骨盤底筋訓練　　家庭でも，外出先でもできる訓練です．毎日続けて行いましょう．

1. 仰向けの姿勢で

●まず仰向けに寝て，足を肩幅に開きます．
●次に，ひざを少し立て，身体の力を抜き，肛門と膣を締め，締めたままゆっくり「1，2，3…」と5つ数えます．　もし途中で力が抜けてしまったら，また締め直します．体操を続けて筋肉が強くなれば，締め続けることができるようになります．仰向けは最もリラックスしやすい姿勢です．また朝晩，布団の中で行えるので，ぜひ毎日続けてください．

2. ひじやひざをついた姿勢で

●床にひざをつき，クッションの上にひじを立てて手にあごをのせます．
●次に，肛門と膣をゆっくり締め，締めたままゆっくり「1，2，3…」と5つ数えます．
●5つ数えたら力を抜き，また締めます．
　新聞紙を床に広げて読むときなど，気軽にできる体操です．新聞を読み終わるまで，締めたりゆるめたり繰り返しましょう．

3. 机にもたれた姿勢で

●机にそばに立ち，足を肩幅に開きます．
●手も肩幅に広げ，机につけます．
●その姿勢で，体重を全部腕にのせます．背中はまっすぐに伸ばし，頭を上げて前をみます．
●肩とお腹の力を抜いて，肛門と膣を締めます．
　骨盤底筋の動きを最も感じやすい姿勢です．台所のシンクやデスクを使っても行えます．

4. 座った姿勢で

●床につけた足を肩幅に開き，背中をまっすぐに伸ばし，頭を上げて前をみます．
●肩の力を抜き，お腹が動かないように，またお腹に力が入らないように気をつけながら，ゆっくり肛門と膣を締めます．
　バスや電車に乗っているときや，家でテレビをみているときにも行えます．

■骨盤底筋訓練の目安
●骨盤底筋訓練のトレーニングの回数は，1人ひとりの筋力の状態で異なります．医師やナースと相談して，自分に適した回数を決めてください．
　骨盤底筋の締まることを自覚できる方なら，速く締める（5回）・締めたままで3～5秒間保つ（5回）を1セットとして，1日10セットが目安です．
●1回に続けてがんばると疲れるので，できるだけこまめに分散して行います．
●効果が現れるまで，少なくとも1～3カ月はかかるので，あきらめずに続けましょう．

図4　骨盤底筋群体操患者指導用パンフレット（文献2より引用）
患者指導用のパンフレットで，現段階では，研究で効果が証明されてはいない．

排尿障害の現状と展望

鳥羽　最後に一言ずつ，言い足りないことがございましたらどうぞ．

岩坪　現場の医療では誤った概念が幅をきかせ，間違った対応がされています．例えば，膀胱機能をきちんと調べていないことと，水分負荷の問題です．脳梗塞予防，脱水予防，便秘，尿路感染の予防治療などが理由にされますが，根拠がありません．介護者の期待通りにならない事実を誤認して認知症のせいにしてしまう傾向もあります．介護はチームワーク，入れ替わり立ち替わり1人の方に接する訳ですから，スタッフの知識レベルや対応がまちまちでは困ります．しかし，人手不足で夜間の介護ができない，インセンティブが働かない保険点数のつけ方など厚生労働省の制度設定が問題です．

真田　褥瘡のハイリスク加算から私自身が学んだことは，排泄学という学問をきちんと体系づけて，1つのアカデミックな部分を多職種チームアプローチとして作り，エビデンスを出し，それによっていろいろなシステムを変えていく大きな取り組みが，今の日本に求められているだろうということです．

　もう1点，看護師の失禁ケアに関する認識を変えることが必要だと考えます．つまり，まずはスキンケアからみて失禁予防という考え方を普及させることです．看護師たちは，褥瘡の患者さんを一生懸命にケアしていますが，入院時に既に失禁があっても，会陰部の皮膚障害には気づかないことがあります．会陰部の皮膚障害，糜爛，膨潤はとても痛いのですが，寝たきりの方々には意識障害のある方も多く，痛くても訴えられない現状があります．看護師たちは，皮膚障害に対処することには最善を尽くしますので，これを予防するためには，まず失禁を予防することだ，と認識することができれば，失禁の予防にもっと積極的に取り組んでいけるのではないかと思います．その1つの手段として，

