3) Grade 3-4 diarrhea

Dose escalations for erlotinib are not allowed after a dose reduction. Erlotinib is terminated if either of the following adverse events occurs.

1) Grade 1 to 4 pulmonary fibrosis

2) Grade 4 non-hematologic toxicity other than pulmonary fibrosis

3) Delay from prior administration over 23 days

The protocol treatment is terminated if the disease progresses, serious adverse events occurs or at the patient's refusal. There is no restriction of maximum number of cycles. There is no restriction of treatment after failure of the protocol treatment.

Endpoints

The primary endpoint is ORR. The secondary endpoints are PFS, OS, disease control rate (DCR) and incidence of adverse events. Patients undergo tumor assessments at baseline and every six weeks by investigators using Response Evaluation Criteria in Solid Tumors version 1.1 [20]. ORR and DCR are defined by the proportion of complete response (CR) and partial response (PR), or the proportion of CR, PR and stable disease (SD), in confirmed best overall response at the time of the primary analysis. OS is defined as the time from registration to death from any cause, and it is censored at the last contact date for living patient. PFS is defined as the time from registration to either the first event of progression of disease or death from any cause, and it is censored at the last date when patient is alive without progression. Adverse events are evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 [21].

Data collection

Patients are followed-up for three months after registration. Schedule of data collection are summarised in Table 1. Radiographic data for tumor assessments is collected every six weeks.

Sample size determination

Simon's minimax two-stage design employing a binomial distribution is used to calculate the required sample size. In the previous trials, the ORR of erlotinib monotherapy ranged 8.9 to 28.3% [9,10]. Thus we consider that an ORR of 20% indicates no value of further investigation of the combination. This trial plans to accrue 42 patients in the first stage and 80 patients in total, which provides 90% power with an alternative hypothesis ORR of 35% and a null hypothesis ORR of 20% using one-sided testing at a 5% significance level.

Statistical consideration

The analysis population for efficacy is the full analysis set. The primary analysis for efficacy is a one-sided binomial test with the null hypothesis of 20% at a 5% significance level in the second stage. A subset analysis according to EGFR mutation status (direct sequence or PNA-LNA PCR clamp methods) is also planned. ORRs with 95% confidence intervals are calculated in the subsets of mutant and wild type, and compared with 20% using the same

Table 1 Schedule of data collection

	Baseline	Under treatment	At termination of treatment	After termination of treatment
Physical examination				
Height	o			
Weight, performance status	o	o	o	
Blood pressure	o	o	o	o
Laboratory test				
Blood count	o	o		
Biochemistry test	o	o		
Urine test	o	o		
SpO_2	o	o		o*1
Electrocardiography	o	o*1		
EGFR gene	o			
Radiology test				
Chest Xp	o	o*1		o*1
Chest CT	o	o*2		o*3
Abdominal CT/Ultra sonography	o	o*2		o*3
Head CT/MRI	o	o*1		o*1
Bone scintigraphy/PET	o	o*1		o*1

*1 If necessary

*2 Every 6 weeks

*3 Six weeks after termination if treatment is terminated for reasons other than progression of disease

binomial test at a 5% significance level separately. Multiplicity is not adjusted for since this is a secondary analysis.

Discussion

We have presented the design of a single arm phase II trial to evaluate the efficacy and safety of combination of bevacizumab and erlotinib in advanced non-squamous NSCLC patients. In particular we are interested in determining the merit of further development of this regimen and whether prospective patient selection using EGFR gene is necessary in future trials.

List of abbreviations used

CR: complete response; CTCAE: the Common Terminology Criteria for Adverse Events; NSCLC: non-small-cell lung cancer; e-CRF: electronic case report form; EGFR-TKI: epidermal growth factor receptor tyrosine kinase inhibitor; ORR: objective response rate; OS: overall survival; PR: partial response; PFS: progression-free survival; SD: stable disease; VEGF: vascular endothelial growth factor.

Acknowledgements and Funding

The authors would like to thank the staff in participating institutes for their support in recruiting eligible patients and the patients who participated in this study.

Author details

[1]Department of Clinical Trial Design and Management, Translational Research Center, Kyoto University Hospital, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan. [2]Department of Respiratory Medicine, Graduate School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Kyoto University, 606-8507, Japan. [3]Outpatient Oncology Unit, Kyoto University Hospital, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan. [4]Department of Translational Clinical Oncology, Graduate School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Kyoto University, 606-8507, Japan.

Authors' contributions

KY conceived of the trial. KY, MH, YS, ST and MN designed the trial. MH searched the literature and drafted the protocol. MN supervised the data management and patient registration. ST is responsible for statistical analysis. ST wrote the final manuscript. All authors have read and approved the final manuscript.

Competing interests

KY received research funding from Taiho Pharmaceutical and Chugai Pharmaceutical. The other authors declare no competing interests.

Received: 14 February 2011 Accepted: 12 May 2011
Published: 12 May 2011

References

1. Wozniak AJ, Crowley JJ, Balcerzak SP, Weiss GR, Spiridonidis CH, Baker LH, Albain KS, Kelly K, Taylor SA, Gandara DR, Livingston RB: **Randomized trial comparing cisplatin with cisplatin plus vinorelbine in the treatment of advanced non-small-cell lung cancer: a Southwest Oncology Group study.** *J Clin Oncol* 1998, **16**:2459-2465.
2. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J, Johnson DH, Eastern Cooperative Oncology Group: **Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer.** *N Engl J Med* 2002, **346**:92-98.
3. Edelman MJ, Clark JI, Chansky K, Albain K, Bhoopalam N, Weiss GR, Giguere JK, Kelly K, Crowley J, Gandara DR: **Randomized phase II trial of sequential chemotherapy in advanced non-small cell lung cancer (SWOG 9806): carboplatin/gemcitabine followed by paclitaxel or cisplatin/vinorelbine followed by docetaxel.** *Clin Cancer Res* 2004, **10**:5022-5026.
4. Yasuda H, Yamaya M, Nakayama K, Sasaki T, Ebihara S, Kanda A, Asada M, Inoue D, Suzuki T, Okazaki T, Takahashi H, Yoshida M, Kaneta T, Ishizawa K, Yamanda S, Tomita N, Yamasaki M, Kikuchi A, Kubo H, Sasaki H: **Randomized phase II trial comparing nitroglycerin plus vinorelbine and cisplatin with vinorelbine and cisplatin alone in previously untreated stage IIIB/IV non-small-cell lung cancer.** *J Clin Oncol* 2006, **24**:688-694.
5. Shepherd FA, Dancey J, Ramlau R, Mattson K, Gralla R, O'Rourke M, Levitan N, Gressot L, Vincent M, Burkes R, Coughlin S, Kim Y, Berille J: **Prospective randomized trial of docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy.** *J Clin Oncol* 2000, **18**:2095-2103.
6. Fossella FV, DeVore R, Kerr RN, Crawford J, Natale RR, Dunphy F, Kalman L, Miller V, Lee JS, Moore M, Gandara D, Karp D, Vokes E, Kris M, Kim Y, Gamza F, Hammershaimb L: **Randomized phase III trial of docetaxel versus vinorelbine or ifosfamide in patients with advanced non-small-cell lung cancer previously treated with platinum-containing chemotherapy regimens. The TAX 320 Non-Small Cell Lung Cancer Study Group.** *J Clin Oncol* 2000, **18**:2354-2362.
7. Pfister DG, Johnson DH, Azzoli CG, Sause W, Smith TJ, Baker S Jr, Olak J, Stover D, Strawn JR, Turrisi AT, Somerfield MR, American Society of Clinical Oncology: **American Society of Clinical Oncology treatment of unresectable non-small-cell lung cancer guideline: update 2003.** *J Clin Oncol* 2004, **22**:330-353.
8. Hanna N, Shepherd FA, Fossella FV, Pereira JR, De Marinis F, von Pawel J, Gatzemeier U, Tsao TC, Pless M, Muller T, Lim HL, Desch C, Szondy K, Gervais R, Shaharyar , Manegold C, Paul S, Paoletti P, Einhorn L, Bunn PA Jr: **Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy.** *J Clin Oncol* 2004, **22**:1589-1597.
9. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, Campos D, Maoleekoonpiroj S, Smylie M, Martins R, van Kooten M, Dediu M, Findlay B, Tu D, Johnston D, Bezjak A, Clark G, Santabárbara P, Seymour L, National Cancer Institute of Canada Clinical Trials Group: **Erlotinib in previously treated non-small-cell lung cancer.** *N Engl J Med* 2005, **353(2)**:123-132.
10. Kubota K, Nishiwaki Y, Tamura T, Nakagawa K, Matsui K, Watanabe K, Hida T, Kawahara M, Katakami N, Takeda K, Yokoyama A, Noda K, Fukuoka M, Saijo N: **Efficacy and safety of erlotinib monotherapy for Japanese patients with advanced non-small cell lung cancer: a phase II study.** *J Thorac Oncol* 2008, **3(12)**:1439-1445.
11. National Comprehensive Cancer Network: **Non-small cell lung cancer.** *NCCN Clinical Practice Guidelines in Oncology* , v.2 2010.
12. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilenbaum R, Johnson DH: **Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer.** *N Engl J Med* 2006, **355(24)**:2542-2550.
13. Reck M, von Pawel J, Zatloukal P, Ramlau R, Gorbounova V, Hirsh V, Leighl N, Mezger J, Archer V, Moore N, Manegold C: **Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAil.** *J Clin Oncol* 2009, **27(8)**:1227-1234.
14. Nishio M, Horai T, Kunitoh H, Ichinose Y, Nishiwaki Y, Hida T, Yamamoto N, Kawahara M, Saijo N, Fukuoka M, JO19907 Study Group: **Randomized, open-label, multicenter phase II study of bevacizumab in combination with carboplatin and paclitaxel in chemotherapy-naive Japanese patients with advanced or recurrent nonsquamous non-small cell lung cancer (NSCLC): JO19907.** *J Clin Oncol* 2009, **27(15S)**:8036.
15. Herbst RS, O'Neill VJ, Fehrenbacher L, Belani CP, Bonomi PD, Hart L, Melnyk O, Ramies D, Lin M, Sandler A: **Phase II study of efficacy and safety of bevacizumab in combination with chemotherapy or erlotinib compared with chemotherapy alone for treatment of recurrent or refractory non small-cell lung cancer.** *J Clin Oncol* 2007, **25(30)**:4743-4750.
16. The World Medical Association: *WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects* [http://www.wma.net/en/30publications/10policies/b3/17c.pdf], accessed on December 16, 2010.
17. The Ministry of Health, Labor, and Welfare: *Ethics Guidelines for Clinical Research* [http://www.mhlw.go.jp/general/seido/kousei/i-kenkyu/index.html], accessed on December 16, 2010.
18. UICC International Union Against Cancer: *TNM classification of malignant tumours*. 7 edition. Wiley-Blackwell, New York; 2009.

19. Herbst RS, Johnson DH, Mininberg E, Carbone DP, Henderson T, Kim ES, Blumenschein G Jr, Lee JJ, Liu DD, Truong MT, Hong WK, Tran H, Tsao A, Xie D, Ramies DA, Mass R, Seshagiri S, Eberhard DA, Kelley SK, Sandler A: **Phase I/II trial evaluating the anti-vascular endothelial growth factor monoclonal antibody bevacizumab in combination with the HER-1/ epidermal growth factor receptor tyrosine kinase inhibitor erlotinib for patients with recurrent non-small-cell lung cancer.** *J Clin Oncol* 2005, **23(11)**:2544-2555.
20. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG: **New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada.** *J Natl Cancer Inst* 2000, **92(3)**:205-216.
21. The Japanese Clinical Oncology Group: *Japanese version of the Common Terminology Criteria for Adverse Events version 4.0* [http://www.jcog.jp/doctor/tool/CTCAEv4J_20100201.pdf], accessed on December 16, 2010.

doi:10.1186/1745-6215-12-120
Cite this article as: Tanaka *et al.*: Design paper: A phase II study of Bevacizumab and Erlotinib in patients with non-Squamous non-small cell lung cancer that is refractory or relapsed after 1-2 previous Treatment (BEST). *Trials* 2011 12:120.

