimulated the food intake dose dependently, and orexin mRNA levels were upregulated on fasting.[15] Besides POMC, which blocks the autonomic, satiety and metabolic effects of leptin *via* the antagonism of MCRs,[16] cocaine- and amphetamine-regulated transcript (CART) in the Arc, which is also regulated by leptin, and corticotropin-releasing hormone (CRH) in the PVN function as anorexigenic neuropeptides.[8,17,18]

Previously, we showed that in animals carrying tumors secreting PTHrP, the levels of mRNA for orexigenic peptides were increased, whereas the levels of mRNA for anorexigenic peptides were decreased,[19–21] under cachectic conditions including HHM, reduced food intake and body weight loss. The administration of a humanized anti-PTHrP antibody raised against the NH_2-terminal 34 amino acids of the human PTHrP ($PTHrP_{1\text{-}34}$) rapidly improved the cachectic symptoms and also normalized the expression of NPY, AgRP, POMC, αMSH, CART and CRH mRNAs.[19–21] Although previous results suggested that HHM rats at least perceived starvation at the hypothalamus, there was no examination that those changes were observed especially in HHM rats secreting PTHrP or in other cachectic rats with or without tumors. There was also no examination of whether rats with hypercalcemia derive those changes.

In this study, we examined whether the increased mRNA expression of the hypothalamic orexigenic peptides and the decreased mRNA expression of the anorexigenic peptides in rats with cachexia are PTHrP-induced or rather general physiological responses under cachectic conditions. We also asked whether such expression of the hypothalamic feeding-regulating-peptides is related to hypercalcemia.

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Material and Methods

Drugs

The humanized anti-PTHrP antibody raised against the NH_2-terminal 34 amino acids of the human PTHrP ($PTHrP_{1\text{-}34}$; Ref. 12) was dissolved in saline. Indomethacin and $1\alpha,25(OH)_2D_3$ were purchased from Sigma-Aldrich (St. Louis, MO) and Calbiochem (San Diego, CA), respectively.

Cells and animal experiments

Group 1. PTHrP-secreting human lung cancer cell line LC-6-JCK originating from human large cell lung cancer was purchased from the Central Institute for Experimental Animals (Kawasaki, Japan). The cells were maintained *in vivo* in nude mice (BALB/cAnN Crj-nu/nu). Small pieces of tumor tissues (~10 mm^3) were subcutaneously (s.c.) implanted into 5-week-old male F344/N Jcl-rnu nude rats. Rats that displayed blood ionized calcium (iCa) levels higher than 1.8 mmol/L and at least 0.5 mmol/L higher than normal (control) rats were used as the HHM rats.[20–23] Nude mice and nude rats were purchased from Charles River Japan (Yokohama, Japan) and Clea Japan (Tokyo, Japan), respectively, and kept in sterilized cages. For treatment with an anti-PTHrP mAb, the rats were given 3 mg/kg of anti-PTHrP antibody intravenously (i.v.) on days 42 and 49 (HHM + vehicle: $n = 6$, HHM + antibody: $n = 6$, and normal: $n = 6$). The body weight of the normal and HHM rats was measured once a week, and iCa was determined on day 51 after implantation of the tumor. Blood was collected from the tail vein, and the concentration of iCa was measured using a Ca^{2+}/pH electrolyte analyzer (Bayer 634, Bayer Diagnostics, Sunbury, UK).

Group 2. A SEKI melanoma cell line which does not express PTHrP was established at the National Cancer Center, Tokyo, Japan.[24] Five-week-old male F344/N Jcl-rnu nude rats were s.c. implanted in the right flank with 1×10^7 of SEKI cells; the rats displayed weight loss without PTHrP secretion or hypercalcemia after implantation of the cells.[25] Body weights (SEKI: $n = 6$, and nontumor-bearing rats: $n = 6$) were measured once a week, and iCa was measured on day 59.