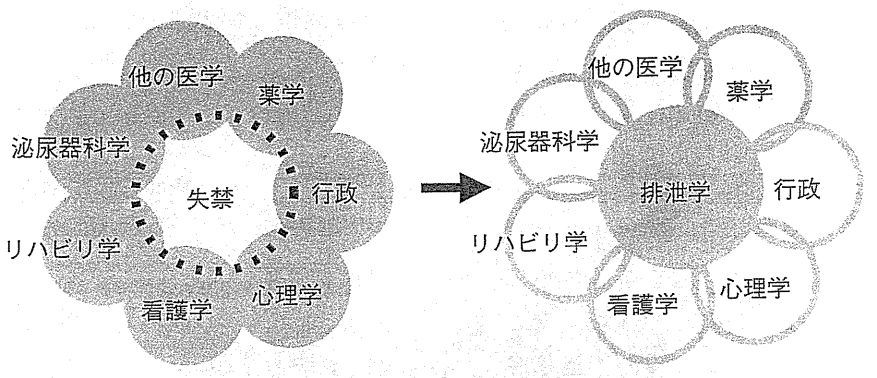

図5　排泄学・コンチネンス学の展開

スキンケアという視点から失禁を減らしていこうという啓発が有効であると考えます．

鳥羽　看護師は目に見えることの方が興味をもちやすいということですか．

真田　はい，皮膚は看護ケアを評価する最も大切な臓器です．

岩坪　オムツの弊害には皮膚のかぶれもありますが，もっと一般的で深刻なのは慢性膀胱炎です．膀胱炎はオムツ患者の80%にもみられ，神経因性膀胱では難治性です．菌の種類によっては急性増悪します．耐えられない痛みと不快感を訴えられない認知症高齢者は，不穏，暴力，せん妄状態になることもみられます．あまり気づかれていないようですね．

真田　尿がアルカリ性であるため，皮膚を傷つけているのです．糜爛は膀胱炎が起因であるということを，看護師たちにもっと伝えていかなければいけないと思います．

本間　実際，皮膚には悪影響なのでしょうか．

真田　はい．すぐに糜爛を起こしてしまいます．

アクティブなシルバーに関しては，失禁が原因で閉じこもりやうつになるということが問題です．失禁と低栄養と転倒が，閉じこもりの原因ですので．失禁に関しては失禁外来というものを立ち上げ，そこで看護師がパッドテストや排尿日誌から失禁をアセスメントするという環境を作り，それに診療報酬がつくようになれば，この領域も変わっていくような気がします．

鳥羽　本間先生，最後に一言．

本間　大学に久し振りに戻って思うことは，やはり医学は強力な科学であるということです．

失禁は個人のプライバシーという属性が強調されがちですが，やはり身体的な疾患なのです．これをわれわれは心理的なバリアで妨げているのです．「排泄学」つまり排泄障害を多領域の関係したサイエンスの分野としてとらえることによって，そのバリアが取り外されるのではないでしょうか．「排泄学」という言葉はなかなか一般に受け入れがたいということで，「コンチネンス医学」という言葉を提唱し，その講座を東京大学に作ろうと思っています(図5)．これは，東京大学だからこそメッセージ性が高いと思います．失禁は科学の対象だ，ということを認めてもらうに努力していきたいと思います．

おわりに

鳥羽　どうもありがとうございました．本日，皆さんに集まっていただき，排尿障害が症状，症候群のようにとらえられていますが，1つの疾患概念として確立すべきです．確立することによって，水分摂取もリスクファクターとしてとらえられますし，睡眠障害や褥瘡，うつといったものを合併症というとらえ方をしていくということがわかりました．

本特集の中では，排尿障害に対する新しいメカニズム，中枢性のもの，様々な機器や薬物療

法についても触れていただいています．本間先生は目覚しい進歩が少ない，とおっしゃいましたが，進歩は少なからずあるが，まだこれからの分野であると感じました．新しくコンチネンス学を確立されるのであれば，泌尿器科だけではなく，多くの内科医や看護師の方がサイエンスの基に集まれるであろうと考えます．さらにその学は，単に医学だけではなく介護現場などにもインパクトをもつということで，本当に今後発展性のある分野だと感じられました．