特集：臨床研究実施の現状と課題

第1部　医師主導臨床試験の推進：各大学の臨床試験支援体制

京都大学病院探索医療センターにおける臨床試験サポート体制

京都大学医学部附属病院探索医療センター探索医療開発部[1)]　同探索医療検証部[2)]
伊藤達也[1)]　新美三由紀[2)]

はじめに

わが国では，基礎研究から臨床応用への橋渡し（以下，トランスレーショナルリサーチ）は新規医薬品や適応外医薬品を用いてヒトへ使用するため，臨床応用研究（以下，臨床試験）の実施の際には守るべき指針や準備必要項目などの規制に従う必要がある。一方で，再生医療のなかで代表される細胞製剤などは技術開発のスピードが速く，技術と並行してガイドラインや法律も整備中であり，臨床試験を実施する環境はこれまでになく複雑である。また，質の高い臨床試験を実施するためにはデータマネジメントや統計解析などで質を担保する必要があり，診療業務を抱える医師を中心とした少人数のグループで質の高い臨床試験を実施することは簡単なことではない。近年，大学や医療機関内などに臨床試験を支援する拠点が設立され，質の高い臨床試験やトランスレーショナルリサーチの実施のための支援体制整備が進められている。本稿では，京都大医学部附属病院探索医療センターにおけるトランスレーショナルリサーチのサポート体制について紹介する。

1　京都大学病院探索医療センターのミッション

京都大学医学部附属病院探索医療センター（以下，当センター）は，わが国初のトランスレーショナルリサーチ（TR）を支援する基盤組織のひとつとして2001年に設立された。当センターは，出口を見据えた開発研究と，科学的にも倫理的にも正当性が高く，質の高い臨床試験，Proof of Concept（POC：概念実証）取得研究の円滑な実施を支援しており，2007年より文部科学省橋渡し研究支援推進プログラムに採択されている。これまで学内外の多くの臨床試験を支援し，特に国内外未承認医薬品や医療機器の医師主導治験を4つ実施した実績がある。

特に探索型研究を進めることを掲げ，基礎研究で発見されたものを早く臨床応用することが目標である。そのため，当センターでは既存の枠を越えた横断的に研究を進めるため，研究者の基礎研究も支援を行い，臨床支援スタッフとの連携により臨床応用も可能とする。若手研究者のオリジナリティや発想を育成し，新たなシーズを発掘する全国拠点を目指している。われわれのねらう臨床開発は，フェーズの浅い段階における探索的治験，厚生労働省の制度下で行う先進的な臨床試験など，POCを取得することや次に臨床展開できるためのエビデンスを立証することである。

2　京都大学病院探索医療センターの体制

支援組織は，探索医療開発部，探索医療検証部，探索医療臨床部の3部より構成され（**図1**），「探索医療開発部」では試験計画書や概要書の作成，規制当局との折衝，臨床試験文書の管理・保管，および試験の進捗管理などを，「探索医療検証部」では試験

Management and Support of Investigator-Initiated Clinical Trials in Translational Research Center, Kyoto University Hospital
Tatsuya Ito：Department of Experimental Therapeutics, Translational Research Center, Kyoto University Hospital；Miyuki Niimi：Department of Clinical Trial Design & Management, Translational Research Center, Kyoto University Hospital

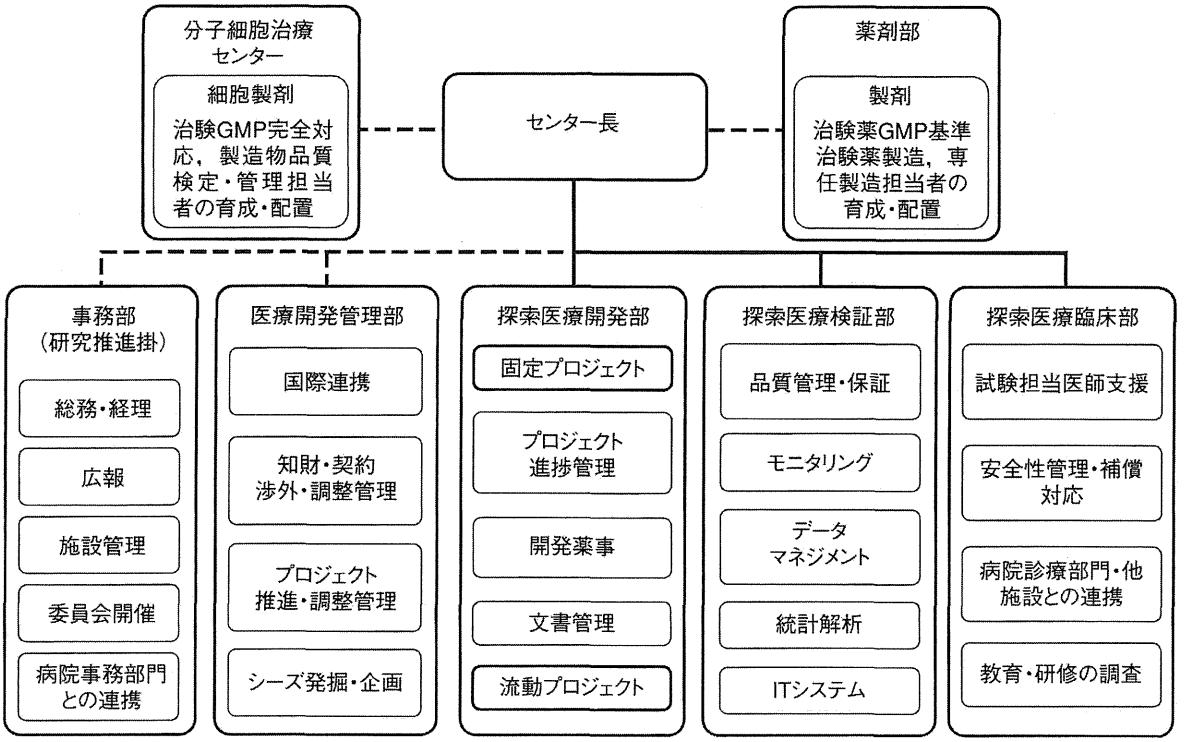

図 1　トランスレーショナルリサーチ（TR）の組織

計画策定時からの統計学的支援，試験データの品質保証とデータ解析を，「探索医療臨床部」では診療科や研究者との連携・調整，被験者の保護，安全性情報などを担当している。さらに TR を推進する 3 つの病院部門，すなわち，知的財産・契約・渉外の調整管理などを担当する「医療開発管理部」，GMP 基準の細胞製剤の調製や品質管理を担当する「分子細胞治療センター」，治験薬管理や治験薬（治験薬 GMP 基準）製造を担当する「薬剤部」とも連携している。

当センターは，支援施設基盤の整備だけでなく，これまでに医師主導治験の手引きおよび当センターにおける業務標準手順書やマニュアルの作成など，円滑な業務遂行をするためのシステム整備が推し進めている。また，研究者の種（シーズ）の採択の際には，当センターは厳選な審査を経て支援を決定する。すなわち，研究者が探索医療センターの要領に従った申請書類を作成し，支援申請の窓口である医療開発部管理部へ提出する。探索医療センター内で協議し，センター長が最終的な採否を決定する。決定後は，3 部により形成した支援チームが研究者側と一体となり臨床試験を進める。また，当センターの 3 部と連携部門を合わせた機能は，単なる「臨床試験調整支援機関」ではなく，実務担当者を設置し育成する「院内完結型支援機関」の位置づけである。

以上のような支援体制により，当センターはハード面とソフト面から研究者（医師）側をサポートし，それぞれのシーズを推進している。

3　質の高い臨床試験実施に必要なもの

当センターは，質の高い臨床試験を実施するために，心がけていることが 2 つある。1 つ目は研究者側とのコミュニケーションである。臨床試験自体は決して短いものではなく，年単位での事業であるため，研究者の熱を冷まさせないようにプロセスとゴールを確認することを常に意識している。2 つ目はチームとしての役割の明確化である。試験の開始から終了までは，多くの業務が発生するため，チームの一人一人が業務に責任をもち，チーム内で連携する。当センターは，これら 2 つのことがうまく機能することを通じて，臨床試験の準備段階から試験スタート，試験の進捗に至るまで円滑な連携を行っている。

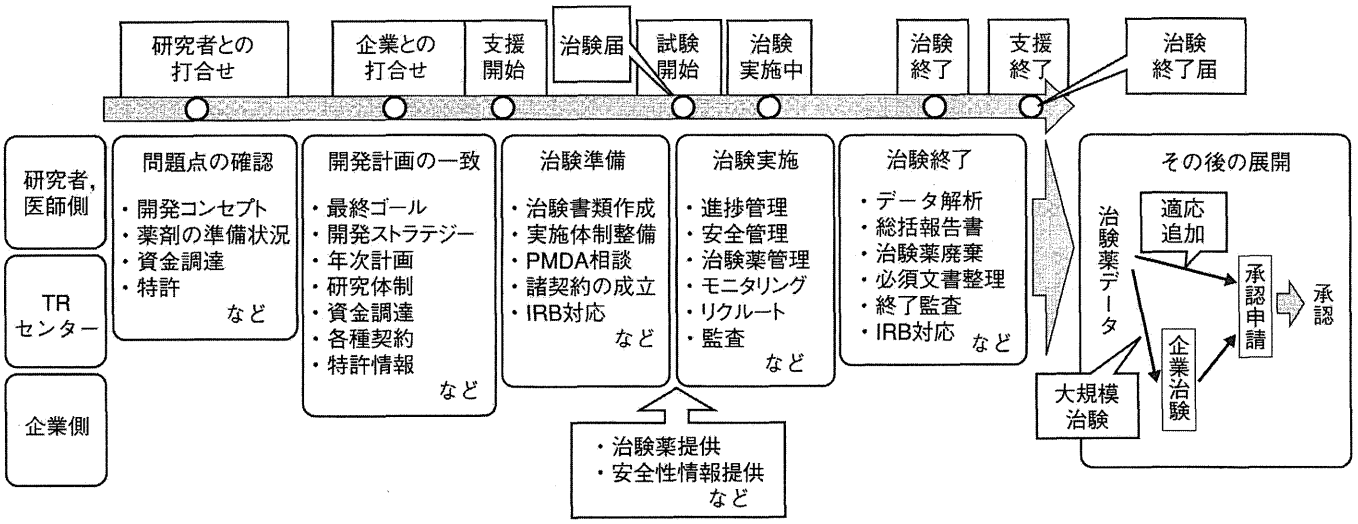

図 2　治験の主な流れ

4　支援の流れ（図 2）

①研究者側との調整

研究者からのシーズ支援の相談があった場合には，探索医療開発部がまず研究者とテーマについて十分な時間をかけて議論する。開発内容が現治療の問題点や競合する医療技術との差別化など，開発根拠を双方が理解したうえで開発を進める。そして，研究の位置づけに基づき，治験，先進医療，高度医療などのいずれのトラックを用いてゴールをめざすのか議論し，ゴールへ向かうために最短ルートを模索する。なお，臨床試験を実施する場合には，探索医療開発部が中心となり，年単位でのスケジュールやプロジェクト経費，そして試験の準備～実施～終了までの実施項目を考えうるものすべてをあげ，その実施時期と作業時間を考慮して，およそのスケジュール表など試験規模を捻出する。また，将来的な開発ロードマップは，臨床研究後の開発方針を企業とも打合せ，一般医療化までの全体の開発ストラテジーのなかで，臨床試験の位置づけを明確にしていく必要がある。また，研究者側が公的資金への申請を検討している場合に，臨床が目標であるのであれば，当センターは積極的に申請計画に加わり共同申請となるようにする。

②企業との折衝

企業とあらかじめ連携している場合には，探索医療開発部が企業側と研究者を交えて早急に折衝をしてプロジェクトとしてのゴールを議論する。企業との共同研究が可能となれば，医療開発管理部が中心となり契約をまとめる。最終的な開発する物が医薬品や医療機器であれば，企業側が臨床研究を引き継ぐこととなるため，企業側がどのような研究成果を期待し，どのように活用するかを最初の時点で製品化までのルートを議論しておくことは非常に重要である。契約内容で特に注意が必要なのは，共同研究のなかで得られた成果の取扱いであり，事前に十分な議論をしたうえで共同研究契約をまとめる。なお，当センターにかかる研究費や成果報酬についても議論が重要である。

③臨床研究のチーム形成と進捗管理

ゴールや目標が決まれば，次に支援組織と研究者側を交えて，具体的な今後のスケジュールや実行項目などを議論し，担当者を決定する。チームによる支援が本格的に開始となる。研究者側を中心に当センターの 3 部とともに試験実施計画書，説明文書・同意文書，概要書の作成などを行う。試験計画書は，臨床試験中で計画の根幹をなすものであり十分な熟慮が必要である。この試験計画書の良し悪しが試験の質を決める。当センターでは，探索医療検証部が統計学的な思考やデータマネジメントなどを中心に担当し，試験計画書の質を担保している。一方では医学部附属病院の各診療科および各部門との協力関係は重要であり，探索医療臨床部が中心となり，準備段階からの綿密な打合せをして臨床試験の効率的な遂行に支援をしている。さらに，臨床研究用製剤