Group 3. Adjuvant-induced arthritic rat (AA) were also used as a cachectic model.[26–29] To induce AA, 8-week-old male Wistar rats (Kyudo Co., Saga, Japan) were intracutaneously (i.c.) injected with 1 mg of heat-killed *Mycobacterium butyricum* (Difco Laboratories, Detroit, MI) in paraffin liquid at the base of the tail. The AA rats were divided into 2 groups: one was orally administered 1 mg/kg of indomethacin in a 0.5 mL suspension of 0.5% methylcellulose daily from day 15 to day 21, and the others was not treated (AA: $n = 6$, AA + indomethacin: $n = 6$, and control: $n = 6$). Body weight and arthritis index were measured every day. The arthritis index was scored by grading each paw from 0 to 4, based on erythema, swelling and deformity of the joints.[28,30]

Group 4. To create nontumor-bearing hypercalcemic rats, 13-week-old male F344/N Jcl-rnu nude rats were orally administered 10 μg/kg of $1\alpha,25(OH)_2D_3$ (active vitamin D3) for three consecutive days.

The animals used for *in situ* hybridization histochemistry were decapitated on day 51 (Group 1), day 59 (Group 2),

day 22 (Group 3) and day 3 (Group 4). The brains were rapidly removed, placed on a glass plate on dry ice and stored at −80°C until use. Trunk blood was collected and the plasma concentration of iCa was measured on the same as above and plasma concentrations of leptin were measured using an ELISA kit (YK051 Rat Leptin-HS, Yanaihara Institute, Shizuoka, Japan). The animals used in the experiment were treated in accordance with the ethical guidelines for animal care, handling and termination promulgated by the Chugai Pharmaceutical Co. (Tokyo, Japan).

In situ hybridization histochemistry

Frozen 12-μm-thick coronal brain sections were prepared in a cryostat at −20°C, thawed, and mounted onto gelatin/chrome alum-coated slides. The PVN, Arc and LHA were determined according to coordinates given by the atlas of Paxinos and Watson.[31] The localization of sections from each rat was checked by microscopic observation. Two sections containing the PVN (plate 24; Ref. 29) and four sections containing the Arc (plate 27 and 28; Ref. 29) and LHA (plate 28; Ref. 29) were used from each rat to measure the density of the autoradiography. *In situ* hybridization was performed as previously described.[32] Hybridization was carried out under a Nescofilm coverslip (Bando Chemical IMD, Osaka, Japan). [^{35}S]3′-end-labeled deoxyoligonucleotides complementary to transcripts coding for NPY (5′-GGA GTA GTA TCT GGC CAT GTC CTC TGC TGG CGC GTC-3′), AgRP (5′-CGA CGC GGA GAA CGA GAC TCG CGG TTC TGT GGA TCT AGC ACC TCT GCC-3′), POMC (5′-CTT CTT GCC CAG CGG CTT GCC CCA GCA GAA GTG CTC CAT GGA CTA GGA-3′), CART (5′-TGG GGA CTT GGC CGT ACT TCT TCT CAT AGA TCG GAA TGC-3′), orexin (5′-TTC GTA GAG ACG GCA GGA ACA CGT CTT CTG GCG ACA-3′) and CRH (5′-CAG TTT CCT GTT GCT GTG AGC TTG CTG AGC TAA CTG CTC TGC CCT GGC-3′) were used as the specific probes. The specificity of the probes was described previously.[19,33–36] Total counts of 6 × 10^5 cpm/slide for NPY, AgPR, POMC, CART and CRH and 4 × 10^5 cpm/slide for orexin were used. Hybridized sections containing the Arc, the LHA and the PVN were exposed to autoradiography film (Hyperfilm; Amersham, Buckinghamshire, UK) for 4 days for orexin and 7 days for NYP, AgRP, POMC, CART and CRH. The autoradiographic images were quantified using an MCID imaging analyzer (Imaging Research, St. Catherines, ON, Canada). The images were captured by a charge-coupled device camera (Dage-MTI, Michigan City, IN) at 40× magnification. The mean absorbance of the autoradiographs was measured and compared with simultaneously exposed ^{14}C microscale samples (Amersham). The standard curve was fitted by the absorbance of the ^{14}C microscale on the same film.

Statistical analysis

All data are given as mean ± SE calculated from the results of the *in situ* hybridization histochemistry. The results of each experimental animal group were compared with those of the control group. The data were analyzed using a one-way factorial ANOVA followed by a Bonferroni correction for multiple comparisons. The changes in body weight, iCa and arthritis index were also statistically analyzed using one-way ANOVA followed by a Bonferroni correction for multiple comparisons. Statistical significance was defined as $p < 0.05$.