本日はお忙しいところ，どうもありがとうございました．

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ISSN 1343-3598
＊本文献は2カード

認知症における精神症状と行動障害

BPSD in Dementia

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■ 認知症における周辺症状と治療の考え方

認知症の全体像の中で、中核症状とされる認知機能障害（記銘力低下、判断力低下、実行機能障害）はごく一部でしかなく、妄想、無欲、自暴自棄、焦燥、行動異常など多様な周辺症状に彩られており、これらは家族の介護負担に大きな影響をもたらし（図1）、不適切な対応は認知症患者が得られるべき安寧に対し不利益にもなる。

周辺症状は「反応性」の症状の部分があり、非侵襲的な非薬物療法はその意味で価値が高い。一方、薬物療法は、激しい症状の緩和には、家族だけではなく、ケアスタッフの安全面からもその要望は強い。しかしながら認知症といえども、心身の予後が重要視されつつあり、日常生活活動度（ADL）を含む包括的な予後を改善する薬物療法が求められている。

■ 用語と定義

1．用語

問題行動（unwanted behavior）という用語については議論が多い。行動障害（behavior disturbance）、異常行動（abnormal behavior）などという単語に置き換えられるべきと考えられる。国際精神医学会では行動の異常と心理学的症状を包括し、認知症の心理行動異常（Behavioral and Psychological Signs and Symptoms of Dementia：BPSD）という用語に統一するよう提言している。

2．定義

広くは周辺症状（associated features）を含むが、狭義には、周辺症状が体現された行動障害の具体的叙述をいう（例：気分の障害；無気力、暴言など）。

■ 分類

周辺症状は、①気分の障害（disorders of mood）、②幻覚、妄想、誤認（delusion、hallucination、misidentification）、③行動障害（behavior disturbance）に分類される。

アルツハイマー病における行動障害は、①攻撃性；暴言・暴力（aggression；verval and physical）、②徘徊（wandering）、③性的抑制の低下（sexal inhibition）、④過食（increased eating）、⑤睡眠障害（sleep disturbance）、⑥異食（hyperorality）で、古い成績では①、②が20%、③～⑥が10%とされている。

