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腸管血流からみた大建中湯の役割
——アメリカ臨床治験薬 TU-100になった理由
Effect of Daikenchuto, an investigational new drug, on intestinal blood flow

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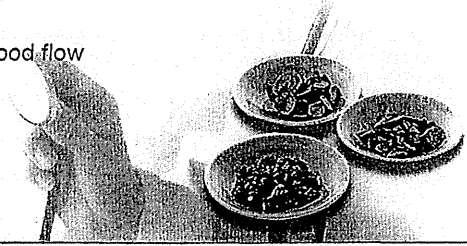
◎日本の伝統的医学である漢方のなかでも大建中湯は山椒，乾姜，人参，膠飴という食材だけで構成されているにもかかわらず，臨床でもっとも高い使用実績と信頼性を得てきた漢方薬である．大建中湯の腸管血流改善作用を検討してきた結果，大建中湯の主要構成生薬である山椒の主成分 hydroxy-α-sanshool，乾姜の主成分 6-shogaol が直接または血中に吸収されて，腸管神経終末からカルシトニン遺伝子関連ペプチド（calcitonin gene related peptide：CGRP），腸管上皮細胞からアドレノメデュリン（adrenomedullin：ADM）という 2 つの強力な微小血管拡張作用を有するカルシトニンファミリーペプチドを動員することを明らかにし，その詳細な作用機序として標的細胞膜上にある transient receptor potential（TRP）チャネルを介していることも明らかにした．また，CGRP 減少が病因論的に関与している Crohn 病への大建中湯の治療的効果を動物レベルで証明し，アメリカ FDA によって臨床治験薬 TU-100 として認知され，現在全米多施設プラセボ二重盲検試験が開始されていることを紹介する．

Keywords　大建中湯，カルシトニン遺伝子関連ペプチド，アドレノメデュリン，
トランジェントレセプターポテンシャルチャネル，Crohn 病，腸管血流

眉唾物扱いからアメリカの臨床治験薬へ

アメリカでもっとも優れた医療施設のひとつとされるメイヨー・クリニックの消化器外科のシニアコンサルタントで併設医科大学の外科教授でもあるサール先生は，2009 年春の第 109 回日本外科学会（会頭：田中雅夫教授/九州大学）に招待され，来日された．以前よりアメリカの主流であるハーバルメディシン中医は眉唾物と信じ込んでいて（「サイドメモ 1」参照），日本に来られたついでに日本のハーバルメディシン漢方が本物か眉唾かどうかを検証しようと乗り込んでこられ，著者らが迎え撃つことになった．著者らは大建中湯の作用機序，とくに腸管血流に関する基礎的エビデンスを分子レベルで紹介．1 時間の予定が数時間にも及ぶディスカッションとなり，漢方が眉唾物ではなくて日本の真の伝統医療であり，その臨床での

可能性を理解しはじめた様子であった．別れる際に，自分が編集している『*Surgery*』誌の巻頭総説に漢方の総説を推薦したいので論文を書くよう

サイドメモ 1

漢方薬と中医薬

漢方薬は 500 年前に中国から伝来した中医を基礎としてはじまり，日本独自の生薬調合が繰り返され，完成された日本の伝統医療である．個々の薬剤名称は変更されることがなかったため，中国で発展してきた中医薬と同名であっても含まれる生薬が異なることに注意を要する．よくマスコミで両者を区別せずに使用しているが，両者はまったく別ものである．大建中湯の山椒は同名の中医では含まれていないし，六君子湯は漢方では 8 種類の生薬が含まれているが，中医では 6 種類である．中医薬の一部はアメリカにてサプリメントとして広く販売されているが，多くの副作用が発生し，その品質が問題となっている．

に，と勧めていただき，力強い握手とともにお約束し数カ月でその約束を果たし，論文は"漢方の代替医療からの脱出"というかなり刺激的な題名で出版された[1]．その後，サール教授はアメリカにおける強力な漢方薬サポーターに大変身された．

当時，伝統医療の主役であるハーバル・メディシン（漢方は含まれていない）は，欧米諸国では代替補完医療の枠組みのなかにあり，エビデンス重視の現代医療のなかに組み込まれることを阻んできた．しかし，高騰する医療費と合成薬剤の限界から，安全性と品質で日本の伝統医療である漢方に対する期待感が巻き起こり，アメリカの食品医薬品局（FDA）が合剤としてはじめて臨床治験薬剤，TU-100（ツムラ大建中湯）を認可するに至った[2]．最近まで日本でも，漢方に対する偏見から多くの医師が漢方に関心を示すことはなかった．しかし，大建中湯のエビデンスレベルの高い基礎研究を契機に現在，全国大学病院の80％が参加する二重盲検プラセボ比較試験が展開されており，大建中湯の有効性が示唆される高いエビデンスレベルをもった臨床データが出はじめている．それは日本よりアメリカで先行している[2]．

大建中湯は医食同源タイプの シンプルな漢方薬

大建中湯に使用されている材料はすべて食材である．抽出生薬は3種類で山椒（サンショウ2.2％），乾燥させた生姜である乾姜（カンキョウ5.6％），朝鮮人参（ニンジン3.3％）で，残りはマルトース（膠飴コウイ）やラクトースでできている[1]．つまり特殊な材料は含まれていない，きわめてシンプルな漢方薬といえる．大建中湯は1回2.5〜5g，1日3回食前に服用することを基本としている．服用しやすくしているのはマルトースのおかげであり，甘く飲みやすい工夫がなされているが，二糖類なので低カロリーで甘みも1/3程度に抑えられている．ちなみに大建中湯の大（ダイ）はきわめてという意味で，建（ケン）は建て直す，中（チュー）は消化管を意味し，湯（トウ）は水溶性

を意味し，合わせて消化管を大きく建て直すという意味で大建中湯と名づけられているそうである．

保険適応症は腹部膨満感，腹部の冷えの改善であるが，実臨床では術後の腸管運動麻痺改善目的で使用されることが多く，漢方薬のなかでもっとも多く使用されているが，保険収載されたのは比較的最近で1986年である．副作用に関してFDAから要請のあった副作用調査でも1％以下で，重篤なものはまったくない，安全性がきわめて高い薬剤である．構成材料が食材であることを考えれば自明である．

腸管粘膜血流の特色と疾患への関与

消化管粘膜のエネルギー消費量は高く，大腸では細菌によってつくられる短鎖脂肪酸を栄養素として，血流からのブドウ糖だけでは不足する栄養素を補っている．そのような絶妙なバランスで保たれている腸管粘膜にいったん虚血状態が発生すれば，腸管バリア機能障害，バクテリアトランスロケーションを経て全身炎症反応に至る．高齢者では動脈硬化による虚血性腸疾患を発症しやすく，若年者に多い炎症性腸疾患では血液凝固系が亢進している状態であり，腸間粘膜血流の血栓などによる血流不全が病因論的に関与していることが指摘され，抗凝固剤の併用が高いエビデンスレベルで推奨されている．また，腸間膜側に縦走潰瘍を形成するCrohn病の発症原因としても，腸間膜側の血流維持の解剖学的脆弱性から説明できるという報告もある．このように腸管粘膜血流は，多くの腸疾患に病因論的に関与している．

大建中湯の成分レベルからみた 腸管粘膜血流改善機序

大建中湯は臨床で高い実績と信用を得た漢方薬であり，とくに術後の麻痺性イレウス改善などを期待して使用されてきた．エビデンスはかならずしも十分とはいえないが，機序として腸管運動神経の関与が示唆され，腸管運動改善が期待できる

というものである[3-7].

ところが，大建中湯の健康保険上の適応は"腹が冷えて痛み，腹部膨満感のあるもの"とある．つまり腸管運動改善効果は腹部膨満感の改善につながると考えられるが，適応の最初にある"腹が冷えて痛む"という点に関しての機序にはつながらない．実際，患者に大建中湯服用後の様子を尋ねると，しばしばお腹が温かくなるという経験談を聞くことができる．そこで，腹の冷えを改善することは消化管の血流改善と置き換えることができると著者らは考え，最初に腸管血流増加機序解明を目的に実験を行い，大建中湯の腸管血流改善機序を薬理学的・分子生物学的に明らかにしてきた．

カルシトニンファミリーペプチド

大建中湯の腸管血流改善機序に関して最初に注目したのは，カルシトニン遺伝子関連ペプチド(calcitonin gene-related peptide：CGRP)である．すでに大建中湯の腸管運動亢進作用にCGRPが関与することは報告されていた[8]．このCGRPは微小血管拡張作用が最強の神経ペプチドであり，神経終末から放出され，血管平滑筋に作用し血管拡張を起こすことが知られていた[9,10]．小動物を用いた実験で腸管血流増加を大建中湯が起こし，その血流増加がCGRP拮抗薬によってほぼ完全に抑制され，その他の血流に関連する神経ペプチド(サブスタンスP，VIPなど)の拮抗薬では抑制されないことから，CGRPが重要な機序因子であることが示唆された[11]．

つぎに受容体レベルでの検討の結果，CGRPの受容体だけでなく，同じカルシトニンファミリーペプチドであるアドレノメデュリン(adreno-medullin：ADM)が大建中湯の血流改善機序に関与していることが示唆された．ADMはCGRPほど強い微小血管拡張作用はないが，相応の微小血管拡張作用がある[12]．産生部位は，CGRPとは大きく異なり非神経組織，上皮細胞や平滑筋などである．さらにCGRPとADMはともに抗炎症性作

用，抗炎症性サイトカイン作用があり，大建中湯の多彩な作用を理解するうえできわめて重要な鍵となると考えられた[1]．

有効成分の同定と薬物動態

有効成分同定のため *in vitro* 研究を進めた結果，大建中湯の山椒と乾姜がADMとCGRPを動員することが観察された．山椒，乾姜の主だった成分をランダム試験で解析を進めると，山椒のhydroxy-α-sanshoolと乾姜の6-shogaolがその有効主成分であることが判明した[13]．