Results

Body weight, arthritis index, iCa and plasma leptin in Normal rats, HHM rats, SEKI rats, AA rats and $1\alpha,25(OH)_2D_3$ rats

Consistent with previous results,[19,20,22,23] the body weight of the HHM rats bearing PTHrP secreting LC-6 significantly decreased after day 30, but significantly increased after the HHM rats were given the anti-PTHrP antibody (Fig. 1*a*). The body weights of the SEKI rats and the AA rats also decreased after day 30 and after day 13, respectively, but significantly increased in the AA rats after they were given indomethacin (Figs. 1*b* and 1*c*). The arthritis index in the AA rats increased sharply from day 10 to 19 and remained the same until day 22, but significantly decreased after they were given indomethacin (Fig. 1*d*). The rats treated with $1\alpha,25(OH)_2D_3$ had a significant decrease in body weight accompanied by an elevation of iCa (Figs. 1*e* and 1*f*). The levels of iCa in the HHM rats were significantly higher than in the normal rats, but it decreased when the HHM rats received the anti-PTHrP antibody (Fig. 1*f*). On the other hand, the SEKI rats had no change in the levels of iCa, though they experienced body weight loss (Figs. 1*b* and 1*f*), and neither did the AA rats (Figs. 1*c* and 1*f*).

Concentration in plasma leptin in HHM rats were significantly lower than that in nontumor-bearing rats and also lower in SEKI and AA rats. Administration of anti-PTHrP antibody to HHM rats increased the plasma leptin level, but it was still lower than that of the nontumor-bearing rats. On the other hand, vitamin D treated rats did not change the plasma leptin concentration (Fig. 2).

Expression of hypothalamic peptides: orexigenic peptides genes in HHM, SEKI, AA and $1\alpha,25(OH)_2D_3$ rats

After the HHM rats developed cachexia, the levels of orexigenic peptide mRNAs, NPY and AgRP in the Arc were significantly higher than in normal rats. Administration of the anti-PTHrP antibody to the HHM rats showed reduced levels of NPY and AgRP mRNAs compared with levels in the untreated HHM rats (Figs. 3*a*–3*d*). Although neither the SEKI rats nor the AA rats showed elevated levels of blood iCa (Fig. 1*f*), similar changes in the mRNA expression of orexigenic peptides were observed in both rat models; the orexigenic peptide mRNAs, such as NPY and AgRP in the Arc, became higher (Figs. 3*a*–3*d*). Administration of indomethacin to the AA rats not only improved their body weight and arthritis index but also restored the mRNA levels

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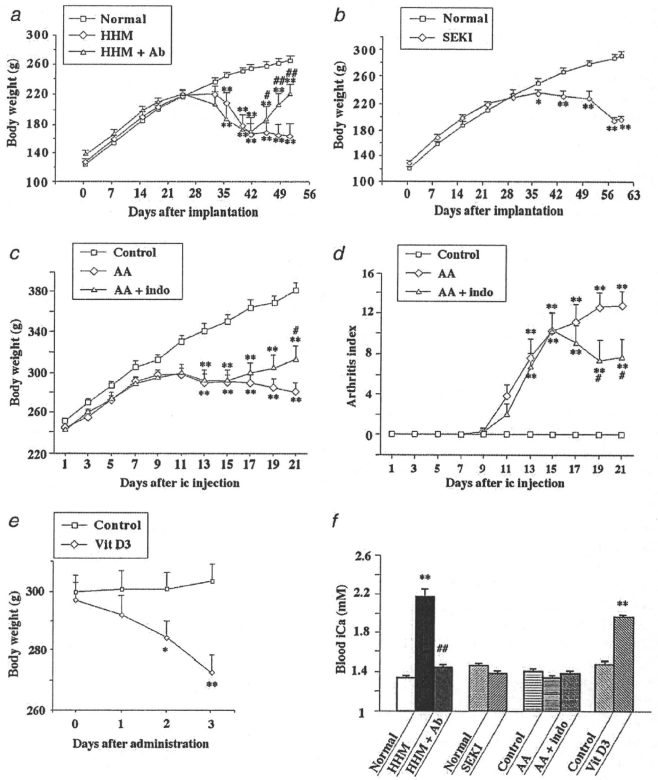