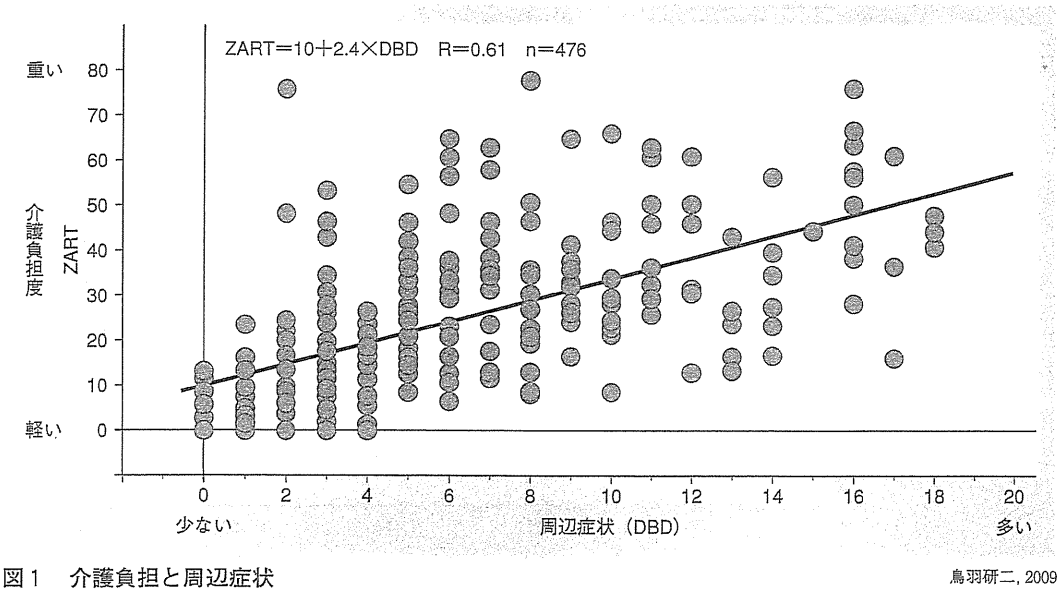

図1　介護負担と周辺症状

鳥羽研二, 2009

■ 評価

より広義の症状を包括した問題行動の評価に「DBD (Dementia Behavior Disturbance) スケール」[1]がある。

その他、主としてアルツハイマー病の行動異常を評価するBEHAVE-AD (Behavioral Pathology in Alzheimer's Disease Rating Scale) は妄想、幻覚、行動障害、攻撃性、日内リズム障害、感情障害、不安・恐怖の下位7尺度から成っている。また、NPI (Neuropsychiatric Inventory) は幻覚、妄想、興奮、うつ、不安、多幸、無為、脱抑制、易刺激性、異常行動の10項目から成る広義の問題行動の尺度である。

■ 頻度

DBDスケールの杏林大学病院もの忘れセンターにおける頻度を示す（図2）。認知機能に関連する「同じことを何度も聞く」といったエピソードが最も多く、物とられ妄想に関連する「物をなくす、隠す」が続き、ムード、意欲の低下に関連する「無関心」、日内リズム障害と意欲に関連する「昼間寝てばかり」「夜間起きだす」といった頻度も高い。興奮や攻撃性に関しては、暴言が30%にみられるが、興奮、暴力などの頻度は高くない。徘徊は約10%にみられる。感情の破壊「感情失禁」「金切り声」などはずっと低く、脱抑制「性的関係」「陰部露出」はごくまれである。

■ 認知症のタイプによる周辺症状の特徴

アルツハイマー型認知症では、初期の焦燥、うつがみられるが、行動障害では、物をなくし大騒ぎして捜す、服薬管理ができず薬が多量に余ったり、足りなくなったりするといったエピソードがみられる。

進行すると、物とられ妄想、嫉妬妄想などが出現し、鏡の中の自分に話しかける「ミラー現象」などが進行例で観察される。末期には、無為無欲、仮面様顔貌となり恍惚状態に見える。