しかし，ここで大きな疑問点が生じた．大建中湯の成分は吸収されるのか，それともされないのかという西洋薬では開発時点で明らかとなっているべき点が，大建中湯では不明であった．そこで，臨床試験と動物試験で薬物動態を検討することにした．その結果，驚くべきことに山椒の主成分であるhydroxy-α-sanshoolは5分以内に血中に大量に存在することが判明，乾姜の6-shogaolは吸収されるが緩徐であり，低レベルであることが判明した[14]．つまり，大建中湯の血流改善効果は直接的だけでなく，血中を介しても起こることが示唆されたのである．薬物動態を詳細に検討された

サイドメモ2

TRPチャネル

1989年にショウジョウバエの眼の光刺激に対して受容体電位が一過性に変化することからtransient receptor potential(TRP)チャネルと名づけられたもので，6回膜貫通型イオンチャネルとしてCa²⁺イオンの高い透過性を有しており，6つのサブファミリーが同定されている．その大きな生理的役割は外界刺激に対応して種々の生理活性物質を活性化することであり，生体"センサー"蛋白質として働くことが知られている．その内容は多岐にわたり，温度刺激・化学刺激・機械刺激・pH・酸化還元・浸透圧などがある．自然物から抽出された物質にTRPチャネル刺激物が多いとされている．
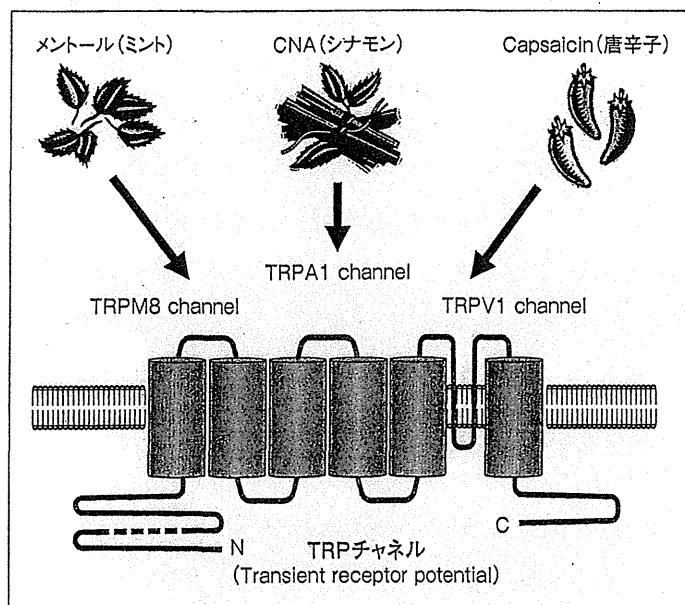
図 1 TRPチャネルとその刺激物
多くの自然物は TRP チャネルの刺激物を含む.

ハーバルメディシンは，大建中湯が世界初である．現在，人工肛門からの便を採集して，どの程度の各成分が大腸に到達するのか検討しており，その結果が待たれる．

トランジェントレセプターチャネル（TRP チャネル）

　大建中湯の有効成分が，どのようにして細胞を刺激して ADM や CGRP を動員するのであろうか，というやっかいな問題を解決するため，著者らは TRP チャネルに着目した（「サイドメモ 2」参照）．TRP チャネルは生体における温度などを感受する生体センサーで，Ca^{2+} イオンを通すチャネルであり，おもに神経組織に発現しているが，最近では腸管上皮細胞にも存在することが報告されている．ミントを含むガムをかむと口のなかが冷たく感じるのは，ミントが冷たいということを感受する TRP チャネル（TRPM8 チャネル）を特異的に刺激する分子だからである．そのほかにも多くの自然物が，TRP チャネルの刺激分子となっている（図 1）．漢方は自然物，植物からできており，

多くの TRP チャネルの刺激分子を含んでいることが容易に想像される．そこで，大建中湯の有効成分である山椒の hydroxy-α-sanshool と乾姜の 6-shogaol が TRP チャネルの刺激分子となっていないか調べたところ，多くの論文ですでに検討されており，TRPA1 と TRPV1 の刺激分子であることがわかっていた．そこで，大建中湯のターゲット細胞のひとつである腸管粘膜上皮細胞に両 TRP チャネルが発現しているか調べたところ TRPA1 のみが発現しており，山椒の hydroxy-α-sanshool と乾姜の 6-shogaol による ADM の分泌が TRPA1 拮抗薬剤でブロックされ，TRPA1 遺伝子を抑制すると ADM の分泌が抑制されることが観察された．さらに，TRPV1 アゴニストで刺激しても ADM の分泌は観察されなかった．これらのことから，大建中湯は山椒の hydroxy-α-sanshool と乾姜の 6-shogaol によって，ターゲット細胞の TRPA1 カルシウムチャネルを介して ADM を分泌させていることが明らかとなった（図 2）．
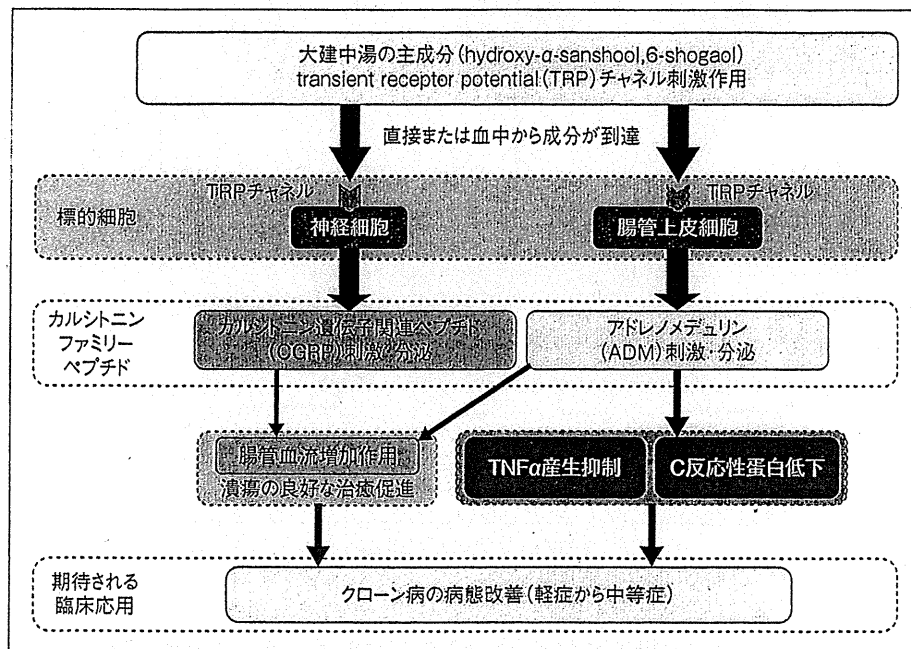
図 2 大建中湯の作用機序と期待される臨床応用

カルシトニンファミリーペプチドと Crohn 病

　大建中湯が神経終末から CGRP，粘膜上皮細胞から ADM という 2 つのカルシトニンファミリーペプチドを放出させる機序を述べてきたが，以前より CGRP および ADM に関して，消化器分野では Crohn 病と強く関連づける報告が臨床的にも実験的にもなされている．とくに，繰り返す炎症による神経組織へのダメージも大きく，神経組織で産生される CGRP の特異的な減少が起こり，Crohn 病腸管の 50％近い血流低下に関与していることが示唆され，動物モデルで外来性 CGRP に治療的効果があると報告されている．また，ADM に関しても動物モデルで外来性に投与することで治療的効果があることが報告されており，CGRP と ADM の Crohn 病治療薬としての可能性が示唆される結果となっている．ところが，外来性にこれらのペプチドを投与することは，全身の循環動態への影響，デリバリーの問題などから不

可能であるとも考えられている．しかし，CGRPや ADM が腸管粘膜血流維持に重要であることは疑いのないことである．

　そこで著者らは大建中湯が，CGRP がうまく働かない状態の Crohn 病腸管において腸管粘膜上皮から ADM を放出させ，CGRP 減少を補う形で腸管血流を正常化させる可能性があるという仮説を得た．さらに，ADM は炎症性サイトカイン TNF-α の産生を抑制する作用が報告されており，現在の Crohn 病治療におけるもっとも有効な治療薬である抗 TNF-α 抗体(インフリキシマブやアダリムマブ)とまったく同じ治療ターゲットであることから，Crohn 病に対して治療効果があるのではないかと考え，Crohn 病動物モデルでこれらのことを検証した結果，大建中湯の治療効果は明らかで，Crohn 病腸管血流改善効果，抗炎症性サイトカイン，とくに TNF-α の抑制効果，さらに C 反応性蛋白 CRP を抑制することが観察された(図 2)[13,15]．

　レミケードは TNF-α に対する抗体で，重症の Crohn 病に開発された薬剤である．一方，Crohn

病患者の75％は軽症から中等症に分類されるが，これらに対する新規薬剤開発は行われてこなかった．そこで，軽症から中等症のCrohn病患者に対する大建中湯(TU-100)の効果を確かめるプラセボ使用の二重盲検臨床治験を，シカゴ大学の炎症性腸疾患センターを中心とした全米多施設(レミケード臨床治験と同じチーム)で2011年秋よりスタートした．詳細はFDAのWebで公開されている．同じく大建中湯を使った二重盲検臨床治験はメイヨー・クリニック，ミネソタ大学などアメリカの超一流の施設で開始されているが，これらの事実はまだ多くの日本の医師たちには知られていない．また，すでにメイヨー・クリニックでの臨床治験で大建中湯の有効性が報告されていることを強調したい[2]．