（院内製剤）が必要の場合には，研究者側と相談のうえ，薬剤部が治験薬 GMP 製剤を，分子細胞治療センターが細胞製剤（GMP 基準）を調製・出荷をする。

試験デザインが決定し，実施計画書もほぼ固定できる段階になれば，対象患者も明確になる。実際に試験が始まると被験者リクルートに苦労することが多いため，実施計画書作成段階から組み入れられそうな被験者を調査する。試験の成功のカギは被験者リクルートを進捗にかかっている。

④規制当局との折衝

「治験」であれば，医薬品や医療機器の承認申請をめざすものであり，独立行政法人医薬品医療機器総合機構（以下，PMDA）との折衝は必要不可欠である。PMDA との対面助言でどのようなことを相談するのか，相談資料の準備も別途必要となるため，当センターは資料作成から対面助言への参加なども支援する。

⑤院内における審査

試験実施計画書，説明文書・同意文書，概要書などが完成できた場合には，研究者側が倫理委員会や治験実施審査委員会などの審査委員会へ申請を行う。当センターは審査委員会からの意見に対する対応も支援する。承認が得られれば開始可能となる。治験の場合には，院内の治験審査委員会にて承認が得られれば，研究者は PMDA へ治験計画届書を提出し，治験が開始となる。

⑥試験期間中～試験終了時の進捗管理

臨床試験がいったん開始となれば，被験者リクルートの進捗管理が重要となる。探索医療臨床部が中心となり，試験期間と被験者リクルート状況について研究者側と定期的に打合せ，今後の対応などを協議している。

臨床試験が順調な進捗をたどり，目標症例数に達成し観察期間など試験期間が終了した場合，あるいは中止した場合には，研究のまとめ作業となる。データマネジメント，統計解析，報告書作成など，研究者側と当センターと今後のスケジュールを立てて，開始段階と同様にチームを形成し作業を打ち合わせる。また，審査委員会などへの報告を行う。さらに，院内関係部署への終了報告を行う。

⑦企業への継承

臨床試験の開発段階で企業とあらかじめ契約された内容を達成可能な場合には，試験終了前に企業を折衝し，今後の動きを具体的に議論する。研究のまとめ作業と並行し，企業へ円滑な移管を行う。

5　課　題

当センターはこれまでの実績から，臨床試験の実施のノウハウは積み上げ，研究者のニーズにあった支援体制を整備してきた。しかし，実際に一般医療まで到達したシーズはなく，企業への継承を含め，最終結果が判明するまでに時間を要する。また，臨床試験の実施に際してのマンパワーが現在不足している。今後当センターの支援体制の質を保つには人材育成が急務の課題である。今後は，これまでの当センターの経験から，今後は他の医療機関とも連携を模索し，さらなる臨床研究のネットワークと人材育成の場を広げ，質の高い臨床試験を次々と実施していくことである。

おわりに

当センターは，学内の有望シーズの臨床開発を通じて，現有の優れた支援機能を増強・拡充・整備し，人材の実地教育による育成をすることによって，学内はもとより外部からのプロジェクトを受け入れて支援する体制を確立すること，さらに，より円滑かつ効率的な橋渡し研究・臨床開発を可能にし，創薬・新規医療開発の国内におけるアカデミア拠点となることが目標としている。また，これらの目標を達成することで，欧米に比し遅れているわが国の臨床試験・治験を推進し，国際競争力の強化に貢献できるものと考える。

〈記録〉第31回 日本臨床薬理学会年会 2010年12月1~3日 京都
シンポジウム6：これからのCRC：臨床試験を支援するスタッフの教育

2．未承認薬・未承認機器の臨床試験を支援するために

新美三由紀* 多田春江* 伊藤達也*

1．はじめに

アカデミアで行う研究者主導臨床試験（臨床研究）は，市販薬を用いてエビデンスを創成する，いわゆる育薬を目的としたものが多い．しかし当学では，産学連携のもとに，基礎医学研究の成果を臨床応用まで一貫して行う，いわゆるトランスレーショナルリサーチを実行する大学病院における先端医療開発拠点として探索医療センターを設置し，未承認薬・未承認機器の臨床試験を多く手がけている．

今回，CRCの専門性やスキルアップを考えるにあたり，企業治験以外の未承認薬・未承認機器を用いた臨床試験における支援という観点から，CRCとしての役割や業務の広がり，さらにはCRCに限らず広く臨床試験専門職（Clinical Research Professionals：CRP）としての役割や業務の広がりを紹介する．とくに，当センターで行っている医師主導治験，高度医療の臨床試験，通常の臨床研究という3つのタイプの違う臨床試験の支援体制を比較しながら，臨床試験支援スタッフぞれぞれのミッションと業務内容，CRCがキャリアアップ，キャリアチェンジとしてそれらの業務を行うために必要なトレーニングについて紹介する．

2．トランスレーショナルリサーチのプロジェクト支援の特徴

当院は全国に7つある橋渡し研究拠点の1つである．これは，医療としての実用化が見込まれる有望な基礎研究の成果を開発している研究機関を対象に，シーズの開発戦略策定や，薬事法を目指した試験物製造のような橋渡し研究の試験を行うアカデミアの機関である．当センターでは，おもに流動プロジェクト[1]，橋渡し研究プロジェクト[1]，スーパー特区（難病創薬スーパー特区，医療機器開発スーパー特区，iPS細胞スーパー特区）[2]という研究プロジェクトの特徴から，医師主導治験，高度医療評価制度下での臨床試験，臨床研究というさまざまな方法で，未承認薬・未承認機器を用いた試験を計画・運営しなければならない．

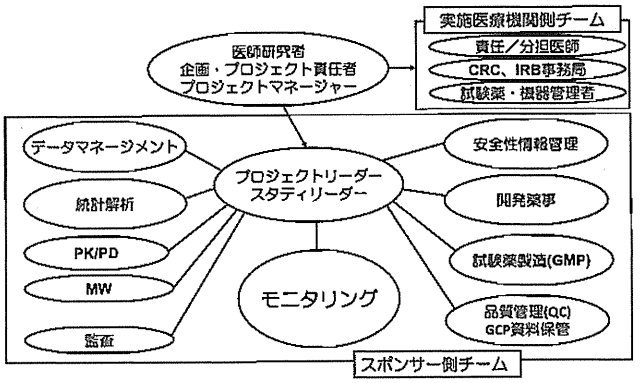

Fig. シーズ・革新的技術開発試験の支援スタッフ

最も大きな特徴は，企業治験では明確に分かれているスポンサー（製薬・医療機器企業側）と実施医療機関が，1つの大学内に存在するということである．Fig.のように，プロジェクトおよび企画の責任者である医師研究者や，プロジェクトマネージャーから，実施医療機関側のチームと，スポンサー側のチームに指示が出される．しかし，この2つのチームは1つの臨床試験において業務上は明確に役割が分けられており，たとえばモニタリングやデータマネジメントのスタッフがCRCを兼任することは決してない．これは，臨床試験における品質を保証するための前提条件であり，リスクの高い試験であるほど，重要となる．仮に，医師やCRCがモニタリング担当者を兼任した場合，実施者がモニタリングという品質チェックをすることになり，公平・公正な第三者的視点が欠如するため，重要な問題点を看過する可能性が高くなるだろう．また，医師やCRCがデータマネジメント担当者を兼任した場合，解析データセットを作成するまでのデータバリデーションの過程で，無意識にバイアスを生じさせているかもしれない．

したがって，これらの役割を明確に分離することが品質保証に繋がるが，大学・医療機関という1つの組

* 京都大学医学部附属病院探索医療センター
〒606-8507 京都市左京区聖護院川原町54

織の中でこれらすべての業務を行うためには，人材（人財）を育成して，その人を適切な役割として効果的に使う必要がある．このため，試験によって実施医療機関側のスタッフからスポンサー側のスタッフへ，つまりCRCから別の役割へ変わることもあるだろう．

3．CRCとしての役割・業務の広がり

当センターのスタッフの中にはCRC経験者は多い．しかし，わが国のCRCはもともと企業治験から臨床試験領域に入る者が多いため，CRCが役割や業務を広げるには，大学内でスポンサー（データセンター・運営管理）機能と，実施医療機関機能の両者を同時に担うという特殊な環境を理解することからはじめる必要がある．また，その基本となるのが，創薬育薬医療チームと創薬育薬医療スタッフ[3]という試験組織全体の理解である．とくに，普段見ることのないスポンサー側にはどのような専門家がいるのか，どのような役割分担をしているのかを詳細に知ろうと努力しなければならない．

CRCの中には，それまでCRC業務には含まれないと思ってきた役割を積極的に担い，場合によっては同じ病院所属で変更はないものの，データマネジャーやモニター等，他の業種にキャリアチェンジしていく人もいるかもしれない．一方，CRC業務をそのまま担う場合でも，企業治験とはアプローチを変えていく必要がある．

説明同意文書の作成を例にとってみよう．企業治験と異なり，トランスレーショナルリサーチをアカデミアで行う場合は，見本として提供される説明同意文書はない．また，すでに海外で販売されている医薬品の国内導入や，適応外使用の場合は，ある程度参考になるものがあるかもしれないが，全く初めてのシーズの場合は，ゼロからの作成となる．また，試験実施計画書と平行作業で作成し，同時完成を目指さなければならないが，もし説明同意文書作成中に試験実施計画書や試験薬概要書に問題があれば修正することも可能である．また，未承認薬・未承認機器の試験であるという点から，補償や医療費の支払いについても交渉しながら説明同意文書を作成していくことになる．

試験薬・試験機器の管理という点では，必ずしもできあがった試験薬を受領するだけとは限らない．院内GMP施設で試験薬を製造する場合もあり，その場合は，トラブルによる追加製造の必要性を判断する情報伝達が重要である．取扱い手順書の策定もゼロからとなるだろう．

できることが広がる分，責任も重くなり，同時にやり甲斐も増え，キャリアアップにも繋がる可能性がある．

4．CRPとしての役割・業務の広がり

次にCRPとしての役割や業務の広がりを考えてみよう．未承認薬・未承認機器を用いたアカデミアベースの臨床試験の場合，スポンサー機能と，実施医療機関機能の両者を院内で同時に担うため，さまざまな役割が生まれる．このような環境では，ゼネラリスト（オールラウンドプレイヤー）は重宝されるだろう．しかし，何でも屋は責任の分散化というリスクに繋がる恐れがある．常に，スポンサー側と医療機関側を明確に分けたうえで，自分の役割の明確化が必要になる．

データマネジメント，モニタリング，プロジェクトマネジメント，メディカルライティング，薬事，品質管理，文書管理，安全性情報管理等々の専門領域で活動することは，役割の明確化としては重要である．しかし，これらのスペシャリストは，その領域に特化した教育とトレーニングを受ける必要がある．これらの専門領域は，CRCの経験は活かせるが，それだけでは足りない．必ず専門家としてのトレーニングが必要である．

5．まとめ

これからのCRCは何を目指すべきだろう．CRCとして極めるのも選択肢の1つである．その場合は，企業治験だけでなく，さまざまな試験を担当することがキャリアアップに繋がるはずである．たとえアカデミア発の臨床応用を目指す未承認薬・未承認機器の臨床試験を担当しなくても，研究者主導臨床試験には自分たちでゼロから作り出す「可能性」が秘められている．

また，CRCからCRPとして展開することも1つの「可能性」である．この場合，より広い概念の臨床試験・研究チームとしてのプレーヤーを意識するだろう．

文　献

1）京都大学医学部附属病院探索医療センター：
http://www.kuhp.kyoto-u.ac.jp/～trc/project/index.html
2）内閣府，文部科学省，厚生労働省，経済産業省：「先端医療開発特区」（スーパー特区）の創設について．平成20年5月23日．
http://www.mhlw.go.jp/houdou/2008/05/h0526-1.html
3）中野重行．創薬育薬医療チームと創薬育薬医療スタッフというコンセプトの重要性．*臨床薬理*．2008：39(4)：75S-76S.