Figure 1. Time course of changes in body weight in Group 1 containing nontumor-bearing rats (Normal), humoral hypercalcemia of malignancy rats (HHM) and HHM rats intravenously (i.v.) injected with anti-PTHrP antibody (HHM + Ab) (*a*), Group 2 containing nontumor-bearing rats (Normal) and SEKI rats (SEKI) (*b*) and Group 3 containing rats injected with vehicle (Control), adjuvant-induced arthritis rats treated with vehicle (AA), and AA rats perorally (po) treated with indomethacin (AA + indo) (*c*). Changes in the arthritis index in Group 3 (*d*). Time course of changes in body weight in Group 4 containing rats treated with vehicle (Control) and rats orally treated with 10 μg/kg of $1\alpha,25(OH)_2D_3$ (Vit. D3) daily from day 0 to 3 (*e*), and changes in iCa of the rats in Group 1 to Group 4 (*f*). For treatment with or without an anti-PTHrP antibody, the rats were given 3 mg/kg of anti-PTHrP antibody or saline i.v. on days 42 and 49 in Group 1. AA rats in Group 3 were not treated or orally administered 1 mg/kg of indomethacin daily from days 15–21. Data points, mean ($n = 6$); bars, SE. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with each control. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with HHM rats or AA rats.

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of the hypothalamic feeding peptides. The level of orexin mRNA in the LHA did not change even under cachectic conditions in the HHM rats, but decreased in the SEKI rats and AA rats when they developed cachexia and, in the AA rats, was not increased by administration of indomethacin (Figs. 3*e* and 3*f*).

Because HHM rats bearing LC-6 concurrently develop cachexia and hypercalcemia, there is a possibility that serum

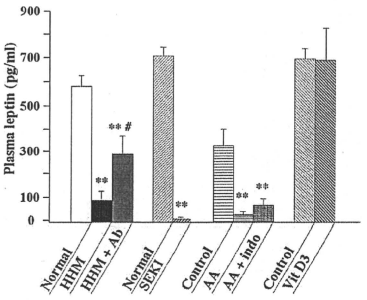

Figure 2. The changes of plasma leptin concentration in Group 1 containing nontumor-bearing rats (Normal), humoral hypercalcemia of malignancy rats (HHM) and HHM rats intravenously (i.v.) injected with anti-PTHrP antibody (HHM + Ab), Group 2 containing nontumor-bearing rats (Normal) and SEKI rats (SEKI), Group 3 containing rats injected with vehicle (Control), adjuvant-induced arthritis rats treated with vehicle (AA) and AA rats perorally (po) treated with indomethacin (AA + indo) and Group 4 containing rats treated with vehicle (Control) and rats orally treated with $1\alpha,25(OH)_2D_3$ (Vit. D3). Data points, mean ($n = 6$); bars, SE. $^{**}p < 0.01$ compared with each control. $^{\#}p < 0.05$ compared with HHM rats.

calcium is also involved in the changes in the expression of hypothalamic feeding-regulating peptides. The SEKI and AA rats showed an increased expression of the orexygenic peptide without an increase in serum iCa. This indicates that changes in the expression of hypothalamic feeding-regulating peptides are not related to an increase in serum calcium. To further confirm the relationship between hypercalcemia and the expression of hypothalamic feeding-regulating peptides, rats were administered a high dose of $1\alpha,25(OH)_2D_3$ to induce hypercalcemia. In the $1\alpha,25(OH)_2D_3$ rats, the serum levels of iCa increased as body weight decreased, but the levels of NPY, AgRP and orexin mRNA did not change significantly.

Expression of hypothalamic peptides: anorexigenic peptides genes in HHM, SEKI, AA and $1\alpha,25(OH)_2D_3$ rats

POMC and CART in the Arc and CRH in the PVN became lower after the HHM rats developed cachexia (Fig. 4). The administration of the anti-PTHrP antibody restored not only their body weight but also restored the mRNA expression of the POMC, CART and CRH.