おわりに

これまでアメリカではハーバルメディシンを単なる代替医療としてとらえ，国立補完代替医療センターなどが多額な予算を使い，エビデンスを得るべく研究活動を行ってきたが，成果はまったく得られず議会から追及されるに至った．そこで，彼らが目をつけたのが日本のハーバルメディシン，漢方であった．高度に医療の発達した日本において漢方が保険収載され，西洋医学中心の医師らによって使われていることに彼らは驚き，同時に安心感ももった．漢方は西洋医学的にとらえれば500年という長期にわたる第Ⅰ相試験を繰り返してきた日本伝統医学であり，漢方は毒性，安全性が担保されたハーバルメディシンであるともいえる．世界的にハーバルメディシンを保険収載薬として西洋薬と同時に処方できる国は日本だけである．つまり西洋医学と漢方を同時に使用できるわれわれ日本人医師が，基礎医学にも臨床医学レベルでエビデンスを構築し世界に向けて発信することはとても意義のあることである．新薬開発に莫大な経費がかかる現代で，漢方薬は新規薬剤開発の手がかりとなる宝庫であり，また漢方薬そのものがこれまでにない適応を発見したりすること

で，大きな展開を生む可能性まである．その先頭に立つのが大建中湯であるといえよう．

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Epithelial transient receptor potential ankyrin 1 (TRPA1)-dependent adrenomedullin upregulates blood flow in rat small intestine

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[1]*Center for Clinical and Biomedical Research, Sapporo Hisgashi Tokushukai Hospital, Hokkaido, Japan;* [2]*Division of Gastroenterologic and General Surgery, Department of Surgery, Asahikawa Medical University, Hokkaido, Japan; and* [3]*Tsumura Research Laboratories, Tsumura and Co., Ibaraki, Japan.*

Kono T, Kaneko A, Omiya Y, Ohbuchi K, Ohno N, Yamamoto M. Epithelial transient receptor potential ankyrin 1 (TRPA1)-dependent adrenomedullin upregulates blood flow in rat small intestine. *Am J Physiol Gastrointest Liver Physiol* 304: G428–G436, 2013. First published December 28, 2012; doi:10.1152/ajpgi.00356.2012.—The functional roles of transient receptor potential (TRP) channels in the gastrointestinal tract have garnered considerable attention in recent years. We previously reported that daikenchuto (TU-100), a traditional Japanese herbal medicine, increased intestinal blood flow (IBF) via adrenomedullin (ADM) release from intestinal epithelial (IE) cells (Kono T et al. *J Crohns Colitis* 4: 161–170, 2010). TU-100 contains multiple TRP activators. In the present study, therefore, we examined the involvement of TRP channels in the ADM-mediated vasodilatatory effect of TU-100. Rats were treated intraduodenally with the TRP vanilloid type 1 (TRPV1) agonist capsaicin (CAP), the TRP ankyrin 1 (TRPA1) agonist allyl-isothiocyanate (AITC), or TU-100, and jejunum IBF was evaluated using laser-Doppler blood flowmetry. All three compounds resulted in vasodilatation, and the vasodilatatory effect of TU-100 was abolished by a TRPA1 antagonist but not by a TRPV1 antagonist. Vasodilatation induced by AITC and TU-100 was abrogated by anti-ADM antibody treatment. RT-PCR and flow cytometry revealed that an IEC-6 cell line originated from the small intestine and purified IE cells expressed ADM and TRPA1 but not TRPV1. AITC increased ADM release in IEC cells remarkably, while CAP had no effect. TU-100 and its ingredient 6-shogaol (6SG) increased ADM release dose-dependently, and the effects were abrogated by a TRPA1 antagonist. 6SG showed similar TRPA1-dependent vasodilatation in vivo. These results indicate that TRPA1 in IE cells may play an important role in controlling bowel microcirculation via ADM release. Epithelial TRPA1 appears to be a promising target for the development of novel strategies for the treatment of various gastrointestinal disorders.

daikenchuto; TU-100; vasodilatation; 6-shogaol; inflammatory bowel diseases

TRANSIENT RECEPTOR POTENTIAL (TRP) channels are nonselective calcium ion channels ubiquitously expressed in many tissues and are known to participate in a broad range of physical, chemical, and environmental stimuli such as taste, temperature, changes in osmolarity, pressure, stretch, and light.

TRP channels are divided into seven subfamilies with 27 different channel types present in humans. Natural products, especially medicinal and culinary herbs such as chili pepper, mustard oil, and menthol, are known to stimulate some of these TRP channels. In recent years there has been a growing interest in elucidating the role of TRP channels in gastrointestinal physiology, including intestinal motility, secretion, and visceral sensation (23, 24, 39, 53). However, the physiological implications of TRP channels in intestinal blood flow (IBF) remain largely unexplored.

Daikenchuto (TU-100), a traditional Japanese herbal medicine (Kampo), is a mixture of extract powders from dried Japanese pepper, processed ginger, ginseng radix, and maltose powder. TU-100 is the most frequently prescribed Kampo medicine in Japan, especially for the treatment of postoperative paralytic and adhesive ileus and ischemic intestinal disorders (28). Basic studies have demonstrated the effect of TU-100 on intestinal motility, adhesion, vasodilatation, inflammation, and bacterial translocation (15, 22, 25, 27, 29, 30, 38, 44–47, 51, 52, 58). In a previous study, we demonstrated that TU-100 increases IBF via enhancement of adrenomedullin (ADM) release from the intestinal epithelial (IE) cells (27). However, the mechanism by which TU-100 enhances ADM release has not been elucidated.

Because some of the major ingredients of TU-100, such as 6-shogaol (6SG) and hydroxy-α-sanshool (HAS), are regarded as TRP vanilloid type 1 (TRPV1)/TRP ankyrin 1 (TRPA1) agonists (21, 31), we hypothesized that TRPV1/TRPA1 stimulation increases IBF via enhancement of ADM release from IE cells, and that the beneficial effect of TU-100 on IBF is mediated by this mechanism. Our results strongly suggest that TRPA1 present in IE cells controls IBF via ADM release and, therefore, the stimulation of intraluminal TRPA1 may be a promising approach for the relief of abdominal symptoms in various intestinal disorders associated with impaired IBF.

MATERIALS AND METHODS

Test sample and reagents. TU-100 is an aqueous extract containing processed ginger, ginseng radix, and Japanese pepper in a ratio of 5:3:2. The dried powdered extract form of TU-100 was obtained from Tsumura and Co. (Tokyo, Japan). The yield of the extract was 12.5%. TU-100 is prepared by mixing TU-100 extract powder and maltose syrup powder (Tsumura and Co.) at a ratio of 1:8. Although the doses of TU-100 in the present study (270–2,700 mg/kg body wt) are higher than the clinical doses used in humans, previous studies in animals have shown that the relevant pharmacological effects occur only in the experimental doses. Furthermore, treatment of rodents with TU-100 at this higher dose range results in blood concentrations of major TU-100 constituents that are similar to those detected in human volunteers treated with TU-100 at clinical dose range (18, 37).

Ginsenoside Rb1, ginsenoside Rg1, ginsenoside Rd, protopanaxadiol, 6SG, 6-gingerol, 10-gingerol, maltose, allyl-isothiocyanate (AITC), and capsaicin (CAP) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Urethane, α-chloralose, cinnamalde-

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hyde (CNA), methyl cinnamate, 2-aminoethoxydiphenyl borate (2-APB), 4α-phorbol 12,13-didecanoate (4α-PDD), H-89, calphostatin C, LY294002, and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma Aldrich (St. Louis, MO). HAS and hydroxy-β-sanshool (HBS) were extracted from Japanese pepper at Tsumura and Co. with purities greater than 97.9%. Xanthoxylin (Tokyo Chemical Industry, Tokyo), butorphanol (Bristol-Myers Squibb, New York), HC-030031 (Biomol International, Plymouth Meeting, PA), and N-(4-tertiarybutylphenyl)-4-(3-chloropyridin-2-yl)-tetrahydropyrazine-1(2H) carboxamide (BCTC; Biomol International) purchased for the study as well the other reagents used for analysis were the highest purity commercially available.

Animals. Seven-week-old male Sprague-Dawley rats weighing 210–230 g were purchased from Japan SLC (Shizuoka, Japan). The animals were allowed free access to water and standard laboratory food, and housed at a temperature of $23 \pm 2°C$ with relative humidity of $55 \pm 10\%$, and a 12:12-h light/dark cycle with lights on from 0700–1900 daily. All experimental procedures were performed according to the Guidelines for the Care and Use of Laboratory Animals of Asahikawa Medical University or Tsumura and Co. Ethical approval for the experimental procedures used in this study was obtained from the Laboratory Animal Committee of Asahikawa Medical University or Tsumura and Co. All animal procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Measurement of intestinal blood flow. Jejunal blood flow was measured by a laser-Doppler flowmeter (ALF21N, Advance, Tokyo) as previously described (30). Briefly, rats were anesthetized with urethane (900 mg/kg ip), α-chloralose (45 mg/kg ip), and butorphanol (1 mg/kg im). A tracheotomy was performed and the rats were artificially ventilated. The left cervical artery was cannulated and connected to a transducer (P23XL, Nihon Kohden, Tokyo) to monitor systemic arterial blood pressure (AP) and heart rate (HR). Body temperature was maintained at $37 \pm 0.5°C$ by a heating pad. After exposing the small intestine by a midline laparotomy, a cannula was inserted into the duodenum to facilitate injection of the test sample. A fiber optic probe was positioned 4 mm above the surface of the midjejunum. Vascular conductance (VC), calculated as the quotient of mean blood flow divided by mean AP, was used as an index of IBF.

Antagonist and antibody studies in vivo. Rabbit polyclonal IgG (50 μg/kg) against rat ADM (Peninsula Laboratory, Belmont, CA), rabbit IgG as an isotype-matched control (Abcam, Cambridge, UK), or the TRPV1 antagonist BCTC (10 mg/kg) was injected at a volume of 1 ml/kg through a polyethylene tube cannulated into the right jugular vein after confirming stable blood flow. TU-100 or a related vasodilator was administered intraduodenally 15 min later. The TRPA1 antagonist HC-030031 prepared in 1% DMSO was administered into the lumen at 1 mg·5 ml^{-1}·kg^{-1} together with the test sample.