Journal of Cardiology (2012) 59, 8–13

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/jjcc

Review

Ghrelin and cardiovascular diseases

Ichiro Kishimoto (MD, PhD)*, Takeshi Tokudome (MD, PhD), Hiroshi Hosoda (MD, PhD), Mikiya Miyazato (MD, PhD), Kenji Kangawa (PhD)

National Cardiovascular Center Research Institute, Osaka, Japan

Received 14 November 2011; accepted 14 November 2011
Available online 16 December 2011

KEYWORDS
Ghrelin;
GHS receptor;
Heart failure;
Myocardial infarction;
Pulmonary hypertension;
Vagus nerve;
Sympathetic nerve

Summary In 1999, a peptide from the stomach called ghrelin was discovered, which exerts potent growth hormone releasing powers. Subsequent studies revealed that it exerts a potent orexigenic action. In addition, the beneficial effects of ghrelin in cardiovascular diseases have been recently suggested. In humans as well as in animals, administration of ghrelin improves cardiac function and remodeling in chronic heart failure. In an animal model for myocardial infarction, ghrelin treatment early after coronary ligation effectively reduces fatal arrhythmia and, consequently, mortality, suggesting the potential therapeutic role of the peptide in acute myocardial infarction. Although how ghrelin may influence the cardiovascular system is not fully understood, the cardiovascular beneficial effects are mediated possibly through a combination of various actions, such as an increase in growth hormone level, an improvement in energy balance, direct actions to the cardiovascular cells, and regulation of the autonomic nervous activity. Of note, current experimental evidence suggests that ghrelin may act centrally to decrease sympathetic nervous system activity through peripheral afferent nerve. Thus, administration of ghrelin might become a unique new therapy for cardiovascular diseases.

Contents

* Corresponding author at: Department of Endocrinology and Metabolism, National Cardiovascular Center Research Institute, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan. Tel.: +81 6 6833 5015; fax: +81 6 6835 5402.
E-mail address: kishimot@hsp.ncvc.go.jp (I. Kishimoto).

0914-5087/$ – see front matter © 2011 Published by Elsevier Ltd on behalf of Japanese College of Cardiology.
doi:10.1016/j.jjcc.2011.11.002

Introduction

Small synthetic compounds called growth hormone secretagogues (GHS) stimulate the release of growth hormone (GH) from the pituitary [1]. In 1996, a receptor for GHS (GHS-R) was cloned from swine and human pituitary gland, and it was revealed that GHS-R is one of the G-protein coupled receptors with seven membrane spanning domains [2]. Since the discovery of the receptor, the quest for searching the endogenous ligand for GHS-R started by many groups worldwide. Most of them thought that the endogenous ligand should be found in the brain and selected the brain as a starting material for the purification of the ligand, based on the assumption that pituitary hormones are regulated by midbrain-pituitary axis. However, although studied thoroughly and extensively, all purification attempts using the brain as a source ended unsuccessfully. Thus, the endogenous ligand was not found until 1999, when it was finally identified from the stomach, the tissue that almost nobody had expected as a source. Using a reverse pharmacology approach, a 28 amino-acid peptide was isolated from the rat stomach and named ''Ghrelin'' derived both from the word root ''ghre'' in Proto-Indo-European languages meaning ''grow'', and from the abbreviation for ''GH-release,'' a characteristic feature of the peptide [3]. Since the discovery of ghrelin, a number of unique features have been identified. First, the discovery of ghrelin from the stomach indicates that the release of GH from the pituitary is regulated not only by the hypothalamus but also by the digestive tract. Second, ghrelin has a unique structural property that is an acylation in its third residue, usually serine. This is the first peptide hormone with acyl modification. Interestingly, the acylation is essential for the ghrelin's ability for the binding to and the activation of its receptor, GHS-R. Third, subsequent studies revealed that exogenously administered ghrelin potently stimulates appetite in humans and in rodents [4,5]. Fourth, not only in the release of GH and in the stimulation of appetite, the roles of ghrelin have also been implicated in the cardiovascular, bone, gastrointestinal, and immune systems [6]. In the present review, we will discuss some of these characteristic features of ghrelin and its possible therapeutic roles in cardiovascular diseases.

Ghrelin is a potent GH secretagogue

Ghrelin was originally discovered as an endogenous ligand for GHS-R and, in fact, has potent GH releasing activity. Intravenous ghrelin administration markedly increases plasma GH levels in humans and in rats. After a bolus ghrelin injection, the level of GH peaks at 15—20 min and the elevation lasts longer than 60 min thereafter [7]. Since the effect of ghrelin on the release of GH is not observed after resection of the gastric branch of the vagus nerve, the vagal afferent nerve is supposed to mediate the effect [8]. The hypothesis is supported by the fact that GHS-R is synthesized in vagal afferent neurons and transported to the afferent nerve terminals [8,9].

Ghrelin as a gastrointestinal hormone

X/A-like cells are among four types of endocrine cells in the oxyntic mucosa of the stomach, and so named as their function had been undefined until recently and as their morphology is similar to pancreatic alpha cells. In situ analysis revealed that ghrelin and its mRNA are mainly localized in X/A-like cells. In the stomach, the 28 amino acids of mature ghrelin are cleaved off from its precursor preproghrelin which is composed of 117 amino acids in rats or in humans. From the submucosal layer of the stomach, ghrelin is secreted into the blood stream (not into the gastrointestinal tract). From plasma ghrelin levels in patients with gastrectomy or gastric bypass surgery, it is demonstrated that the stomach is a major organ secreting the circulating ghrelin [10]. Although the contents are much less, cells producing ghrelin are also found in the intestines and in specific regions of the brain such as the arcuate nucleus.

Octanoyl modification

The distinguished structural feature of ghrelin is its fatty acid modification at the third residue (serine in most species including humans) [3,11] (Fig. 1). Interestingly, the acylation, particularly n-octanoyl modification, is conserved among many species including mammals, fish, birds, and

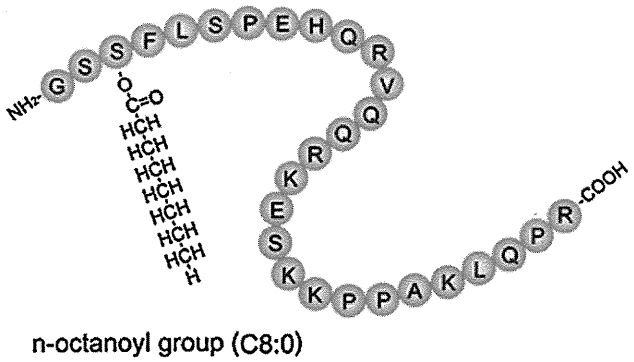

Figure 1 The structure of human ghrelin. Ghrelin is a 28 amino acid peptide discovered from the stomach. A distinguished structural feature of ghrelin is its n-octanoylation at the third serine residue, which is necessary for its receptor binding and function.

amphibians and is necessary for full binding of ghrelin to its receptor, GHS-R, and thus for expressing the biological function. The attachment of octanoate to the third serine residue of ghrelin is catalyzed by a membrane-bound enzyme, named ghrelin O-acyltransferase (GOAT) [12].

Localization of ghrelin receptor, GHS-R

In the brain, gene expression of the receptor for ghrelin, GHS-R, is detected predominantly in the arcuate nuclei, in the ventromedial nuclei, and in the hypocampus [2]. To a lesser extent, it is also detected in pituitary and in detate gyrus. Outside of the brain, various organs including lung, liver, kidney, pancreas, and gastrointestinal tract expressed GHS-R gene. In the cardiovascular system, GHS-R is expressed in the heart and in the aorta. It is also reported that, GHS-R gene can be detected in cultured cardiomyocyte cell line and in human vascular endothelial cells.

Ghrelin as a hunger hormone

Exogenously administered ghrelin has a potent appetite-stimulating effect [4]. Since the orexigenic effect of ghrelin can be observed in GH-deficient dwarf rats, the appetite-promoting effect is independent of GH release. The plasma level of ghrelin and mRNA level in the stomach are increased by fasting and decreased by feeding [10,13]. Oral or intravenous administration of glucose decreases plasma ghrelin level. Since ghrelin has a potent orexigenic action, ghrelin can serve as a ''hunger hormone.'' In addition, fasting plasma ghrelin level is low in obese people [14] and high in lean people and in patients with anorexia nervosa. Since ghrelin induces weight gain by promoting appetite and by reducing fat utilization [15], the nutritional state seems to be a major determinant of release of ghrelin from the stomach.

Multiple actions of ghrelin

Since its discovery, many studies were conducted and it has been demonstrated that ghrelin has multiple biological actions, all of which could affect the cardiovascular system.

Activation of GH/IGF-1 pathway

Ghrelin activates the pathway of GH and its mediator, insulin-like growth factor-1 (IGF-1), both of which are anabolic hormones necessary for skeletal and myocardial growth and for metabolic homeostasis. Since GH/IGF-1 exerts effects on cardiac structure and function, ghrelin can affect the cardiovascular system through the elevation of plasma GH levels.

Stimulation of appetite

Endogenous ghrelin and its receptor are involved in the regulation of food intake and adiposity. Intravenous infusion of ghrelin is reported to increase food intake and body weight in healthy subjects [16–18] and to stimulate appetite and food intake in patients with congestive heart failure [19], chronic obstructive pulmonary disease [20], cancer [21], functional dyspepsia [22], and anorexia nervosa [23]. Recently, in a prospective randomized, placebo-controlled, clinical trial, it was suggested that administration of ghrelin after esophagectomy increased oral food intake, attenuated weight loss, and improved decreased lean body weight after operation [24]. Cachexia, which is a catabolic state characterized by weight loss and muscle wasting, is associated with hormonal changes and cytokine activation in severely sick patients. Since ghrelin causes a positive energy balance through GH-dependent and independent mechanisms, it could improve cachexia due to severe pathological conditions as seen in many end-stage diseases. In fact, in ghrelin-treated cachectic patients with congestive heart failure, increases in body weight, in lean body mass, and in muscle strength are reported [19]. Therefore, it is conceivable that ghrelin administration can be a novel therapeutic approach for cachexia in humans.

Direct cardiovascular action

Ghrelin is demonstrated to dilate human artery [25] and the action is endothelium-independent. In addition, ghrelin inhibits apoptosis of cultured cardiomyocytes and endothelial cells possibly through activation of extracellular signal-regulated kinase-1/2 and Akt serine kinases [26]. Together with the localization of GHS-R in the cardiovascular system, these results suggest that ghrelin may act directly on the cardiovascular system.

Anti-inflammatory action

Ghrelin suppresses the production of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α. In addition, ghrelin inhibits the activation of nuclear factor-κB (NF-κB), a transcriptional factor regulating the gene expression of pro-inflammatory cytokines [27]. In patients with pulmonary infections, it is reported that chronic administration of ghrelin is reported to decrease inflammatory cytokine levels. The inhibition of the release of proinflammatory

cytokines could be mediated at least partly by the ghrelin-induced activation of the vagus nerve. Since cardiovascular diseases are often accompanied by an augmented inflammatory response, ghrelin may exert its protective actions through these anti-inflammatory potentials.

Suppression of sympathetic nerve

As described below, ghrelin potently inhibits sympathetic nerve which is often over-activated in cardiac diseases.

Cardiovascular actions of ghrelin

Since the administration of ghrelin has been demonstrated to decrease blood pressure, reduce cardiac afterload, and increase cardiac output without affecting heart rate in humans and in animals, the therapeutic potentials of ghrelin in cardiac diseases have been speculated. In addition, ghrelin potently stimulates GH release from the pituitary gland, improves energy balance, and modulates the autonomic nervous system, all of which could have beneficial effects on the cardiovascular system. Moreover, the receptor for ghrelin, GHS-R, can be demonstrated in the cardiac ventricles and in the blood vessels, suggesting that ghrelin might have direct cardiovascular actions.

Ghrelin in cardiovascular diseases

Heart failure

In rats with heart failure, chronic ghrelin treatment improved cardiac systolic dysfunction [28]. In addition, in patients with congestive heart failure, intravenous administration of ghrelin (2 μg/kg, twice a day) for three weeks significantly improved left ventricular ejection fraction (from 27% to 31%; $p < 0.05$), and increased peak workload and peak oxygen consumption during exercise, which was accompanied by a dramatic decrease in plasma norepinephrine (from 1132 to 655 pg/mL; $p < 0.001$) [19]. The therapeutic potential of ghrelin is, therefore, suggested in heart failure patients.

Myocardial infarction

Left ventricular remodeling after myocardial infarction is often associated with subsequent heart failure, which could lead to a fatal outcome. In a rat model of experimental myocardial infarction, peripheral ghrelin administration attenuated left ventricular dysfunction and remodeling was examined as described below.