The SEKI rats and the AA rats also showed similar changes in the mRNA expression of anorexigenic peptides; the mRNA levels of POMC and CART in the Arc and CRH in the PVN were lower after they developed cachexia. On the other hand, $1\alpha,25(OH)_2D_3$ rats did not experience a change in the levels of POMC, CART and CRH mRNAs similar to orexigenic peptides such as NPY, AgRP and orexin mRNAs. Taken together, the results demonstrate that the upregulation of orexigenic peptides and downregulation of anorexigenic peptides are not specific to PTHrP-induced cachexia but rather to other physiological responses under cachectic conditions. Furthermore, such changes in the expression of hypothalamic feeding-regulating peptides occur independently of hypercalcemia.

Discussion

Tumor and host tissues containing macrophages often secrete proinflammatory cytokines and elevated levels of cytokines have been thought to directly or indirectly transmit signals to the hypothalamus, repressing the feeding center and activating the satiety center. In fact, it was reported that macrophage inhibitory cytokine-1 (MIC-1), which causes cachexia in cancer and renal disorders, binds to TGFβ Type II receptors and downregulates NPY and upregulates POMC.[37] In addition, MIC-1 and leptin have similar effects on the expression of hypothalamic feeding peptides that act at different sites of the hypothalamus.

However, in this study, the expression of orexigenic peptide mRNAs was upregulated and anorexigenic peptide mRNAs expression was downregulated in several cachectic models. Particularly, nontumor-bearing cachectic AA rats revealed the same mRNA changes in the hypothalamus as did HHM and SEKI rats. In addition to the observation that those mRNA changes were restored in HHM rats after they were treated with an anti-PTHrP antibody, the AA rats treated with indomethacin, which suppressed the synthesis of prostaglandins, had a partial restoration of those mRNA changes accompanied by a decrease of the arthritis index. It is possible that under cachectic conditions, the feeding center is activated and the satiety center is repressed, and yet, inflammatory cytokines, hormones and bioactive substances affect the orexigenic and anorexigenic peptide mRNA expression downstream of the feeding and satiety centers. In addition, we have previously reported that the body weight loss in HHM rats was accompanied by reduced amounts of muscle as well as fat.[23] Indeed, the concentration of plasma leptin in the HHM rats was significantly lower and administration of the anti-PTHrP antibody increased the plasma leptin level. A recessive mutation in the mouse *ob* genes results in obesity, and the *ob* gene encodes a hormone leptin that is expressed in adipose tissue.[38] Leptin regulates energy balance in part by suppressing NPY neurons and activating POMC neurons in the Arc[39] and it would be possible that leptin deficiency partly affects the changes of the gene expression in this study.

Although the mechanisms that explain the differences in the expression of hypothalamic feeding-regulating peptides between MIC-1-induced cachexia and our models remain to be elucidated, one possibility is that downstream processes of