Quantitation of ADM. Plasma ADM levels were assayed using enzyme immunoassay (EIA) kits specific for rat ADM according to the procedure provided by the manufacturer (Phoenix Pharmaceuticals, Burlingame, CA). Briefly, 5 ml blood was collected from the portal vein at 15, 30, 60, and 120 min after administration of TU-100 (2,700 mg/kg), and plasma was separated immediately. The plasma was then applied to ADM extraction using a C18 Sep-Column. The detection limit for ADM was 10 pg/ml. ADM release was assayed using an IEC-6 rat intestinal epithelial cell line (DS Pharmaceuticals, Osaka, Japan). IEC-6 cells were grown in DMEM supplemented with 10% heat-inactivated FBS, 2 mmol/l L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10 mmol/l HEPES. Cells between the 30th and 37th passage were plated in 96-well flat-bottom microtiter plates at 2×10^4 cells/well in DMEM supplemented with the same additives as described above, allowed to settle overnight, and then culture fluids were replaced with HBSS containing 0.1% BSA, 0.1–0.3% DMSO. TU-100 was added to the culture after being passed through a 0.45-μm filter. Cells were incubated for 6 h, and ADM in the culture fluids was quantified using EIA kits specific for rat ADM.

To investigate functional expression of TRPA1 in IEC-6 cells, the cell was exposed to the TRPA1-selective antagonist HC-030031 (100 μmol/l) 30 min before addition of TRPA1 activators.

Preparation of IE cells from small intestine. Segments of the small intestine were everted, end-ligated, and preincubated in HBSS containing 1 mmol/l DTT and 10% FBS to remove mucus. The sacs were then incubated for 10 min at 37°C in chelating digestive buffer (70 mmol/l NaCl, 5 mmol/l KCl, 20 mmol/l NaHCO$_3$, 0.5 mmol/l NaH$_2$PO$_4$, 1 mmol/l Na$_2$HPO$_4$, 50 mmol/l HEPES, 11 mmol/l glucose, 1 mmol/l EDTA, 0.5% BSA, and 0.05 mmol/l DTT), followed by collection of the supernatant. The incubation was repeated twice, and the supernatants of each were pooled. The cell pellets obtained by centrifugation at 300 g for 10 min were suspended in 0.1% BSA HBSS and passed through a nylon mesh filter. The cell suspension was applied to a 25% gradient of Percoll (GE Healthcare, Piscataway, NJ). After centrifugation at 710 g for 30 min, the interface containing enriched IE cells was collected. IE cells were separated into negative fractions using a BD IMag cell separation system (BD Biosciences, San Jose, CA) with rabbit anti-nerve growth factor receptor p75 antibody (Millipore, Bedford, MA), followed by biotinylated anti-rabbit Ig (BD Bioscience) and biotinylated anti-CD45 antibody (clone, OX-1; BD Bioscience), and thereafter incubated with streptavidin-labeled magnetic beads. Further, purified IE cells were stained with various cell-marker antibodies following a cytospin. Antibodies and positive cell percentages were wide cross-reactivity anti-cytokeratin (DAKO, Carpinteria, CA) at >90%, and anti-E-cadherin (clone, 36/E-cadherin; BD Bioscience) at >95%. Positive staining with anti-CD45 (clone, OX-1; BD Bioscience), anti-PGP9.5 (clone, 13C4/I3C4; Abcam), or anti-GFAP (clone, GF12.24; Progen, Heidelberg, Germany) was not detected.

Gene expression. The pellets of IEC-6 cells, enriched IE cells obtained from the small intestines, and L1 to L6 dorsal root ganglia (DRG) isolated from normal rats were homogenized in QIAzol reagent (Qiagen, Valencia, CA), and total RNA was isolated using an RNeasy kit (Qiagen) according to the manufacturer's recommendations. The respective cDNA was prepared using a high-capacity RT kit (Applied Biosystems, Warrington, UK). The sequences of the sense and antisense primers for rat TRPA1 were 5′-TTTGCCGCCAGCTATGGGCG-3′ and 5′-TGCTGC-CAGATGGAGAGGGGT-3′ to obtain a 117-bp product. Those for rat TRPV1 were 5′-GGTGTGCCTGCACCTAGC-3′ and 5′-CTCT-TGGGGTGGGGACTC-3′ to obtain a 107-bp product. Those for rat ADM were 5′-CTCGACACTTCCTCGCAGTT-3′ and 5′-GCTG-GAGCTGAGTGTGTCTG-3′ to obtain a 446-bp product. Those for rat β-actin were 5′-CCTGGGTATGGAATCCTGTGGCAT-3′ and 5′-GGAGCAATGATCTTGATCTTC-3′ to obtain a 198-bp product. An aliquot of the RT reaction product served as a template in 30 cycles with 10 s of denaturation at 98°C, 30 s of annealing at 60°C, and 30 s of extension at 68°C using the DNA polymerase KOD FX (TOYOBO, Osaka, Japan). A portion of the PCR mixture was electrophoresed on 2% agarose gel in Tris-acetate-EDTA buffer (pH 8.0), and the gel was stained with ethidium bromide and imaged on a Typhoon 9410 imager (GE Healthcare). Sample-to-sample variation in RNA loading was controlled by comparison with β-actin.

Flow cytometry. Single cells were suspended in Cytofix/Cytoperm solution (BD Biosciences) for 20 min at 4°C, washed, and then preincubated for 5 min at 4°C with goat polyclonal IgG antibody (Abcam) to reduce nonspecific binding of antibodies. Next, cells were incubated for 20 min at 4°C with rabbit polyclonal IgG antibody (4 μg/ml) against rat ADM, rat TRPA1 (Abcam), TRPV1 (Alomone Labs, Jerusalem, Israel), or isotype control IgG (Abcam). Cells were washed, incubated for 20 min with the Alexa Fluor 488-labeled goat polyclonal antibody against rabbit IgG (Invitrogen, Carlsbad, CA), and subjected to flow cytometry analysis using a FACScalibur analyzer and CellQuest Pro software (BD Biosciences). In some experiments, a control peptide for TRPA1 or TRPV1 (Abcam) was added at 4 μg/ml with antigen-specific antibody.

Calcium influx in rat TRPA1-transfected cells. A rat TRPA1-expressing cell line was generated using a tetracycline-inducible T-Rex expression system (Life Technologies, Grand Island, NY). T-Rex293 cell (Life Technologies) was transfected stably with plasmids encoding rat TRPA1 (pcDNA4/TO-rat TRPA1) using FuGENE HD Transfection Reagent (Roche, Indianapolis, IN) according to the manufacturer's instructions. Control cell was transfected with the pcDNA4/TO vector alone. Intracellular calcium was measured 1 day after induction with tetracycline (1 μg/ml). Cells were washed with an assay buffer (115 mmol/l NaCl, 5.4 mmol/l KCl, 13.8 mmol/l glucose, 2.5 mmol/l probenecid, 20 mmol/l HEPES, pH 7.6) and then loaded with Fluo-4 dye (Dojindo, Kumamoto, Japan). After 30 min incubation, cells were washed with the assay buffer. Then the test compound was added to each well. Fluorescence intensity was measure by FlexStation3 (Molecular Devices, Sunnyvale, CA). Concentration-response curves were fitted using Prism 3.0 with a Hill equation model.

Statistical analysis. All values are expressed as means ± SE. The statistical significance was evaluated by one- or two-way analysis of variance (ANOVA) followed by Dunnett's test or Student's *t*-test. A probability of less than 0.05 was considered significant.

RESULTS

Upregulation of IBF by TRPV1 and TRPA1 stimulation. We first investigated the vasoactive effect of TRPV1 and TRPA1 agonists administered into the lumen of the small intestine. The TRPV1 agonist CAP (3 mg/kg) caused a rapid increase in IBF, which peaked 15 min after administration and remained at high levels throughout data acquisition (Fig. 1A). The TRPA1 agonist AITC (0.002 mg/kg) produced a gradual increase in vasodilatation which peaked at 120 min or later (Fig. 1B). Neither of the agonists influenced systemic circulation (data not shown), and therefore, the effects were limited to the local microcirculation. The TRPV1-selective antagonist BCTC and the TRPA1-selective antagonist HC-030031 diminished the vasodilatory effect of CAP and AITC, respectively. Both antagonists had no effect by themselves (data not shown).

Involvement of TRPA1 and ADM in the vasodilatory effect of TU-100. Past studies have shown that TU-100 increases blood flow in the small intestine of normal rats (38) and potentiates the production of vasoactive ADM by IE cells (27, 30). Accordingly, we sought to identify the TRP channel involved in the vasodilatory effect of TU-100. IBF increase by administration of TU-100 (2,700 mg/kg) was largely attenuated by pretreatment with HC-030031, but BCTC showed no effect (Fig. 2, A and B). We next addressed whether ADM is critical for the vasodilatory effect of TU-100. As shown in Fig. 2C, vascular conductance at 90, 105, and 120 min was decreased significantly ($P < 0.01$) by pretreatment with antibody against

ADM. In accordance with the above findings, ADM concentrations in plasma of the portal vein (Fig. 2D) were elevated significantly at 15, 30, and 60 min by administration of TU-100 (2,700 mg/kg). Finally, the vasodilatory effect by AITC was also abrogated by anti-ADM treatment (Fig. 2E).

Expression of TRPA1 and ADM in IEC-6 and purified IE cells. We previously reported immunohistochemical identification of ADM in the mucosal epithelium of the small and large intestines of SD rats, the same strain used in the present study (30). Here we examined the expression of TRPA1 and TRPV1 mRNAs in IEC-6 cells and purified IE cells obtained from the intestines. The expression of TRPA1 mRNA was clearly detected in these cells, as was DRG (Fig. 3A), while gene expression of TRPV1 was below the detection limit. TRPA1 protein levels in these cells were evaluated by flow cytometric analysis. As shown in Fig. 3B, the fluorescence intensities for anti-TRPA1 and anti-ADM antibody were higher than those of the subtype-control antibody. Marked reduction of fluorescence intensity by coexistence of the epitope peptide of TRPA1 antigen indicated that both of these cells types expressed TRPA1 protein.