Chronic treatment: subcutaneous administration of ghrelin at a dose of 100 μg/kg twice a day for two weeks significantly improved left ventricular enlargement induced by myocardial infarction. In addition, there was a substantial improvement in cardiac function parameters in ghrelin-treated rats compared with saline-treated controls. Furthermore, ghrelin attenuated an increase in interstitial fibrosis in the non-infarct region. Importantly, the infarction-induced increase of heart rate was suppressed completely in ghrelin-treated animals [29].

Acute treatment: whether one bolus subcutaneous injection of ghrelin 1 min after the coronary ligation leads to a beneficial effect during the acute phase was next examined using a rat model of myocardial infarction [30]. Surprisingly, the high mortality rate after myocardial infarction was significantly reduced by the early bolus of ghrelin administration [61% in saline-treated rats vs 23% in ghrelin-treated rats ($p < 0.05$)]. In addition, mortality due to fatal arrhythmias was also improved by the ghrelin treatment. Furthermore, the ghrelin-treated group had significantly fewer arrhythmic insults by the second to third hour after myocardial infarction [30]. The results show that one bolus of ghrelin treatment early after myocardial infarction improves survival after myocardial infarction by preventing the increase in frequency of ventricular arrhythmias.

Myocardial ischemia/reperfusion injury

It is reported that administration of ghrelin protects the heart against ischemia/reperfusion injury [31]. The cardioprotective effects of ghrelin are independent of GH release and likely involve binding of the peptide to receptors in the heart. The anti-apoptotic effect of ghrelin via the ERK 1/2 and PI3K/Akt-dependent pathway could potentially contribute to the beneficial effect of ghrelin infusion on myocardial ischemia/reperfusion injury.

Pulmonary hypertension

Whether ghrelin would impede pulmonary arterial hypertension during chronic hypoxia has been examined [32]. Conscious male Sprague Dawley rats were housed in a hypoxic chamber (10% oxygen) and received daily subcutaneous injection of ghrelin. While, in saline-treated rats, chronic hypoxia significantly elevated pulmonary arterial pressure and increased wall thickness of peripheral pulmonary arteries, the hypoxia-induced development of pulmonary arterial hypertension (110% increase in control vs 48% increase in ghrelin group), pulmonary vascular remodeling was significantly attenuated in ghrelin-treated animals. Therefore, the therapeutic benefits of ghrelin for pulmonary hypertension are suggested, particularly in subjects prone to chronic hypoxia.

Sympathetic inhibitory action of ghrelin

Recently, the effects of ghrelin on blood pressure, sympathetic nervous system activity, and mental stress responses were investigated in lean and overweight or obese individuals and it was found that stress-induced significant increase in these parameters were significantly reduced by 1 h intravenous infusion of ghrelin irrespective of obese phenotype [33]. In addition, administration of ghrelin significantly suppressed heart rate increase and ghrelin significantly suppressed plasma norepinephrine level in both humans and animals. Furthermore, it has been reported that the intracerebroventricular administration of ghrelin inhibited the sympathetic nerve activity [34].

Using a rat model of myocardial infarction, we investigated the beneficial effect of peripheral subcutaneous

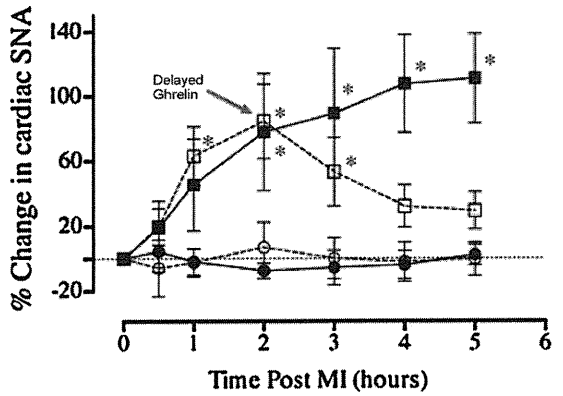

Figure 2 The sympathetic inhibitory effect of ghrelin after myocardial infarction. Transient responses in cardiac sympathetic nervous activity (SNA; percent increase in cardiac SNA of integrated area of the raw nerve signal) in sham rats (closed circle) and three groups of myocardial infarction (MI) rats: untreated (closed square); ghrelin treated immediately after myocardial infarction (open circle); and ghrelin treated 2 h after MI (open square). Either immediately after MI or 2 h after MI, ghrelin treatment effectively reduces the up-regulated SNA. *Significantly different from before MI (time ''0'') ($p < 0.05$). Adapted from Schwenke et al. [30].

ghrelin administration. Direct recording of cardiac sympathetic nerve activity revealed that ghrelin administration prevents an increase in cardiac sympathetic nerve activity as shown in Fig. 2. Importantly, the effect of ghrelin was accompanied by a reduction in mortality [30]. In acute myocardial infarction, the initial increase in the cardiac sympathetic nervous activity often leads to the fatal ventricular arrhythmia. The results, therefore, suggest that ghrelin-induced attenuation of the early increase in cardiac sympathetic nerve activity could potentially improve cardiac prognosis.

Ghrelin signaling through cardiac vagal afferent pathway

Interestingly, the orexigenic effect of peripherally administered ghrelin was suppressed by ligation of the gastric branch of the vagal nerve [8] or by pre-treatment with capsaicin, a neurotoxin specific for sensory afferent, indicating that vagal sensory afferent mediates the appetite promoting effect of peripherally administered ghrelin [8]. Furthermore, when ghrelin was microinjected into the nucleus of the solitary tract, the brain region important for controlling the autonomic nervous system, there was observed significant decreases in heart rate and mean arterial pressure [35]. In addition, GHS-R is shown to localize on the nerve terminals within the heart [9]. Furthermore, the sympathoinhibitory effect of intravenous administration of ghrelin was abolished in post-gastrectomy vagotomized patients, suggesting the vagus nerve is important for the effects of peripheral ghrelin [36]. Taken together, by acting on the vagal afferent nerve, which sends signals to the vasomotor center of the medulla via the nucleus of the solitary tract, ghrelin might exert its potent sympathetic inhibitory action

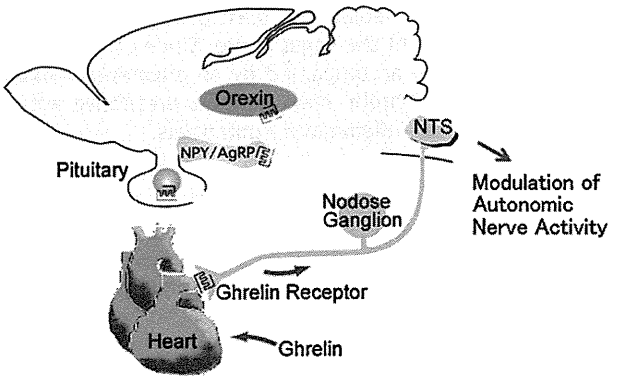

Figure 3 The signaling pathway in autonomic modulatory actions of ghrelin. Ghrelin acts on the cardiac vagal afferent nerve terminals, which send signals to the vasomotor center of the medulla through the nucleus of the solitary tract (NTS), which inhibits the sympathetic nerve activity and protects the heart from excessive damage.
Adapted from Kishimoto et al. [9,37].

resulting in decreases in sympathetic activity and in heart rate elevated after myocardial infarction (Fig. 3).

Conclusion

As described above, ghrelin has potent cardioprotective actions in diseases such as heart failure, myocardial infarction, pulmonary hypertension, and fatal arrhythmias through various mechanisms including GH release, direct actions on cardiovascular cells and inhibition of the sympathetic nervous activity. Since ghrelin is an endogenous hormone, it has advantages over other medications. It is, therefore, suggested that ghrelin can be a promising new treatment for cardiac diseases.

References

[1] Smith RG, Cheng K, Schoen WR, Pong SS, Hickey G, Jacks T. A nonpeptidyl growth hormone secretagogue. Science 1993;260:1640–3.
[2] Howard AD, Fieghner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI. A receptor in pituitary and hypothalamus that functions in growth hormone release. Science 1996;273:974–7.
[3] Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999;402:656–60.
[4] Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. Nature 2001;409:194–8.
[5] Horvath TL, Diano S, Sotonyi P, Heiman M, Tschop M. Minireview: ghrelin and the regulation of energy balance – a hypothalamic perspective. Endocrinology 2001;142:4163–9.
[6] Kojima M, Kangawa K. drug insight: the functions of ghrelin and its potential as a multitherapeutic hormone. Nat Clin Pract Endocrinol Metab 2006;2:80–8.
[7] Nagaya N, Kojima M, Uematsu M, Yamagishi M, Hosoda H, Oya H, Hayashi Y, Kangawa K. Hemodynamic and hormonal effects of human ghrelin in healthy volunteers. Am J Physiol 2001;280:R1483–7.
[8] Date Y, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, Kangawa K, Nakazato M. The role of the gastric afferent

vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. Gastroenterology 2002;123:1120–8.
[9] Kishimoto I, Tokudome T, Schwenke DO, Soeki T, Hosoda H, Nagaya N, Kangawa K. Therapeutic potential of ghrelin in cardiac diseases. Exp Rev Endocrinol Metab 2009;4:283–9.
[10] Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, Purnell JQ. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N Engl J Med 2002;346:1623–30.
[11] Kojima M, Kangawa K. Ghrelin: structure and function. Physiol Rev 2005;85:495–522.
[12] Yang J, Brown MS, Liang G, Grishin NV, Goldstein JL. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. Cell 2008;132: 387–96.
[13] Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 2001;50:1714–9.
[14] Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. Diabetes 2001;50:707–9.
[15] Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 2000;407:908–13.
[16] Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR. Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab 2001;86:5992–5.
[17] Akamizu T, Takaya K, Irako T, Hosoda H, Teramukai S, Matsuyama A, Tada H, Miura K, Shimizu A, Fukushima M, Yokode M, Tanaka K, Kangawa K. Pharmacokinetics, safety, and endocrine and appetite effects of ghrelin administration in young healthy subjects. Eur J Endocrinol 2004;150:447–55.
[18] Druce MR, Wren AM, Park AJ, Milton JE, Patterson M, Frost G, Ghatei MA, Small C, Bloom SR. Ghrelin increases food intake in obese as well as lean subjects. Int J Obes (Lond) 2005;29:1130–6.
[19] Nagaya N, Moriya J, Yasumura Y, Uematsu M, Ono F, Shimizu W, Ueno K, Kitakaze M, Miyatake K, Kangawa K. Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure. Circulation 2004;110:3674–9.
[20] Nagaya N, Itoh T, Murakami S, Oya H, Uematsu M, Miyatake K, Kangawa K. Treatment of cachexia with ghrelin in patients with COPD. Chest 2005;128:1187–93.
[21] Neary NM, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C, Frost GS, Ghatei MA, Coombes RC, Bloom SR. Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. J Clin Endocrinol Metab 2004;89:2832–6.
[22] Akamizu T, Iwakura H, Ariyasu H, Hosoda H, Murayama T, Yokode M, Teramukai S, Seno H, Chiba T, Noma S, Nakai Y, Fukunaga M, Nakai Y, Kangawa K, FD Clinical Study Team. Repeated administration of ghrelin to patients with functional dyspepsia: its effects on food intake and appetite. Eur J Endocrinol 2008;158:481–98.
[23] Hotta M, Ohwada R, Akamizu T, Shibasaki T, Takano K, Kangawa K. Ghrelin increases hunger and food intake in patients with restricting-type anorexia nervosa: a pilot study. Endocr J 2009;56:1119–28.
[24] Yamamoto K, Takiguchi S, Miyata H, Adachi S, Hiura Y, Yamasaki M, Nakajima K, Fujiwara Y, Mori M, Kangawa K, Doki Y. Randomized phase II study of clinical effects of ghrelin after esophagectomy with gastric tube reconstruction. Surgery 2010;148:31–8.
[25] Okumura H, Nagaya N, Enomoto M, Nakagawa E, Oya H, Kangawa K. Vasodilatory effect of ghrelin, an endogenous peptide from the stomach. J Cardiovasc Pharmacol 2002;39:779–83.
[26] Baldanzi G, Filigheddu N, Cutrupi S, Catapano F, Bonissoni S, Fubini A, Malan D, Baj G, Granata R, Broglio F, Papotti M, Surico N, Bussolino F, Isgaard J, Deghenghi R, et al. Ghrelin and des-acyl ghrelin inhibit cell death in cardiomyocytes and endothelial cells through ERK1/2 and PI 3-kinase/AKT. J Cell Biol 2002;159:1029–37.
[27] Li WG, Gavrila D, Liu X, Wang L, Gunnlaugsson S, Stoll LL, McCormick ML, Sigmund CD, Tang C, Weintraub NL. Ghrelin inhibits proinflammatory responses and nuclear factor-kappaB activation in human endothelial cells. Circulation 2004;109:2221–6.
[28] Nagaya N, Uematsu M, Kojima M, Ikeda Y, Yoshihara F, Shimizu W, Hosoda H, Hirota Y, Ishida H, Mori H, Kangawa K. Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. Circulation 2001;104:1430–5.
[29] Soeki T, Kishimoto I, Schwenke DO, Tokudome T, Horio T, Yoshida M, Kangawa K. Ghrelin suppresses cardiac sympathetic activity and prevents early left ventricular remodeling in rats with myocardial infarction. Am J Physiol 2008;294:H426–32.
[30] Schwenke DO, Tokudome T, Kishimoto I, Horio T, Shirai M, Cragg PA, Kangawa K. Early ghrelin treatment after myocardial infarction prevents an increase in cardiac sympathetic tone and reduces mortality. Endocrinology 2008;149:5172–6.
[31] Chang L, Ren Y, Liu X, Li WG, Yang J, Geng B, Weintraub NL, Tang C. Protective effects of ghrelin on ischemia/reperfusion injury in the isolated rat heart. J Cardiovasc Pharmacol 2004;43:165–70.
[32] Schwenke DO, Tokudome T, Shirai M, Hosoda H, Horio T, Kishimoto I, Craig P, Kangawa K. Exogenous ghrelin attenuates the progression of chronic hypoxia-induced pulmonary hypertension in conscious rats. Endocrinology 2008;149:237–44.
[33] Lambert E, Lambert G, Ika-Sari C, Dawood T, Lee K, Chopra R, Straznicky N, Eikelis N, Drew S, Tilbrook A, Dixon J, Esler M, Schlaich MP. Ghrelin modulates sympathetic nervous system activity and stress response in lean and overweight men. Hypertension 2011;58:43–50.
[34] Matsumura K, Tsuchihashi T, Fujii K, Abe I, Iida M. Central ghrelin modulates sympathetic activity in conscious rabbits. Hypertension 2002;40:694–9.
[35] Lin Y, Matsumura K, Fukuhara M, Kagiyama S, Fujii K, Iida M. Ghrelin acts at the nucleus of the solitary tract to decrease arterial pressure in rats. Hypertension 2004;43:977–82.
[36] Huda MS, Mani H, Dovey T, Halford JC, Boyland E, Daousi C, Wilding JP, Pinkney J. Ghrelin inhibits autonomic function in healthy controls, but has no effect on obese and vagotomized subjects. Clin Endocrinol (Oxf) 2010;73:678–85.
[37] Kishimoto I, Tokudome T, Hosoda H, Miyazato M, Nishikimi T. Ghrelin and Cardiovascular Diseases. In: Kastin AJ, editor. Handbook of the Biologically Active Peptides. 2012, in press.