脳血管性認知症では、涙もろくなったり、落ち込んだりするpost stroke depressionがみられる。進行期には、易怒性、興奮などもみられる。

レビー小体型認知症では、前駆症状として睡眠時の異常行動である「レム睡眠行動障害」があり、ベッ

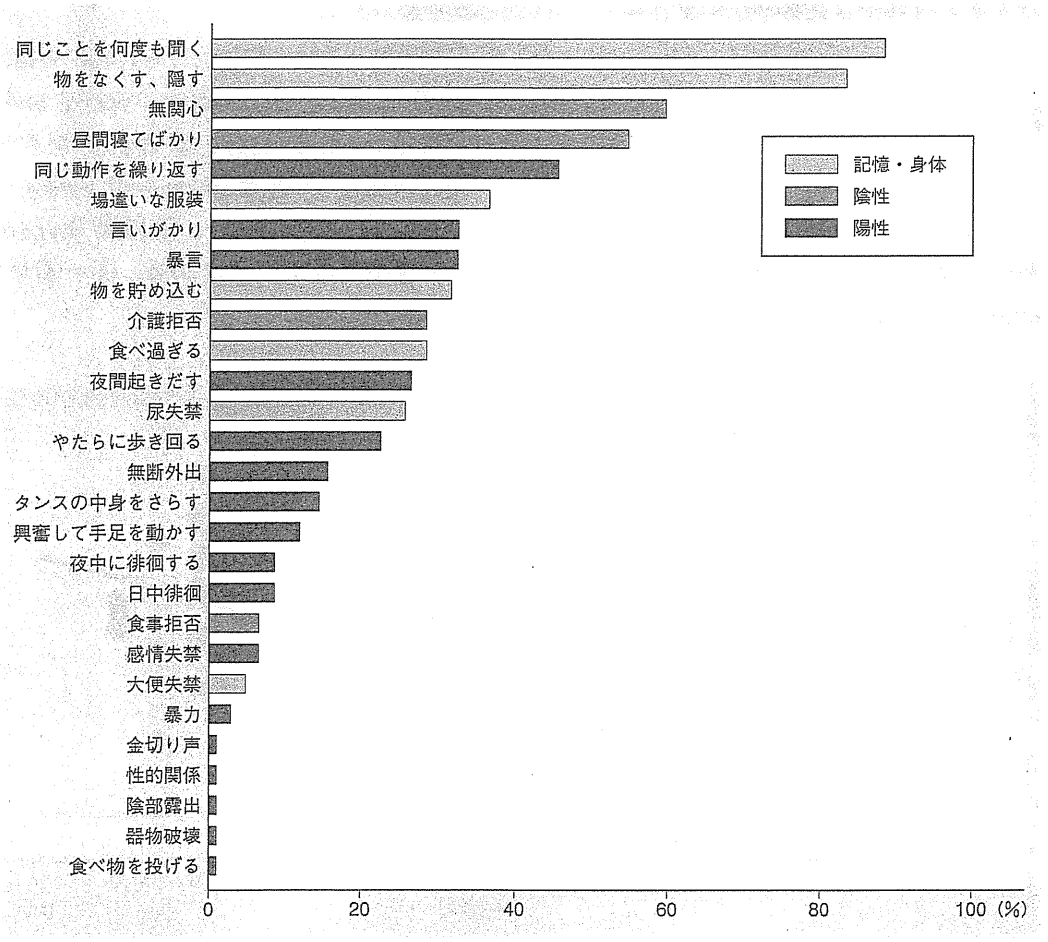

図2　問題行動の頻度（杏林大学病院もの忘れセンター）

鳥羽研二, 2009

＊PTM 9 (2 - 2) NOV., 2009へ続く

ドパートナーへの暴力、夜間突然起き上がって身振り手振りでわめくなどがみられる。早期に「知らない人が横にいる」「犬がそばに来ている」などといった具体的、写実的な幻視がみられる。また軽度のうつ症状を合併することが多い。

前頭側頭葉型認知症は、性格的に傍若無人「我が道をゆく」態度で、暴言などでの周囲とのトラブルも少なくない。一方、初期から意欲の低下が顕著である。

決まった時間に同じ行動を繰り返す「常同行動・時刻表的活動」もみられる。甘いものが極端に好きになる「食行動障害」が初期中期にみられ、進行例では過食もみられる。

■対応と対策

非薬物療法（いわゆる広義の行動療法やスタッフ教育）と薬物療法に大別される。

1．非薬物療法

1）問題行動全般

Rogersらは84人の認知症患者に対する15日間の観察研究で行動訓練によって有意な問題行動の減少を報告している[2]。鳥羽らは、6カ月間のグループホームで、DBDスコアの減少（p＝0.14）を示した（効果的医療技術の確立推進臨床研究2003年度報告書）。

平成19年度から介護保険で認められた、認知症短期集中リハビリテーション（1回20分以上、個人療法、週3回、3カ月間）では、周辺症状が介入群のみ有意に認められ（DBD）、記憶、昼夜逆転、介護拒否、暴言など易介護負担に直結する多くの項目に改善がみられ（表）、平成21年度からはデイケアでも認められることになった。

2）興奮、攻撃性

興奮、攻撃性に関しては非薬物療法の有効性が多く示されている。

a）**活動療法、運動療法**：活動療法は無作為対照試験により30%以上対照群より興奮を改善し[3]、運動療法は安眠療法に比べ有意に興奮を改善した（−20%対+150%）[4]。

観察研究では、散歩によって有意に暴力行為（staff incident reports of aggression）が減少（−30%）している[5]。

b）**レクリエーション療法**：8週間のレクリエーション療法で、興奮のエピソードが50%減少し[6]、73%のスタッフがやや有効と判定している。

表　認知症短期集中リハビリテーションによる周辺症状下位項目の前後の値の有意差

	対照群（63）	認知リハ群（203）
物をなくす	ns	p=0.003
昼間寝てばかり	ns	p=0.0023
介護拒否	NA	p=0.0072
何度も同じ話	ns	p=0.022
暴言	NA	p=0.0097
言いがかり	NA	p=0.0006
場違いな服装	NA	p=0.0023
貯め込み	ns	ns
無関心	ns	p=0.0072
昼夜逆転	ns	p=0.0593
常同行動	p=0.08	ns
散らかし	ns	ns
徘徊	ns	ns