ADM releasing activity of TRPA1 agonists and TU-100. Considering the expression of TRPA1 and ADM in IE cells, we investigated the ability of TRP channel agonists to release ADM. Samples tested were CAP, AITC, and CNA (TRPA1 agonists), 2-APB (agonist of TRPV1, TRPV2, and TRPV3), and 4α-PDD (TRPV4 agonist). As shown in Fig. 4A, the ADM concentrations in the culture fluids from rat IEC-6 cells treated with AITC (3–30 μmol/l) or CNA (100 μmol/l) were several times greater than control. On the other hand, CAP, 2-APB, and 4α-PDD were inactive in the test. As for TU-100 (Fig. 4B), the ADM concentrations in the culture fluids from IEC-6 cells with 270, 900, or 2,700 μg/ml of TU-100 were 16 ± 1, 17 ± 1, and 19 ± 1 pg/mL, respectively. These concentrations were 1.44, 1.60, and 1.74 times greater than control (11 ± 1), respectively. We then sought to identify the active ingredients responsible for the enhancement of ADM release. Twelve main ingredients were tested (Fig. 4, C–E). 6SG at concentrations of 10 and 30 μmol/l dramatically increased ADM release (2.27 and 8.30 times greater than control, respectively) with no cytotoxic effects. HAS significantly enhanced ADM release at concentrations of 30 and 100 μmol/l (1.49 and 1.83 times, respectively), although its activity was weaker than that of 6SG. 6-Gingerol was inactive in this test. Considering the intensity of ADM release activity and the high 6SG content in TU-100, 6SG appears to be the main active ingredient responsible for the vasodilatory effect of TU-100.

Fig. 1. Intraluminal transient receptor potential (TRP) vanilloid type 1 (TRPV1) and TRP ankyrin 1 (TRPA1) agonists increase blood flow in the small intestine. Capsaicin (CAP, 3 mg/kg body wt) or allyl isothiocyanate (AITC, 0.002 mg/kg body wt) was administered intraduodenally, and vascular conductance (VC) in the midjejunum was monitored. *A*: TRPV1 antagonist N-(4-tertiarybutylphenyl)-4-(3-chloropyridin-2-yl)tetrahydropyrazine-1(2H)-carboxamide (BCTC) (10 mg/kg) was given intravenously 15 min before CAP administration; $N = 3$; *B*: TRPA1 antagonist HC-030031 (1 mg/kg) was administrated intraluminally together with AITC; $N = 5$–6. *$P < 0.05$, **$P < 0.01$ vs. water + vehicle (*A*) or vehicle (*B*). #$P < 0.05$, ##$P < 0.01$ vs. agonist alone, respectively.
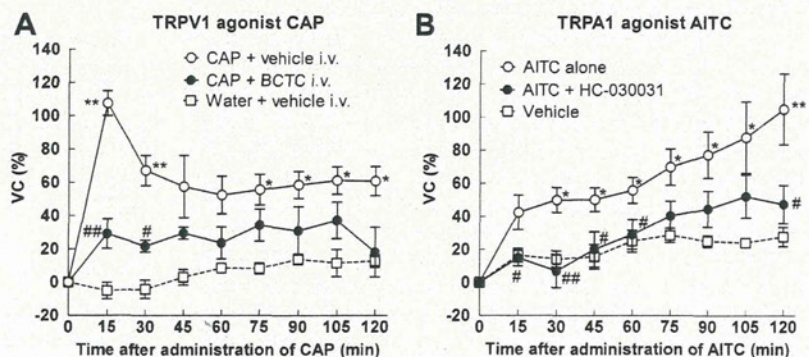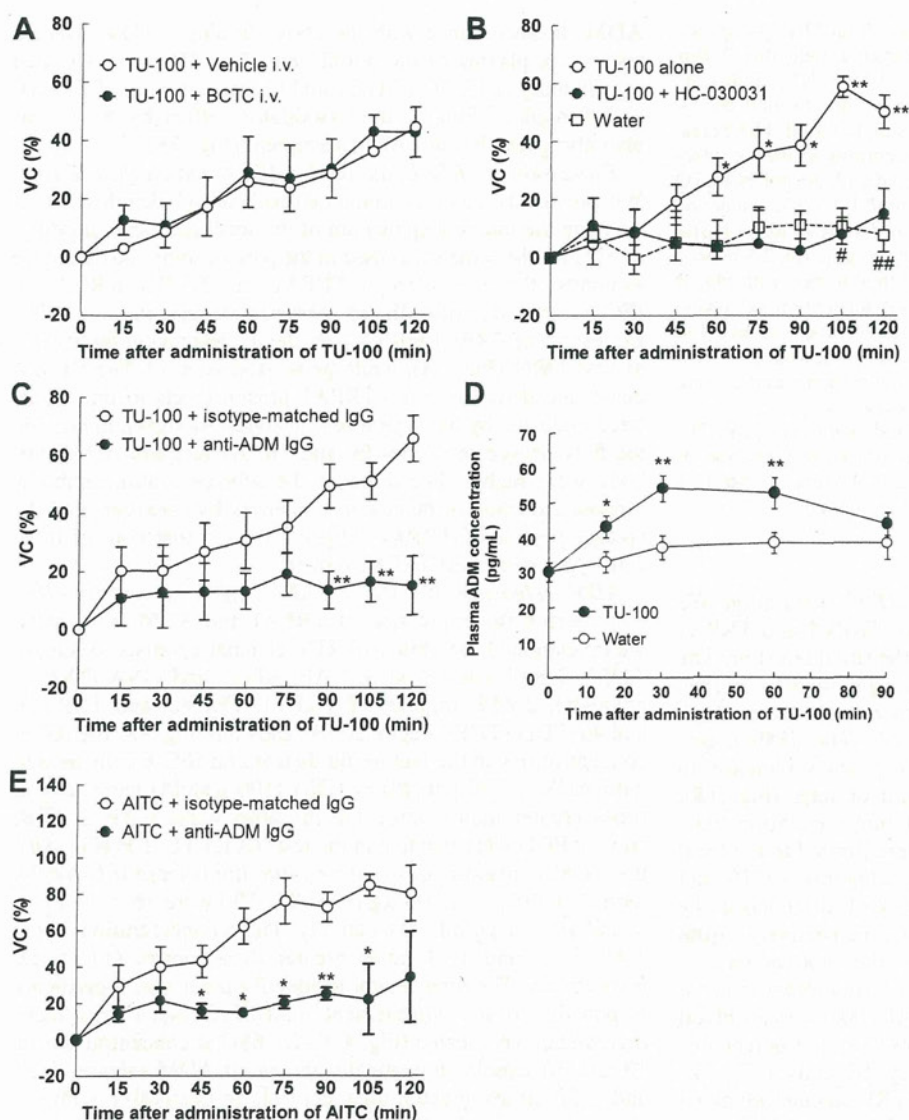
Fig. 2. TU-100 (daikenchuto) increases blood flow via the TRPA1-adrenomedullin cascade. TU-100 was administered intraduodenally at a dose of 2,700 mg/kg. Blood flow was monitored every 15 min after administration of TU-100. *A*: BCTC (10 mg/kg) was given intravenously 15 min before TU-100 administration; $N = 3–5$; *B*: HC-030031 (1 mg/kg) was administrated intraluminally together with TU-100; $N = 6–7$. *C*: antibody to adrenomedullin (ADM) was injected intravenously at a dose of 50 μg/kg 15 min before the administration of TU-100; $N = 7–8$. *D*: ADM content in the portal veins was measured using an EIA kit after purification on a C18 Sep-column; $N = 16$. *E*: AITC (0.002 mg/kg body wt) was administered intraduodenally and anti-ADM antibody was injected as described above; $N = 4$. $*P < 0.05$, $**P < 0.01$ vs. water (*B*), no-antibody control (*C*), water (*D*), or no-antibody control (*E*). $\#P < 0.05$, $\#\#P < 0.01$ vs. TU-100 alone, respectively.

Investigation of signal pathways linking TRPA1 to ADM release. The functional interaction of TRPA1 activators intrinsic to TU-100 with the TRPA1 molecule was investigated in two assays: blockage of ADM release using HC-030031 in IEC-6 cells and calcium influx in TRPA1-transfected cells. The influence of coaddition of HC-030031 was first examined with respect to ADM-releasing activity of TU-100, AITC, and 6SG. As shown in Fig. 5*A*, ADM release by these activators was significantly abolished by HC-030031. In addition, the ADM-releasing activity of these activators was not detected in calcium-free buffer (data not shown). T-Rex293 cells stably expressing rat TRPA1 were incubated with various concentrations of AITC and 6SG (Fig. 5*B*). Calcium influx was clearly evoked after their addition, while mock-transfected cells showed no response (data not shown). Finally, the involvement of the kinase pathway in ADM release by TRPA1 activators was examined. This was accomplished by evaluating the effects of the cAMP-dependent protein kinase (PKA) inhibitor H-89, the protein kinase C (PKC) inhibitor calphostin C, and the phosphatidylinositol 3-kinase (PI3K) inhib-

itor LY294002 in an ADM release test of AITC and 6SG. As shown in Fig. 5*C*, ADM-releasing activity of AITC and 6SG was reduced by the addition of calphostin C. On the other hand, the activity of 6SG but not AITC was enhanced by the addition of H-89, while LY294002 had no effect. Moreover, the PKC-specific activator PMA significantly augmented ADM release (Fig. 5*D*).

Vasodilatory effect of 6SG. After confirming that 6SG was the main active ingredient of TU-100 that stimulates TRPA1 and ADM release, we evaluated its effect on IBF. As shown in Fig. 6*A*, the dose-dependent vasodilatory effect by 6SG was quantified using the area under curve of vascular conductance from 0 to 120 min. The effect of 6SG was completely abolished by pretreatment with HC-030031 (Fig. 6*B*).