European Journal of Internal Medicine 23 (2012) 197–202

Contents lists available at SciVerse ScienceDirect

European Journal of Internal Medicine

journal homepage: www.elsevier.com/locate/ejim

Review article

The physiological significance and potential clinical applications of ghrelin

Takashi Akamizu [a,b,*], Kenji Kangawa [a,c]

[a] Ghrelin Research Project, Department of Experimental Therapeutics, Translational Research Center, Kyoto University Hospital, Kyoto University School of Medicine, Kyoto 606-8507, Japan
[b] The First Department of Medicine, Wakayama Medical University, Wakayama 641-8509, Japan
[c] Department of Biochemistry, National Cerebral and Cardiovascular Center Research Institute, Osaka 565-8565, Japan

ARTICLE INFO

Article history:
Received 16 November 2011
Received in revised form 30 November 2011
Accepted 6 December 2011
Available online 30 December 2011

Keywords:
Cachexia
Anorexia nervosa
GH deficiency
Clinical trial
Ghrelin
Gastrectomy

ABSTRACT

Ghrelin, a natural ligand for the growth hormone (GH)-secretagogue receptor (GHS-R), is now known to play a role in a number of different physiological processes. For example, ghrelin increases GH secretion, feeding, and body weight when administered centrally or peripherally. These unique effects of ghrelin should be invaluable for the development of novel treatments and disease diagnostic techniques. Clinical trials have already been performed to assess the utility of ghrelin for the treatment of several disorders including anorexia, cachexia, and GH-related disorders. This review summarizes the recent advances in this area of research.

1. Introduction

Ghrelin is a peptide hormone that was discovered in 1999 as an endogenous ligand for the growth hormone (GH)-secretagogue receptor (GHS-R) [1]. Ghrelin is a 28-amino-acid peptide and possesses a unique fatty acid modification, *n*-octanoylation, at Ser 3. There are two circulating forms of ghrelin, acylated and unacylated (desacyl), and the acylated form is essential for ghrelin's biological activity through GHS-R. Recently, however, desacyl ghrelin was reported to influence both cell proliferation and adipogenesis through another unknown receptor [2–5]. Ghrelin is produced primarily in the stomach and circulates in the blood at a considerable plasma concentration. Expression of ghrelin is also detectable in the hypothalamus, intestine, pituitary, placenta, and other tissues [1,6–8]. Ghrelin is now known to play a role in a number of different physiological processes; for example, ghrelin increases GH secretion and feeding, and decreases insulin secretion [1,9–19].

These unique effects of ghrelin and growth hormone secretagogues (GHS) should be invaluable for the development of novel treatments and disease diagnostic techniques [20–22]. Clinical trials have already been performed to assess the utility of ghrelin for the treatment of various disorders including anorexia [23–26], cachexia [27–29], malnutrition [30], GH-related disorders [31], and postgastrectomy/esophagectomy [32,33]. Because many excellent reviews concerning basic and clinical research on ghrelin have already been published, we will summarize and discuss recent clinical trials of ghrelin in this work.

2. Physiological actions of ghrelin

2.1. Orexigenic action

Ghrelin has a well-established role in stimulating appetite and increasing food intake [34,35]. Peripheral administration of ghrelin stimulates GH secretion and appetite in both animals and humans [10,18]; it is the only hormone known to have this effect. Ghrelin increases c-fos expression in the arcuate nucleus, and also activates hypothalamic neuropeptide Y (NPY)/Y1 receptors and agouti-related peptide (AgRP) pathways [36–38]. In addition, ghrelin induces food intake via the orexin pathway [39]. These functions are mediated at least in part by vagal nerve pathways [40]. Repeated administration of ghrelin resulted in significant weight gain in rats [41] and patients with chronic obstructive pulmonary disease (COPD) [28].

2.2. Stimulation of GH secretion

Ghrelin strongly stimulates GH secretion in humans [12,16,17,42], several-fold more potently than GHRH under similar conditions. Furthermore, ghrelin and growth hormone releasing hormone (GHRH) synergistically increase GH release [17]. Ghrelin might also play a role in GH release in a non-acute setting [43,44]. GH regulates IGF-I levels, promotes anabolism, and increases muscle strength [45,46].

* Corresponding author at: Ghrelin Research Project, Department of Experimental Therapeutics, Translational Research Center, Kyoto University Hospital, 54 Shogoin-Kawaharacho, Sakyo-ku, Kyoto 606-8507, Japan. Tel.: +81 75 751 4723; fax: +81 75 751 4731.
E-mail address: akamizu@kuhp.kyoto-u.ac.jp (T. Akamizu).

0953-6205/$ – see front matter © 2011 European Federation of Internal Medicine. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.ejim.2011.12.001

While GH enhances lipolysis, IGF-1 stimulates protein synthesis, myoblast differentiation, and muscle growth.

2.3. Anti-inflammatory action

Evidence that ghrelin exerts anti-inflammatory actions has been accumulating. Ghrelin suppresses the production of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α both *in vitro* [47,48] and *in vivo* [49–51]. In clinical trials, daily administration of ghrelin for 3 weeks decreased inflammatory cytokine levels and neutrophil density in sputum from patients with chronic respiratory infections [52]. In contrast, ghrelin induces the anti-inflammatory cytokine IL-10 [49,53].

Ghrelin inhibits the activation of NF-κB, a transcription factor known to control the production of multiple pro-inflammatory cytokines during inflammatory insults [48,50,53]. Although the molecular mechanisms and cellular targets mediating ghrelin inhibition of NF-κB activation remain to be determined, the vagus nerve may play an important role in the ghrelin-mediated inhibition of pro-inflammatory cytokine release [50,54]. Cachexia and muscular wasting occur via protein degradation by the ubiquitin—proteasome pathway [55]. Two muscle-specific ubiquitin ligases, muscle RING-finger protein-1 (MuRF1) and atrogin-1/muscle atrophy F-box (MAFbx), are up-regulated under catabolic conditions. NF-κB activation may regulate skeletal muscle proteasome expression and protein degradation. The elevation in MuRF1 and MAFbx expression seen in skeletal muscle after thermal injury, arthritis, and dexamethasone administration was normalized, attenuated, and prevented, respectively, by ghrelin or GHS administration [56–58]. IGF-1 prevents the expression of MuRF1 and MAFbx by inhibiting Forkhead box O (FOXO) transcription factors via stimulation of the phosphatidylinositol-3-kinase (PI3K)/Akt pathway. The IGF-1 receptor triggers activation of several intracellular kinases, including phosphatidylinositol-3-kinase (PI3K) [59]. Thus, the effects of ghrelin on NF-κB activation and IGF-1 synthesis are favorable for minimizing inflammatory responses and sarcopenia in patients with cachexia.

2.4. Other actions

The role of ghrelin in stimulating gastric emptying and acid secretion is well-established [60]. This effect may ameliorate gastrointestinal symptoms in patients with anorexia—cachexia syndrome. Ghrelin also increases endogenous nitric oxide (NO) release [61,62], which may influence its orexigenic and anti-inflammatory actions [63,64].

3. Potential clinical applications of ghrelin

3.1. Appetite-related disorders

3.1.1. Anorexia nervosa (AN) and related disorders

Anorexia nervosa (AN) is an eating disorder characterized by chronically decreased caloric intake, resulting in self-induced starvation. Plasma ghrelin levels are elevated in lean patients with anorexia nervosa, consistent with a state of negative energy balance [65–67]. Only a few preliminary studies have been performed to examine the effects of ghrelin in individuals with AN. Miljic et al. infused ghrelin (300-min intravenous infusion of 5 pmol/kg/min ghrelin) into nine AN patients with very low body weights, six AN patients who had partially recovered their body weights but who remained amenorrheic, and ten constitutionally thin female subjects [68]. The fifteen AN patients felt significantly less hungry compared with the constitutionally thin subjects, suggesting that AN patients are less sensitive to the orexigenic effects of ghrelin than healthy controls. In another paper, however, six of nine patients with restrictive AN were reported to have been hungry after ghrelin administration (1.0 μg/kg as an intravenous bolus), a similar ratio to that seen in normal subjects (five of seven) [69]. We examined the effects of ghrelin on appetite, food intake, and nutritional parameters in AN patients [26]. Five female patients who met the Diagnostic and Statistical Manual IV (DSM-IV) criteria for restricting-type AN [70] and desired to recover from the disorder participated in this study. The patients were hospitalized for 26 days (6 days pre-treatment, 14 days ghrelin infusion, and 6 days post-treatment). The patients received an intravenous infusion of 3 μg/kg ghrelin twice a day (before breakfast and dinner). Attitudes toward food were evaluated by visual analogue scale (VAS) questionnaires and daily energy intake was calculated by dieticians. Ghrelin infusion improved epigastric discomfort or constipation in four patients, whose hunger scores on VAS also increased significantly after ghrelin administration. Daily energy intake during ghrelin administration increased by 12–36% compared with the pre-treatment period. The change in body weight of the five patients ranged from +1.5 to 2.4 kg. Nutritional parameters such as total protein and triglyceride levels improved. There were no serious adverse effects, including psychological symptoms. All patients who did not gain weight during hospitalization did so after discharge. These findings suggest that ghrelin may have therapeutic potential in AN patients who cannot gain weight because of gastrointestinal dysfunction. Clearly, further studies, including randomized controlled trials, are needed to determine whether ghrelin is useful for the treatment of AN.