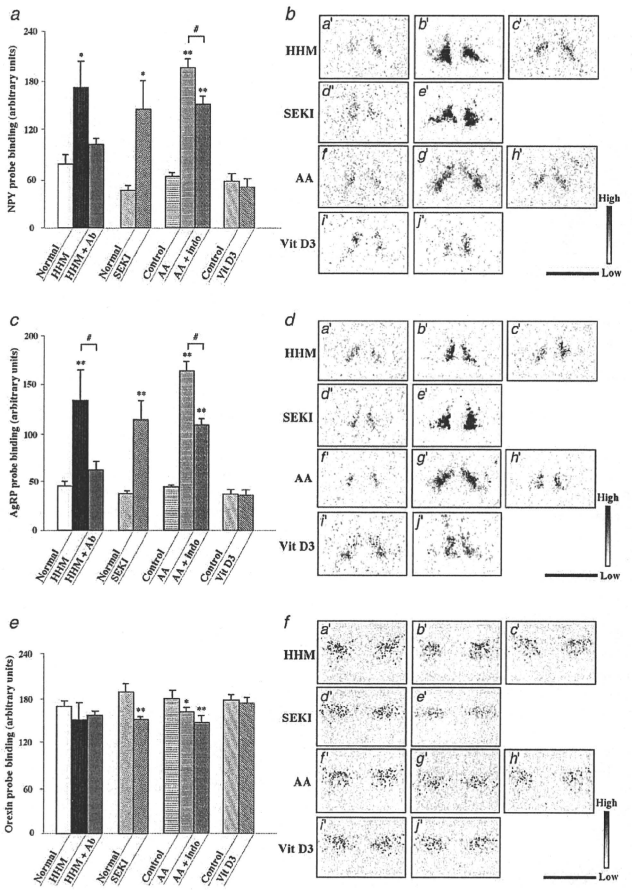

Figure 3. Expression of mRNA for neuropeptide Y (NPY) (*a* and *b*), agouti-related protein (AgRP) (*c* and *d*) in the arcuate nucleus (Arc) and orexin (*e* and *f*) in the lateral hypothalamic area (LHA) of Group 1 containing nontumor-bearing rats (Normal; *b-a'*, *d-a'*, *f-a'*), HHM rats (HHM; *b-b'*, *d-b'*, *f-b'*) and HHM rats injected with anti-PTHrP antibody (HHM + Ab; *b-c'*, *d-c'*, *f-c'*), Group 2 containing nontumor-bearing rats (Normal; *b-d'*, *d-d'*, *f-d'*) and SEKI rats (SEKI; *b-e'*, *d-e'*, *f-e'*), Group 3 containing rats injected with vehicle (Control; *b-f'*, *d-f'*, *f-f'*), adjuvant-induced arthritis rats treated with vehicle (AA; *b-g'*, *d-g'*, *f-g'*), and AA rats treated with indomethacin (AA + indo; *b-h'*, *d-h'*, *f-h'*) and Group 4 containing rats treated with vehicle (Control; *b-i'*, *d-i'*, *f-i'*) and rats treated with $1\alpha,25(OH)_2D_3$ (Vit. D3; *b-j'*, *d-j'*, *f-j'*). Representative autoradiographs of sections hybridized by a ^{35}S-labeled oligodeoxynucleotide probe complementary to mRNA for NPY (*b-a'*-*j'*), AgRP (*d-a'*-*j'*) and orexin (*f-a'*-*j'*). Signal intensity ranges from high (black) to low (white). Bar, 1 mm. Columns, mean ($n = 6$); bars, SE. *$p < 0.05$ and **$p < 0.01$ compared with each control. $^{\#}p < 0.05$ compared with HHM rats or AA rats.

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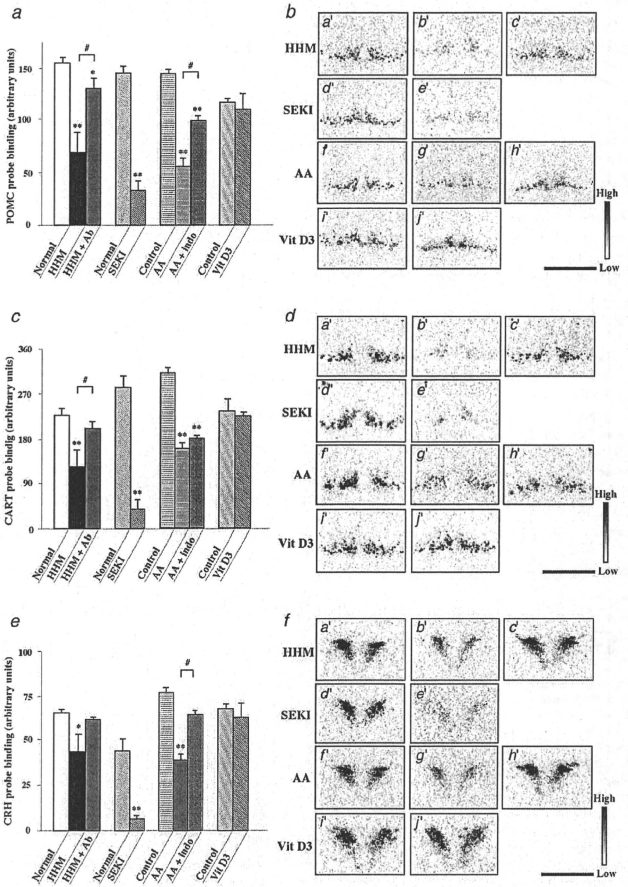