DISCUSSION

In this study we demonstrated that *1*) freshly purified rat IE cells and the rat intestinal epithelial cell line IEC-6 expressed
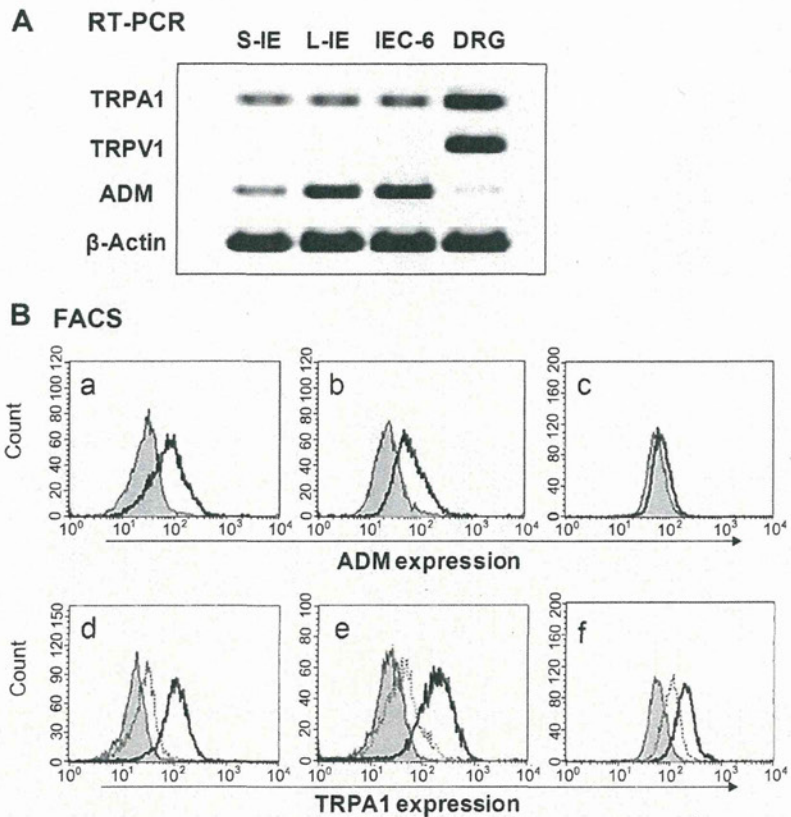
A   RT-PCR

B   FACS

Fig. 3. TRPA1 and ADM expression in intestinal epithelial cells. *A*: RT-PCR analysis was performed for TRPA1, TRPV1, ADM, and β-actin in rat intestinal epithelial (IE) cells of the small intestine (S-IE), those of the large intestine (L-IE), dorsal root ganglion (DRG) cells, and the rat IE cell line IEC-6. The PCR products were resolved on a 2% agarose gel electrophoresis. *B*: flow cytometric analysis. *a–c* ADM images. *d–f*: TRPA1 images. *a* and *d*: S-IE. *b* and *e*: L-IE. *c* and *f*: IEC-6. Thin solid line: control Ab (rabbit IgG); thick solid line: antigen specific Ab; broken line: antigen specific Ab + epitope peptide. Data shown represent the results of 3 experiments.

mRNAs and proteins of ADM and TRPA1, 2) TU-100 increased IBF via ADM release, 3) AITC, TU-100, and 6SG increased IBF in a TRPA1-dependent manner, and 4) AITC, TU-100, and 6SG stimulated ADM release/production in IE cells via stimulation of TRPA1. These data that suggest the activation of the epithelial TRPA1-ADM system in the small intestine as a potent factor in regulating IBF are a novel and important finding to understand intestinal physiology, and
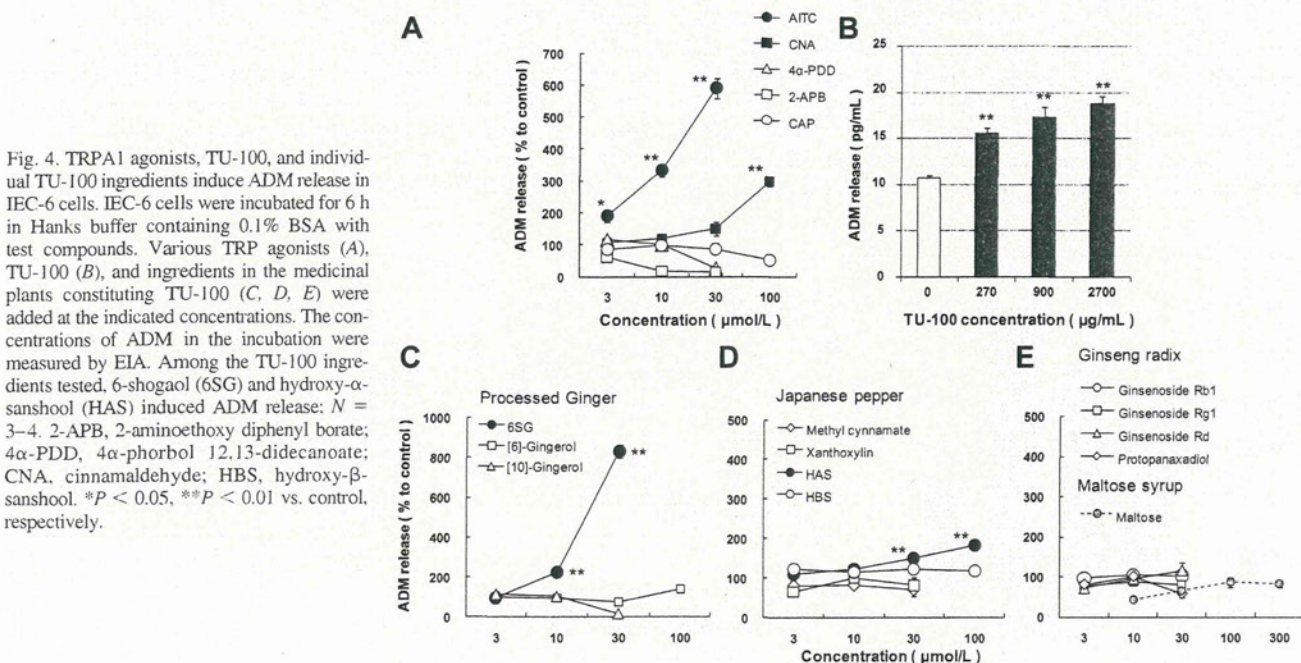

Fig. 4. TRPA1 agonists, TU-100, and individual TU-100 ingredients induce ADM release in IEC-6 cells. IEC-6 cells were incubated for 6 h in Hanks buffer containing 0.1% BSA with test compounds. Various TRP agonists (*A*), TU-100 (*B*), and ingredients in the medicinal plants constituting TU-100 (*C, D, E*) were added at the indicated concentrations. The concentrations of ADM in the incubation were measured by EIA. Among the TU-100 ingredients tested, 6-shogaol (6SG) and hydroxy-α-sanshool (HAS) induced ADM release; *N* = 3–4. 2-APB, 2-aminoethoxy diphenyl borate; 4α-PDD, 4α-phorbol 12,13-didecanoate; CNA, cinnamaldehyde; HBS, hydroxy-β-sanshool. *$P < 0.05$, **$P < 0.01$ vs. control, respectively.
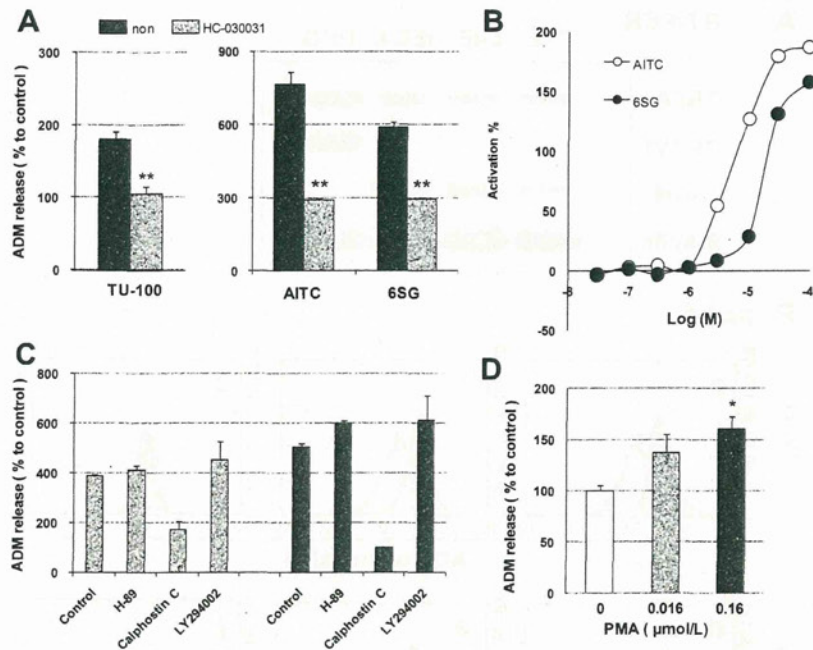
Fig. 5. AITC and 6SG stimulate TRPA1 to induce ADM release via protein kinase C. ADM release by TU-100 (2,700 µg/ml), AITC (30 µmol/l), and 6SG (30 µmol/l) was abrogated by cotreatment with 100 µmol/l of HC-030031 (A). AITC and 6SG induce calcium influx in T-Rex293 cells stably transfected with rat TRPA1 (B). Among the kinase inhibitors tested, the protein kinase C (PKC) inhibitor calphostin C potently inhibited AITC- and 6SG-induced ADM release (C). The PKC activator phorbol 12-myristate 13-acetate (PMA) induced ADM release (D); $N = 3$. $**P < 0.01$ vs. control.

pathobiology of various intestinal disorders with impaired intestinal microcirculation.