Functional dyspepsia (FD) is a disorder characterized by the presence of chronic or recurrent symptoms of upper abdominal pain or discomfort [71]. Although no known specific organic abnormalities are present in FD, abnormalities in gastrointestinal motility and sensitivity are thought to play a role in a substantial subgroup of patients. In addition, some patients suffer from anorexia and body-weight loss. We found that levels of plasma acylated, but not desacyl, ghrelin correlated with a subjective symptom score in FD patients, suggesting that acylated ghrelin may play a role in the pathophysiology of FD [72]. We attempted to evaluate the clinical response to repeated ghrelin administration in patients with anorexia caused by functional disorders, such as FD and 'other eating disorders' or 'unspecified eating disorders' [24]. The inclusion criteria in this study were subjects who 1) were diagnosed with functional anorexia, including FD or other eating disorders with the exception of anorexia nervosa, 2) were lean (BMI<22 kg/m^2), and 3) exhibit decreased food intake. Subjects received an intravenous infusion of ghrelin for 30 min twice a day (before breakfast and dinner) for 2 weeks, and we investigated the effects on food intake, appetite, hormones, and metabolic parameters. Six patients with FD were enrolled in this study. Ghrelin administration tended to increase daily food intake in comparison to levels before and after completion of treatment, but this difference, which was the primary endpoint of the study, did not reach statistical significance. Hunger sensation was significantly elevated at the end of drip infusion. No severe adverse effects were observed. These results suggest that ghrelin administration is safe and that this treatment has stimulatory effects on appetite in patients with FD. Further studies remain necessary to confirm the efficacy of ghrelin treatment for anorexia-related disorders.

3.1.2. Cachexia and related disorders

A number of trials seeking to utilize ghrelin for the treatment of cachexia have recently been performed [73]. These studies have sought to evaluate ghrelin as a treatment for patients with cachexia associated with congestive heart failure (CHF), COPD, cancer, and End-stage renal disease (ESRD). Cachexia manifests as excessive weight loss in the setting of an underlying chronic disease [74], and is typically associated with anorexia as a major cause of weight loss. Weight loss and decreased appetite are the major causes of morbidity and mortality in patients with anorexia—cachexia syndrome. There is an immediate need for effective, well-tolerated treatments to

stimulate appetite [75], prompting several trials to explore the application of ghrelin as a treatment for patients with cachexia.

3.1.2.1. CHF-associated cachexia. Ghrelin induces a positive energy balance state through both GH-dependent and -independent mechanisms and has protective cardiovascular effects [76]. GH treatment may be especially useful in a subgroup of patients with cardiac cachexia [77]. Ghrelin stimulates food intake, induces adiposity, regulates the central nervous system to decrease sympathetic nerve outflow, and inhibits apoptosis of cardiomyocytes and endothelial cells in a GH-independent manner. Nagaya et al. investigated the effects of ghrelin on cardiac cachexia in 10 patients with CHF [27] (Table 1). Daily administration of ghrelin for 3 weeks increased both food intake and body weight. This study also demonstrated improvements in patient exercise capacity, muscle wasting, and left ventricular function. Ghrelin treatment also resulted in significantly decreased plasma norepinephrine levels. Although this study was neither randomized nor placebo-controlled, the eight CHF patients who did not receive ghrelin (control group) were followed to rule out any time-course effects during hospitalization. None of the aforementioned parameters changed in patients with CHF who did not receive ghrelin therapy. Further studies will be necessary to identify the pathways involved in this use of ghrelin and to determine the best therapeutic strategies for ghrelin use to combat the wasting process found in cardiac cachexia patients [77]. Clinical trials are currently attempting to reproduce these data in a double-blind, placebo-controlled fashion.

3.1.2.2. COPD-associated cachexia. Patients with COPD often exhibit some degree of cachexia [78], which is an independent risk factor for mortality in COPD; GH treatment increases muscle mass in such patients. COPD and CHF are both associated with multiple pathophysiological disturbances, including anemia and neurohormonal activation [79]. In COPD patients, ghrelin exhibits anti-inflammatory effects. Chronic respiratory infections, characterized by neutrophil-dominant airway inflammation, lead to end-stage cachexia [80]. The cytotoxicity of accumulated neutrophils against bronchial and alveolar epithelial cells induces a deterioration of pulmonary function in COPD, resulting in excess energy expenditure and weight loss in patients. Intravenous ghrelin treatment for 3 weeks reduced neutrophil counts in sputum samples as well as the volume of sputum, suggesting that ghrelin suppressed excess neutrophil influx [52].

An open-label pilot study examined the ability of ghrelin to improve cachexia and functional capacity in patients with COPD; ghrelin was administered intravenously for 3 weeks to seven cachectic patients with COPD [28]. Repeated ghrelin administration significantly increased food intake, body weight, lean body mass, and peripheral and respiratory muscle strength. Ghrelin treatment ameliorated exaggerated sympathetic nerve activity, as indicated by marked decreases in plasma norepinephrine levels. Subsequently, another placebo-controlled trial demonstrated that ghrelin increased both appetite and body weight with an apparent dose-dependent trend towards improved physical performance (chair stand score) [81]. A larger clinical trial is currently being conducted to confirm these data in a double-blind, placebo-controlled fashion. Comparisons of this treatment to current standard medications will be required [79].

3.1.2.3. Cancer cachexia. Anorexia is frequently encountered in cancer patients, and is one of the major causes of malnutrition and cachexia in this patient population. Ghrelin administration resulted in significant increases in weight and food intake in rodent models of cancer-associated cachexia [82–84]. DeBoer et al. determined that weight gain resulted from a reversal in the loss of lean body mass, a critical component of cachexia [82].

Several randomized, double-blind placebo-controlled trials have demonstrated the efficacy and safety of ghrelin or GHS in patients with cancer-associated cachexia [23,25,85]. Nearry et al. performed a randomized, placebo-controlled, cross-over clinical trial to determine whether ghrelin could stimulate appetite in seven cancer patients with severe anorexia [23]. Ghrelin infusion resulted in a marked increase in energy intake in comparison to saline-treated controls; all patients in the study demonstrated increased food consumption. The meal appreciation score was also higher in ghrelin-treated individuals. Strasser et al. detailed a randomized, double-crossover, phase 1/2 study in 21 patients with advanced cancer [25]. They infused a low or high dose of ghrelin or placebo before lunch daily for 4 days in each course. Nutritional intake and eating-related symptoms did not differ between the ghrelin- and placebo-treated groups. More patients, however, preferred ghrelin to placebo at the middle and end of the study, although this finding was not dose-dependent. In contrast to the results of Neary et al., this study did not demonstrate any increases in food intake. As the patient characteristics and study designs were very different in the two studies, further investigation is required.

An important concern regarding the use of ghrelin in cancer-associated cachexia is that ghrelin may increase the levels of growth factors, such as GH and IGF-1, that stimulate tumor growth. Additionally, ghrelin itself may have mitogenic potential. As far as we know, no *in vivo* data has examined the differences in tumor growth following ghrelin or GHS treatment. Long-term, large-scale clinical trials are required to determine whether ghrelin treatment promotes tumor growth.

3.1.2.4. End-stage renal disease (ESRD). ESRD is a chronic condition frequently associated with nutritional dysfunction [86]. This type of malnutrition is highly resistant to intervention and is a major predictor of morbidity and mortality for patients on either peritoneal dialysis (PD) or hemodialysis. Wynne et al. sought to determine whether a single injection of ghrelin could enhance food intake in patients with evidence of malnutrition receiving maintenance peritoneal dialysis [30]. Nine PD patients exhibiting mild to moderate malnutrition were subcutaneously administered either ghrelin or a saline placebo in a randomized, double-blind, cross-over protocol. Ghrelin

Table 1
Clinical studies of ghrelin.

Diseases	Reference	Published year	Study design	Number of patients	Ghrelin administration
CHF	[27]	2004	Open-label pilot study	10	2 μg/kg b.i.d. for 3 wks, i.v.
COPD	[28]	2005	Open-label pilot study	7	2 μg/kg b.i.d. for 3 wks, i.v.
Cancer cachexia	[23]	2004	Acute, randomized, placebo-controlled, cross-over study	7	5 pmol/kg/min i.v. for >180 min
Cancer cachexia	[25]	2008	Randomized, placebo-controlled, cross-over study	21	2 or 8 μg/kg, i.v. for 4 days, once a day
ESRD	[65]	2005	Acute, randomized, placebo-controlled, cross-over study	9	3.6 nmol/kg, s.c.
ESRD	[8]	2009	Randomized, placebo-controlled, cross-over study	12	12 μg/kg, s.c. for 1 wk, once a day
AN	[26]	2009	Open-label pilot study	5	3 μg/kg b.i.d. for 2 wks, i.v.
FD	[24]	2008	Open-label pilot study	6	3 μg/kg b.i.d. for 2 wks, i.v.
THR for OA	[31]	2008	Randomized, placebo-controlled, double-blind study	32	2 μg/kg b.i.d. for 3 wks, i.v.
Total gastrectomy	[32]	2010	Randomized, placebo-controlled, double-blind study	21	3 μg/kg b.i.d. for 10 days, i.v.
Esophagectomy	[33]	2010	Randomized, placebo-controlled, double-blind study	20	3 μg/kg b.i.d. for 10 days, i.v.

CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; FD, functional dyspepsia; ESRD, End-stage renal disease; THR, total hip replacement; OA, osteoarthritis.

administration significantly increased mean absolute energy intake during the study meals and non-significant increases in energy intake were observed over the first 24 h without a subsequent rebound. This research group subsequently sought to analyze the efficacy of repeated ghrelin administration in malnourished dialysis patients [87] by performing a double-blind randomized cross-over study of a week of daily subcutaneous ghrelin injections in a group of 12 malnourished dialysis patients. Ghrelin administration significantly increased appetite, with increases in energy intake noted at the first study meal. Persistence of this effect throughout the week was confirmed by food diaries and final study meals, indicating that daily ghrelin treatment resulted in a sustained positive change in energy balance in malnourished dialysis patients. In support of this data, experiments using a nephrectomized rat model of renal cachexia demonstrated that daily treatment for 2 weeks with ghrelin or two GHS agents (BIM-28125 and BIM-28131) resulted in increased food intake, improved lean body mass accrual, and decreased circulating inflammatory cytokines [88]. Long-term studies are needed to demonstrate efficacy in improving appetite, weight gain, lean body mass, and quality of life.

3.2. GH deficiency-related disorders

Strong stimulation of GH secretion by ghrelin has been well documented in humans [12–16,21,42]. As with GHS, ghrelin may be useful for the diagnosis and treatment of short stature and GH deficiency. Elderly individuals may be particularly suitable candidates for ghrelin treatment, as aging is associated with progressive decreases in GH secretion, appetite, and energy intake [89–92]. This reduced GH secretion is called "somatopause" and may be a cause of age-related metabolic and physiologic changes including reduced lean body mass and expansion of adipose mass. Sarcopenia is associated with functional decline and death. Altered blood lipid profiles also favor the development of vascular diseases that may increase overall mortality. The age-related reduction in energy intake has been termed "the anorexia of aging" and predisposes to the development of under-nutrition, which has been implicated in the development and progression of chronic diseases commonly affecting the elderly, as well as in increasing mortality. Growth hormone therapy increases IGF-I levels, promotes anabolism, and increases muscle strength in healthy elderly individuals, as well as in selected patient groups [93–95]. Therefore, ghrelin and GHS may also have therapeutic potential to assist in the recovery of frail patients who require nutritional support and conventional rehabilitation [96]. We evaluated the effects of ghrelin administration on physical performance and body composition in patients undergoing elective total hip replacement (THR) as treatment for osteoarthritis (OA) in a randomized, double-blind, placebo-controlled, phase II study [31]. Thirty-two patients were assigned to two groups of 16 subjects each; the ghrelin group received intravenous injections of 2 μg/kg ghrelin twice daily for 3 weeks beginning 1 week before surgery, while the placebo group received vehicle alone. While ghrelin significantly increased lean body mass after the three-week injection period, it did not affect muscle strength or walking ability. Significant decreases in fat mass and GH responses to ghrelin injection were also observed. No severe adverse effects occurred in response to ghrelin treatment. Despite increased lean tissue reserves, ghrelin administration using this study protocol did not provide any favorable effect on physical performance in patients with OA undergoing THR. Further studies are necessary to examine the efficacy of ghrelin treatment in such patients.

We found that plasma levels of acylated ghrelin in healthy elderly female subjects tended to be low and were correlated positively with IGF-1 levels, suggesting that negative feedback mechanism does not function properly in elderly subjects [97]. Further, acylated ghrelin concentrations in elderly females correlated with both systolic blood pressure and the frequency of bowel movements. These findings suggest that, in elderly females, acylated ghrelin may play a role in the regulation of the GH/IGF-1 axis, blood pressure, and bowel movements.