Figure 4. Expression of mRNA for proopiomelanocortin (POMC) (*a* and *b*) and cocaine-and amphetamine-regulated transcript (CART) (*c* and *d*) in the arcuate nucleus (Arc) and corticotropin-releasing hormone (CRH) (*e* and *f*) in the paraventricular nucleus (PVN) of Group 1 containing nontumor-bearing rats (Normal; *b-a'*, *d-a'*, *f-a'*), HHM rats (HHM; *b-b'*, *d-b'*, *f-b'*) and HHM rats injected with anti-PTHrP antibody (HHM + Ab; *b-c'*, *d-c'*, *f-c'*), Group 2 containing nontumor-bearing rats (Normal; *b-d'*, *d-d'*, *f-d'*) and SEKI rats (SEKI; *b-e'*, *d-e'*, *f-e'*), Group 3 containing rats injected with vehicle (Control; *b-f'*, *d-f'*, *f-f'*), adjuvant-induced arthritis rats treated with vehicle (AA; *b-g'*, *d-g'*, *f-g'*) and AA rats treated with indomethacin (AA + indo; *b-h'*, *d-h'*, *f-h'*) and Group 4 containing rats treated with vehicle (Control; *b-i'*, *d-i'*, *f-i'*) and rats treated with $1\alpha,25(OH)_2D_3$ (Vit. D3; *b-j'*, *d-j'*, *f-j'*). Representative autoradiographs of sections hybridized by a ^{35}S-labeled oligodeoxynucleotide probe complementary to mRNA for POMC (*b-a'-j'*), CART (*d-a'-j'*) and CRH (*f-a'-j'*). Bar, 1 mm. Columns, mean ($n = 6$); bars, SE. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with each control. $^{\#}p < 0.05$ compared with HHM rats or AA rats.

NPY action are affected by humoral factors such as cytokines and tumor-derived factors.[40] In fact, intracerebroventricular injection of TNF-α reportedly increased the NPY mRNA level in the hypothalamus but reduced food intake,[41] and proinflammatory signals decreased the secretion of AgRP but increased the transcription of the AgRP gene.[42]

On the other hand, the rats treated with $1\alpha,25(OH)_2D_3$ had reduced body weight without changes in feeding-regulating peptide gene expression. One possible explanation is that changes of metabolic rates and locomotor activity related to $1\alpha,25(OH)_2D_3$-induced hypercalcemia may be involved in the decrease of body weight without affecting feeding. Another possible explanation is that body fluid balance related to drinking and urine volume will change and cause dehydration. The reason why body weight was reduced after $1\alpha,25(OH)_2D_3$ treatment without affecting the feeding-regulating peptide genes should be clarified by further study.

Previously, using HHM rats treated with anti-PTHrP antibody, we have demonstrated: (*i*) body weight gain accompanied by restoration of locomotor activity and food and water intake, (*ii*) restoration of plasma calcium levels and (*iii*) restoration of feeding-regulating peptide genes.[19,23] It could be possible that proinflammatory cytokines such as IL-1, IL-6 and TNFα are responsible for the changes in feeding-regulating peptide gene expression, to be sure, but PTHrP might also be responsible for those changes. In this study, there were no effects of hypercalcemia induced by $1\alpha,25(OH)_2D_3$ treatment on the hypothalamic feeding-regulating peptide gene expression. Consequently, not PTHrP-induced hypercalcemia but hormonal effects of PTHrP might have brought about the changes in feeding-regulating peptide gene expression.

In the HHM rats, the level of orexin mRNA that enhanced feeding was not significantly increased but rather decreased in the LHA. Because orexin is involved not only in feeding behavior but also in sleep regulation and narcolepsy,[43–45] orexin expression may be regulated in a more complex manner. Especially, orexin increases the proportion of time spent awake through projecting fibers for the locus coeruleus that is a key modulator of attentional state.[46] Previously, Onuma *et al.* reported that there was an approximately double increase in the locomotor activity of the HHM rats after they received the anti-PTHrP antibody.[23] Consequently, in tumor-bearing cachextic rats and AA rats it can be presumed that locomotor activity and waking state are reduced and result in reduced orexin gene expression. Further studies are necessary both to clearly understand the mechanisms of orexigenic and anorexigenic peptide regulations in response to cachectic conditions and the mechanisms by which orexigenic and anorexigenic peptide regulations could cause the cachectic conditions.

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