In the gastrointestinal tract, TRPA1 is predominantly expressed in a subset of TRPV1-expressing extrinsic sensory nerves, especially the DRG neurons (6, 32). The distribution of TRPA1 appears related to its physiological and pathophysiological roles such as mechanosensation (6, 9, 59), chemosensation (9) and inflammatory hyperalgesia (7, 36, 57). In addition, a recent study has also verified the presence of TRPA1 in several types of enteric nerves including inhibitory motoneurons, descending interneurons, and intrinsic primary afferent neurons (43). Furthermore, a subtype of enteroendocrine cells has been shown to express abundant TRPA1 whose stimulation induces 5-HT release that can activate intrinsic nerves and vagal endings (39). These reports strongly suggest that TRPA1 may play a role in the regulation of gut motility as confirmed by several motility studies of experimental animals using TRPA1 ligands and gene-manipulation (11, 12, 26, 42, 43). More recently, considerable attention has been given to the

presence of TRPA1 in IE cells. Kaji et al. (23, 24) detected TRPA1 mRNA and protein by RT-PCR and immunohistochemistry in human and rat epithelium isolated from intestinal mucosa, and Pool et al. (43) reported on TRPA1 immunosignals in mouse IE cells. The former study showed that AITC and an herbal ingredient, thymol, evoked electrogenic anion secretion from colonic epithelium segments in a TRPA1-dependent manner, although it is still unclear which cell types were stimulated by TRPA1 agonists because the study used unpurified epithelial preparations. The latter study did not examine the biological effect of TRPA1 in IE cells. In contrast, our study clearly showed that the stimulation of epithelial TRPA1 induces endogenous ADM release, which in turn participates in the regulation of IBF. Determining the specificity and mechanistic pathways of the epithelial TRPA1-ADM axis is an important area for further investigation, and studies using siRNA and knockout approaches remain to be done.

We also found that 6SG was the main active ingredient in TU-100 with ADM-releasing activity on that basis that 1) 6SG
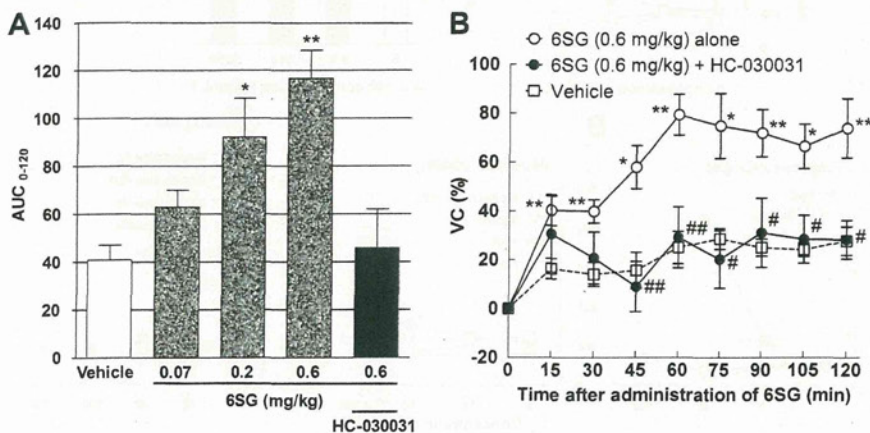
Fig. 6. Intraluminal 6SG increases blood flow in the small intestine. 6SG was administrated intraduodenally at a dose of 0.07, 0.2, or 0.6 mg/kg body wt and VC in the midjejunum was monitored. HC-030031 (1 mg/kg) was administered intraluminally together with 0.6 mg/kg of 6SG. Quantitation by area under curve (A) and time-dependent changes (B) are shown; $N = 4$-6, $*P < 0.05$, $**P < 0.01$ vs. vehicle, $\#P < 0.05$, $\#\#P < 0.01$ vs. 6SG alone, respectively.

potently induced vasodilatation in vivo, ADM release in vitro, and calcium influx in vitro in a TRPA1-dependent manner, and *2*) the amount of 6SG in TU-100 was sufficient to explain most of the vasodilatation produced by TU-100 (a 2,700 mg/kg dose of TU-100 contains about 0.6 mg/kg of 6SG). Yet given that a small amount of 6SG in TU-100 has been reported to enter systemic circulation (19), our concern was whether TU-100 may affect systemic blood flow. A recent examiner-blinded randomized crossover trial investigating the effects of TU-100 on cardiac output and blood flow volume in the superior mesenteric artery in humans showed that a significant increase in blood flow in this artery occurred after TU-100 administration without any increase in systemic circulation (48). Another study indicated that TU-100 administration increased portal blood flow in healthy volunteers, cirrhotic patients, and liver-transplant patients without any significant changes in the systemic blood pressure and heart rate (40). These clinical findings are in good agreement with those of other basic studies using experimental animals. Thus TU-100 was surmised to increase IBF by affecting the regulatory mechanism of local blood circulation and thereby alleviate the detrimental effects of intestinal ischemia without causing cardiovascular complications.

ADM is known to have anti-inflammatory and vasodilatory effects, which have been confirmed by multiple colitis models induced by trinitrobenzenesulfonic acid and dextran sulfate sodium, the commonly used experimental models of inflammatory bowel diseases (4, 13). Some of the reported activities of ADM include suppression of certain proinflammatory cytokine production and release (16), antimicrobial effects (56), and enforcement of endothelial barrier function (49). These lines of evidence are consistent with the conjecture that intestinal ADM release via epithelial TRPA1 stimulation is involved in the maintenance and protection of gut functions. Although the results of these studies collectively suggest a novel approach to the treatment of colitis with ADM, exogenous administration of ADM is impractical because of its rapid clearance and potential systemic effects (35, 55). Meanwhile, as described in the previous paragraph, TU-100 appears to affect endogenous ADM system and IBF only locally and not systemically. In fact, we previously demonstrated that oral administration of TU-100 exerted an anti-colitis effect in trinitrobenzenesulfonic acid-induced colitis model via upregulation of intestinal ADM. Such localized increase in endogenous ADM by TU-100 may be advantageous because the potent biological effect of ADM is more or less confined to the diseased sites. On the basis of a number of reports indicating the ameliorating effect of TU-100 in various animal GI disease models (1, 8, 17, 20, 27, 30, 51), several double-blind, placebo-controlled, randomized trials in patients with postoperative paralytic ileus, refractory functional constipation, irritable bowel syndrome, and Crohn's disease are currently being conducted in Japan (JFMC39–0902, JFMC40–1001 and JFMC42–1002 funded by the Japanese Foundation For Multidisciplinary Treatment of Cancer) and the United States (NCT00871325, NCT01139216, NCT01388933, and NCT01348152). Among these studies, one recent study reported that TU-100 has a prokinetic effect in healthy volunteers (33).

The present study has addressed the possibility that PKC and/or PKA/cAMP may play a role in TRPA1-related ADM release. This was of interest because a role of these molecules in TRPA1

signaling has not been reported except for the sensitization of TRPA1, an event that occurs upstream of TRPA1 signaling (2, 34, 54). Clarifying the PKC isoform(s) and molecular pathways involved in the effect is a priority for future research. As to the possible involvement of PKA/cAMP, it should be noted that H89 affected only 6SG-induced ADM release. Furthermore, there was no detectable change in cAMP levels in AITC- and 6SG-treated IEC6 cells (unpublished observations). These results suggest that PKA/cAMP may not be involved in vasodilatation induced by either 6SG or AITC. However, the enhancement of effect of 6SG by H89 suggests the possible involvement of mitogen-activated kinases (MAPKs): i.e., H89 inhibits not only PKA but also mitogen- and stress-activated kinase 1 (MSK1), which plays a critical role in NFκB-related inflammatory responses including production of prostaglandins, interleukin(IL)-8, and IL-10 (3, 10, 50). Multiple studies have shown that 6SG inhibits inflammatory responses (e.g., prostaglandin E2 synthesis) concomitant with potent suppression of the activation of certain mitogen-activated kinases (MAPKs) (5, 14, 41) including ERK1/2, which is typically located upstream of MSK1. Although the effect of 6SG on MSK has not been reported, it would be worthwhile to determine whether the MAPK system plays a role in the stimulation by 6SG.

In conclusion, our study revealed that epithelial TRPA1-ADM axis constitutes a possible regulatory system of IBF. In the gastrointestinal tract, TRPA1 appears to modulate digestive functions in at least three ways: induction of nociception via neuropeptide release from sensory neurons, facilitation of motility via 5-HT release from enterochromaffin cells, and promotion of vasodilatation via ADM release from IE cells (Fig. 7). Emerging physiological implications of TRPA1, especially its activity on the intestinal epithelium, identify TRPA1 ligands as promising drug targets for the management of gastrointestinal disorders with aberrant microcirculation.
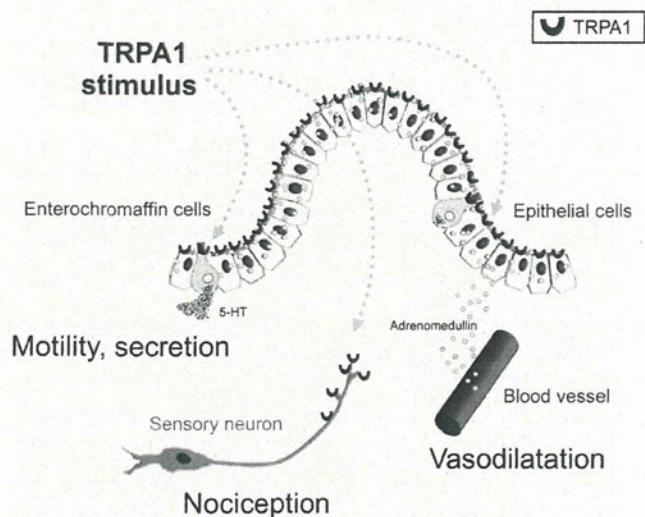

Fig. 7. Gut TRPA1 elicits physiological and pathophysiological responses in 3 ways. TRPA1 activators have 3 potential target cells: intestinal epithelial (IE) cells, enterochromaffin (EC) cells, and TRPA1-positive sensory neurons. As a result of TRPA1 stimulation, TRPA1 agonists stimulate IE cells to release ADM, EC cells to release 5-HT, and sensory neurons to release neuropeptides/neurotransmitters, respectively, resulting in physiological and biodefensive responses in vasodilatation, motility, secretion, and pain signaling.

DISCLOSURES

T. Kono received a research grant from Tsumura and Co. A. Kaneko, Y. Omiya, K. Ohbuchi, N. Ohno, and M. Yamamoto are employees of Tsumura and Co. Tsumura and Co. manufactures TU-100.