3.3. Post-gastrectomy and -esophagectomy

Body weight loss is common and is a serious outcome in patients who have undergone total gastrectomy and esophagectomy. Such weight loss correlates with decline in postoperative quality of life and is the most reliable indicator of malnutrition, which impairs immune function, susceptibility to infection, and survival [32,33]. Plasma ghrelin levels decreased after total gastrectomy and esophagectomy [65,98,99]. Moreover, a significant correlation between ghrelin concentration and postoperative weight loss suggested a role for loss of ghrelin. To examine this, Adachi et al. evaluated the efficacy of ghrelin in 21 patients undergoing total gastrectomy [32]. Food intake and appetite were significantly higher in the ghrelin group (3 μg/kg, twice daily for 10 days after starting oral food intake following surgery) compared with the placebo group, and BW loss was significantly lower in the ghrelin group than in the placebo group. Fat mass, lean body mass, and basal metabolic rate decreased significantly in the placebo group; however, the reductions in lean body mass and basal metabolic rate were not significant in the ghrelin group, although that of fat mass was significant. Thus, short-term administration of synthetic ghrelin successfully lessened postoperative body weight loss and improved appetite and food intake after total gastrectomy. Subsequently, the same research group performed a similar study in 20 patients who underwent esophagectomy [33]. Again, they found that administration of ghrelin after esophagectomy increased oral food intake and attenuated weight loss together with maintenance of lean body weight. Thus, ghrelin administration may be useful in minimizing the side effects of these operations.

3.4. Other disorders

Reflecting the wide expression patterns of both ghrelin and its receptor, this peptide is now known to play a role in a number of different physiological processes including cellular proliferation and differentiation, pancreatic exocrine and endocrine function, glucose metabolism, sleep and behavior, immune regulation, and cardiovascular function. For example, as discussed above, repeated administration of ghrelin in patients with CHF significantly improved left ventricular function as well as food intake. A large number of studies have been performed by investigators worldwide to elucidate the various activities of ghrelin. We believe that some of these may lend support to the development of clinical applications of ghrelin to disorders other than those described above in the future.

4. Conclusions

More than ten years have passed since the discovery of ghrelin, and abundant evidence now indicates that it plays a role in a variety of physiological functions. In parallel, clinical trials have proceeded to exploit these activities in the treatment and diagnosis of human disease. There are several characteristic features of the clinical applications of ghrelin: 1) the multiplicity and uniqueness of its function, 2) its unique structure and fatty acid modification, and 3) the paucity of severe adverse effects [100]. These characteristics should allow us to develop novel and unique therapies for a variety of disorders, including many currently intractable and serious diseases. Indeed, research on clinical applications of ghrelin is a challenging and potentially rewarding avenue for the future.

5. Learning points

- Ghrelin plays a critical role in a variety of physiological processes, including the stimulation of food intake and growth hormone secretion.
- The effects of ghrelin should be invaluable for the development of novel treatments and disease diagnostic techniques.
- Clinical trials have already been performed to assess the utility of ghrelin for the treatment of several disorders including anorexia, cachexia, and GH-related disorders.
- This review summarizes the recent advances in this area of research.

Conflict of interest statement

The authors state that they have no conflicts of interest.

Acknowledgments

Research in the authors' laboratory was supported in-part by funds from the Ministry of Education, Culture, Sports, Science and Technology of Japan; the Ministry of Health, Labour and Welfare of Japan; the Program for the Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO); the Smoking Research Foundation; and the Tokyo Biochemical Research Foundation and the Foundation for Growth Science.

References

[1] Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999;402:656–60.
[2] Cassoni P, Papotti M, Ghe C, Catapano F, Sapino A, Graziani A, et al. Identification, characterization, and biological activity of specific receptors for natural (ghrelin) and synthetic growth hormone secretagogues and analogs in human breast carcinomas and cell lines. J Clin Endocrinol Metab 2001;86:1738–45.
[3] Bedendi I, Alloatti G, Marcantoni A, Malan D, Catapano F, Ghe C, et al. Cardiac effects of ghrelin and its endogenous derivatives des-octanoyl ghrelin and des-Gln(14)-ghrelin. Eur J Pharmacol 2003;476:87–95.
[4] Thompson NM, Gill DA, Davies R, Loveridge N, Houston PA, Robinson IC, et al. Ghrelin and des-octanoyl ghrelin promote adipogenesis directly *in vivo* by a mechanism independent of the type 1a growth hormone secretagogue receptor. Endocrinology 2004;145:234–42.
[5] Broglio F, Gottero C, Prodam F, Gauna C, Muccioli G, Papotti M, et al. Non-acylated ghrelin counteracts the metabolic but not the neuroendocrine response to acylated ghrelin in humans. J Clin Endocrinol Metab 2004;89:3062–5.
[6] Korbonits M, Bustin SA, Kojima M, Jordan S, Adams EF, Lowe DG, et al. The expression of the growth hormone secretagogue receptor ligand ghrelin in normal and abnormal human pituitary and other neuroendocrine tumors. J Clin Endocrinol Metab 2001;86:881–7.
[7] Gualillo O, Caminos J, Blanco M, Garcia-Caballero T, Kojima M, Kangawa K, et al. Ghrelin, a novel placental-derived hormone. Endocrinology 2001;142:788–94.
[8] Gnanapavan S, Kola B, Bustin SA, Morris DG, McGee P, Fairclough P, et al. The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. J Clin Endocrinol Metab 2002;87:2988.
[9] Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 2000;407:908–13.
[10] Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, et al. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 2000;141:4325–8.
[11] Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, et al. A role for ghrelin in the central regulation of feeding. Nature 2001;409:194–8.
[12] Takaya K, Ariyasu H, Kanamoto N, Iwakura H, Yoshimoto A, Harada M, et al. Ghrelin strongly stimulates growth hormone release in humans. J Clin Endocrinol Metab 2000;85:4908–11.
[13] Arvat E, Di Vito L, Broglio F, Papotti M, Muccioli G, Dieguez C, et al. Preliminary evidence that Ghrelin, the natural GH secretagogue (GHS)-receptor ligand, strongly stimulates GH secretion in humans. J Endocrinol Invest 2000;23:493–5.
[14] Peino R, Baldelli R, Rodriguez-Garcia J, Rodriguez-Segade S, Kojima M, Kangawa K, et al. Ghrelin-induced growth hormone secretion in humans. Eur J Endocrinol 2000;143:R11–4.
[15] Nagaya N, Kojima M, Uematsu M, Yamagishi M, Hosoda H, Oya H, et al. Hemodynamic and hormonal effects of human ghrelin in healthy volunteers. Am J Physiol Regul Integr Comp Physiol 2001;280:R1483–7.
[16] Arvat E, Maccario M, Di Vito L, Broglio F, Benso A, Gottero C, et al. Endocrine activities of ghrelin, a natural growth hormone secretagogue (GHS), in humans: comparison and interactions with hexarelin, a nonnatural peptidyl GHS, and GH-releasing hormone. J Clin Endocrinol Metab 2001;86:1169–74.
[17] Hataya Y, Akamizu T, Takaya K, Kanamoto N, Ariyasu H, Saijo M, et al. A low dose of ghrelin stimulates growth hormone (GH) release synergistically with GH-releasing hormone in humans. J Clin Endocrinol Metab 2001;86:4552.
[18] Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, et al. Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab 2001;86:5992.
[19] Dezaki K, Sone H, Yada T. Ghrelin is a physiological regulator of insulin release in pancreatic islets and glucose homeostasis. Pharmacol Ther 2008;118:239–49.
[20] Casanueva FF, Dieguez C. Growth hormone secretagogues: physiological role and clinical utility. Trends Endocrinol Metab 1999;10:30–8.
[21] Ghigo E, Arvat E, Camanni F. Orally active growth hormone secretagogues: state of the art and clinical perspectives. Ann Med 1998;30:159–68.
[22] Petersenn S. Growth hormone secretagogues and ghrelin: an update on physiology and clinical relevance. Horm Res 2002;58(Suppl 3):56–61.
[23] Neary NM, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C, et al. Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. J Clin Endocrinol Metab 2004;89:2832–6.
[24] Akamizu T, Iwakura H, Ariyasu H, Hosoda H, Murayama T, Yokode M, et al. Repeated administration of ghrelin to patients with functional dyspepsia: its effects on food intake and appetite. Eur J Endocrinol 2008;158:491–8.
[25] Strasser F, Lutz TA, Maeder MT, Thuerlimann B, Bueche D, Tschop M, et al. Safety, tolerability and pharmacokinetics of intravenous ghrelin for cancer-related anorexia/cachexia: a randomised, placebo-controlled, double-blind, double-crossover study. Br J Cancer 2008;98:300–8.
[26] Hotta M, Ohwada R, Akamizu T, Shibasaki T, Takano K, Kangawa K. Ghrelin increases hunger and food intake in patients with restricting-type anorexia nervosa: a pilot study. Endocr J 2009;56:1119–28.
[27] Nagaya N, Moriya J, Yasumura Y, Uematsu M, Ono F, Shimizu W, et al. Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure. Circulation 2004;110:3674–9.
[28] Nagaya N, Itoh T, Murakami S, Oya H, Uematsu M, Miyatake K, et al. Treatment of cachexia with ghrelin in patients with COPD. Chest 2005;128:1187–93.
[29] Garcia JM, Cata JP, Dougherty PM, Smith RG. Ghrelin prevents cisplatin-induced mechanical hyperalgesia and cachexia. Endocrinology 2008;149:455–60.
[30] Wynne K, Giannitsopoulou K, Small CJ, Patterson M, Frost G, Ghatei MA, et al. Subcutaneous ghrelin enhances acute food intake in malnourished patients who receive maintenance peritoneal dialysis: a randomized, placebo-controlled trial. J Am Soc Nephrol 2005;16:2111–8.
[31] Akamizu T, Iwakura H, Ariyasu H, Murayama T, Sumi E, Teramukai S, et al. Effects of ghrelin treatment on patients undergoing total hip replacement for osteoarthritis: different outcomes from studies in patients with cardiac and pulmonary cachexia. J Am Geriatr Soc 2008;56:2363–5.
[32] Adachi S, Takiguchi S, Okada K, Yamamoto K, Yamasaki M, Miyata H, et al. Effects of ghrelin administration after total gastrectomy: a prospective, randomized, placebo-controlled phase II study. Gastroenterology 2010;138:1312–20.
[33] Yamamoto K, Takiguchi S, Miyata H, Adachi S, Hiura Y, Yamasaki M, et al. Randomized phase II study of clinical effects of ghrelin after esophagectomy with gastric tube reconstruction. Surgery 2010;148:31–8.
[34] Cummings DE. Ghrelin and the short- and long-term regulation of appetite and body weight. Physiol Behav 2006;89:71–84.
[35] Wren AM, Bloom SR. Gut hormones and appetite control. Gastroenterology 2007;132:2116–30.
[36] Chen HY, Trumbauer ME, Chen AS, Weingarth DT, Adams JR, Frazier EG, et al. Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. Endocrinology 2004;145:2607–12.
[37] Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein mRNA levels and body weight in rats. Diabetes 2001;50:2438–43.
[38] Shintani M, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K, et al. Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. Diabetes 2001;50:227–32.
[39] Toshinai K, Date Y, Murakami N, Shimada M, Mondal MS, Shimbara T, et al. Ghrelin-induced food intake is mediated via the orexin pathway. Endocrinology 2003;144:1506–12.
[40] Date Y, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, et al. The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. Gastroenterology 2002;123:1120–8.
[41] Wren AM, Small CJ, Abbott CR, Dhillo WS, Seal LJ, Cohen MA, et al. Ghrelin causes hyperphagia and obesity in rats. Diabetes 2001;50:2540–7.
[42] Akamizu T, Takaya K, Irako T, Hosoda H, Teramukai S, Matsuyama A, et al. Pharmacokinetics, safety, and endocrine and appetite effects of ghrelin administration in young healthy subjects. Eur J Endocrinol 2004;150:447–55.
[43] Nass R, Pezzoli SS, Oliveri MC, Patrie JT, Harrell Jr FE, Clasey JL, et al. Effects of an oral ghrelin mimetic on body composition and clinical outcomes in healthy older adults: a randomized trial. Ann Intern Med 2008;149:601–11.
[44] Nass R, Farhy LS, Liu J, Prudom CE, Johnson ML, Veldhuis P, et al. Evidence for acyl-ghrelin modulation of growth hormone release in the fed state. J Clin Endocrinol Metab 2008;93:1988–94.
[45] Gibney J, Healy ML, Sonksen PH. The growth hormone/insulin-like growth factor-I axis in exercise and sport. Endocr Rev 2007;28:603–24.
[46] Velloso CP. Regulation of muscle mass by growth hormone and IGF-I. Br J Pharmacol 2008;154:557–68.