AUTHOR CONTRIBUTIONS

Author contributions: T.K. conception and design of research; T.K., A.K., Y.O., K.O., N.O., and M.Y. interpreted results of experiments; A.K., Y.O., K.O., and N.O. performed experiments; A.K., Y.O., K.O., and N.O. analyzed data; A.K., Y.O., K.O., and N.O. prepared figures; M.Y. drafted manuscript.

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3 消化管疾患に対する漢方医療の実際
(4) イレウス

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Key words：漢方，大建中湯，イレウス，山椒，乾姜

── 要 旨 ──

　エビデンス重視の現代医療で漢方は注目を集めてきている．その契機となったのが大建中湯の薬効機序に関する分子レベルの研究で，腸管運動に関与する神経伝達物質以外にも山椒や乾姜の成分が腸管粘膜上皮細胞から内因性ペプチドやセロトニンを動員し，腸管血流や腸管運動を改善，術後の炎症反応を抑制することが明らかとなった．これを契機に米国，日本で複数のプラセボ二重盲検臨床試験が行われ，大建中湯の腸管運動改善効果が証明されつつある．さらに，薬物動態を明らかにするため大建中湯の吸収試験が行われ，山椒や乾姜の有効成分が吸収されることも明らかとなった．大建中湯を術後早期に使用することでイレウス発症を抑制することが期待される．

はじめに ── 麻痺性イレウスと癒着性イレウス

　語源がギリシア語であるとされるイレウス（腸閉塞）は腸管内容物が滞る病気の総称で，腸管閉塞機転の有無により，機械的イレウスと機能的イレウスに大別される．日本において2万人以上いるイレウス患者の全国集計によると癒着性イレウスが60％でそのうち，手術既往のある者が98％以上，その大半に消化管手術既往が

あった．米国でも癒着性イレウスがもっとも多く，本邦と同様である．術後イレウスは早期の麻痺性イレウスとその後の癒着性イレウスが含まれるが，創感染とともに入院期間延長原因として医療経済学的にも解決すべき重要な問題点として最近クローズアップされてきている．

　本稿で取り上げるイレウスはおもに開腹手術後のイレウスである．術後ほぼ全例に発生する麻痺性イレウスは腸管運動が消失し，腸管内容物が停滞することで腸管拡張が生じるものである．腸管運動は内輪，外縦の二つの平滑筋層によって起こる蠕動である．アウエルバッハ神経叢とマイスナー神経叢を中心にカハール介在細胞がペースメーカーとなり制御されている．手術ストレスによって交感神経優位となり腸管運動が抑制されると考えられている．また，手術による用手的操作，器械的操作などによって腸管壁に炎症が起こり，一酸化窒素やプロスタグランディン E_2（PGE_2）が高度に生成され平滑筋収縮能を抑制することも原因であるともいわれている[1]．

　一方，癒着性イレウスに関して炎症に伴うインターフェロンγが主原因のサイトカインであるという動物モデルでの報告もあるが[2]，未だに本質的な機序解明には至っていない．腸管の

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用手的擦過など物理的刺激，乾燥による影響などが要因ではないかと考えられ，物理的刺激や乾燥を起こしにくい腹腔鏡手術が推奨されているのもこれらの点を配慮したためである．

また，早期離床が癒着性イレウスに有利であることは経験的にわかっていたことだが，その理由として炎症を起こした腸管同士や他臓器との接触時間が長くなると癒着が発生するリスクが高まることから体位変換することで接触時間を短縮し，癒着発生を防いでいると考えられている．したがって，術後の麻痺性イレウスが遷延することは癒着発生の点からも不利であり，早期に麻痺性イレウスを改善することは癒着性イレウスを軽減することに役に立つことは明らかである．

I．歴史的経緯

この項のポイント
● 成分レベルおよび分子レベルまでの機序解明は行われてこなかった．

イレウスの治療に大建中湯（だいけんちゅうとう）が使用され始めたのは 1990 年代である．大建中湯が薬価基準収載されたのが 1986 年であり，当時から腸管運動改善作用が期待されて使用されてきた．とくに，イレウス管から大建中湯溶解液を流し込むことでイレウスが改善される症例が多く，数多くの症例報告がなされたのもこの時期である．同時期に機序解明も始まり，腸管運動に関与するアセチルコリン，サブスタンス P，カルシトニン遺伝子関連ペプチドなど神経伝達物質やモチリン分泌作用などが相次いで発表された[3]～[8]．

しかしながら，基礎的研究において成分レベルおよび分子レベルまでの機序解明は行われてこなかった．臨床研究においてもエビデンスレベルとしては低く，プラセボ対照の二重盲検試験は行われてこなかった．さらに，医学部での漢方教育も広まっておらず，多くの医師たちにとってハリやお灸などと同じ代替医療として位置づけられてきた．また，中国の中医と漢方の違いが理解されていないことから，安全性や品質に関して懐疑的な意見も多かった．さらに，1990 年代に漢方のなかで肝炎の特効薬的扱いを受け一世を風靡していた小柴胡湯（しょうさいことう）が間質性肺炎で死亡例を出してから，「漢方は効かないかもしれないが安全である」というそれまでの神話が大きく崩れ，一気に漢方熱は冷めてしまった．その風潮を打破したのが大建中湯である[9]．

II．大建中湯

この項のポイント
● 日本独自に変遷してきた大建中湯は，中国の中医にある大建中湯とはまったく異なる生薬内容である．

日本独自に 500 年かけて変遷してきた大建中湯の語源は中国の中医（四千年の歴史）に始まり，消化管（中）を大きく建て直す（大建）という意味である[9]．含まれる生薬（しょうやく）はすべて食材で，中医にある大建中湯とはまったく異なる．人参（にんじん）・山椒（さんしょう）・乾姜（かんきょう）・膠飴（こうい）の 4 種類から構成されており，保険適用は腹部の冷えと腹部膨満感の二つである．

III．薬理作用機序

この項のポイント
● 腸管粘膜上皮細胞から ADM とセロトニンを放出させることで腸管血流増加と腸管運動亢進作用を発現させている．

これから述べていく大建中湯はツムラで抽出されたものである．漢方薬の特色として抽出方法には企業努力によるということがあり，また，品質管理においても各社にバラツキがあることも事実である．大建中湯の薬効生薬（山椒，乾姜，

人参)はあわせても10%未満で，残り90%はマルトースやラクトースなどの糖類である．

1．CGRPへの作用

最初にわれわれが着目したのは，大建中湯によって刺激される神経ペプチド，カルシトニン遺伝子関連ペプチドのCGRP（calcitonin gene related peptide）である．CGRPはヒトが有するもっとも強い血管拡張作用をもつ神経ペプチドとして知られている[10]．そこで機序解明の突破口としてこの神経ペプチドCGRPが大建中湯の腹部の冷えの改善作用に関与しているという仮説をもとに研究を進めた．すぐにその仮説は立証されることになったが，受容体に関してCGRPだけでなくCGRP受容体関連因子も大建中湯によって刺激を受けることが明らかとなった．

2．CGRP受容体

CGRPの受容体は恒常的に存在せず，未成熟な受容体であるCRLR（calcitonin receptor-like receptor）が成熟化するプロセスが必要で，その成熟化にはRAMP（receptor activity-modifying membrane protein）が必須である．RAMPには3種類のタイプがあり，RAMP1が出現し成熟化に関与するとCGRP受容体になるが，RAMP2，RAMP3が出現し成熟化に関与するとCGRPと同じカルシトニン・ファミリー・ペプチドであるADM（adrenomedullin）の受容体に変化することが報告されていた．

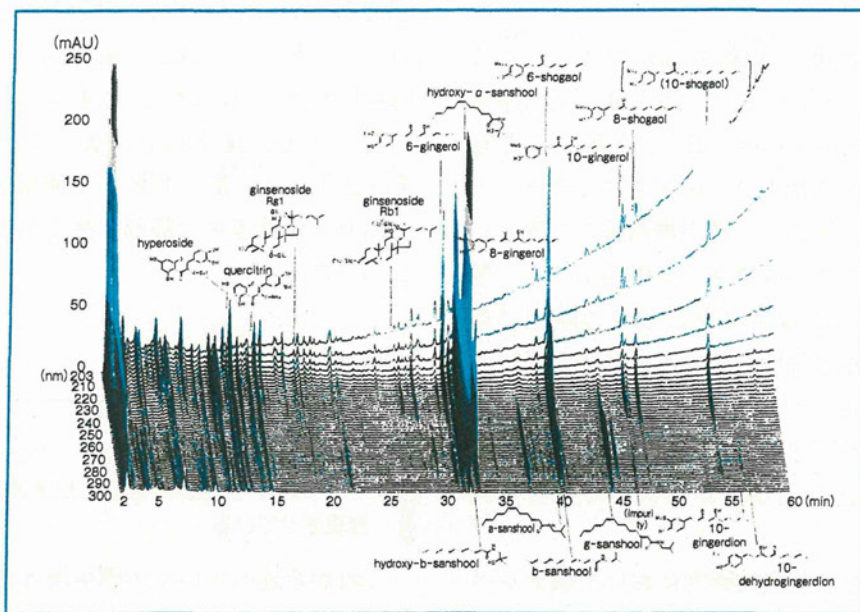われわれの実験結果から大建中湯によって3種のRAMPいずれも増加することが明らかとなり，カルシトニン・ファミリー・ペプチドの二つのペプチド，CGRPとADMおよびその受容体関連因子が大建中湯の血流改善機序に関

図1 大建中湯の主成分の3D-HPLC（3D高速液体クロマトグラフィー）による解析
hydroxy-α-sanshool（Japanese pepper），6-shogaol（processed ginger），ginsenoside Rb1（ginseng radix），maltose（maltose powder）
毒素，殺虫剤，微生物は検出されなかった．

〔Kono, T., et al.：J. Gastroenterol. 46；1187-1196, 2011[13]より引用〕