鳥羽研二，2009

c）**ペット療法**：28人に対する1時間のペット療法の観察研究で、定性的ではあるが興奮の改善が示されている[7]。

d）**音楽療法**：18週間交差試験（crossover trial）で65%の興奮の改善がみられ[8]、観察研究でも、9〜63%の興奮症状の改善が報告されている[9-12]。

e）**スタッフ教育**：看護補助者教育によっても20%の興奮が有意に改善した[13]。観察研究では、2カ月の抑制廃止プログラムによって、抑制減少と興奮症状改善（agitation scores）がみられ[14]、患者との交わり増加（刺激療法）によって興奮が85%減少した成績もある[15]。

3）徘徊

有効な報告はほとんどない。

個別対応強化によって50〜80%徘徊が減少したという報告があるが対象症例数が少ない（4人）。

環境改善では外出欲求には無効であったという報告[16]、30人に対する15週間の音楽療法は徘徊に無効[17]。スタッフ教育に関する報告はない。

4）支離滅裂言語

個別社会適応訓練[18]や、ビデオによる模擬再現[19]が支離滅裂言語減少に有効であるとされている。

5）無気力、意欲の低下

a）**行動療法**：対照群をおいた前向き観察研究で、中等度以上の認知症で、排尿誘導による意欲の向上が認められている[20]。認知症症例でデイケアの利用者は在宅単独者に比べ、意欲の保持が有意に優れている。

b）**音楽療法**：音楽療法など感覚刺激療法は、無気力など陰性症状に対し有効な成績は報告されてい

ない。

c）スタッフ教育：看護補助者教育で、陰性症状に対して無効であった[13]。

2．薬物療法

1）抗精神病薬

625人に対する12週の無作為対照試験でリスペリドンが用量依存的に有意に問題行動の改善を認めている（プラセボ：－33%、1mg：－45%、2mg：－50%）[21]。ハロペリドールとの二重盲検比較試験でも有意に問題行動（BEHAVE-AD）を抑制した[22]。

オランザピンも無作為比較対照試験でプラセボ（25%）の2倍程度の改善を認めている。

チオリダジン塩酸塩はハロペリドールと同等という成績である。

非定形精神病薬に関して、The Cochrane Libraryの9つのコントロールスタディのメタアナリシスによるレビューによれば、リスペリドンとオランザピンはプラセボに比し有意にBPSDを改善した。しかし脳血管障害、錐体外路症状、転倒を有意に増やし、死亡率は質の高い研究に絞っても1.54倍であったと報告している[23]。また、2mg以上のリスペリドンや5～10mgのオランザピンでは脱落も有意に多く、認知機能の改善は証明されていない。米国食品医薬品局（FDA）では、死亡率が1.7倍になるデータを引用して、原則的に使用を控えるべきと勧告している。本邦でもアルツハイマー病に対する適応は認められておらず、少なくとも長期の投与には、家族の薬物有害作用の理解とそれをもってしてもどうしても投与して欲しいときにのみ限定されるだろう。

2）漢方薬

岩崎らは、ADLをも改善しながら、NPIで詳細に測定した認知症の周辺症状の有意な改善を報告した[24]。この効果はレビー小体病のドネペジル塩酸塩抵抗性の幻覚にも有効であるとも報告した[25]。

われわれも、無作為交差試験を関東の多施設で100例以上に行い、興奮や幻覚などを中心に、有意な有効性を認めた。今回の新知見では、休薬後も一定期間BPSDの改善を認めており、家族の安らぎが、反応性のBPSDの改善効果を延長した可能性がある。

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各　論：後期高齢者に多い老年症候群

認知症

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治療（J.Therap.）別刷
Vol.92, No.01〈2010.1〉
株式会社 南山堂

各　論：後期高齢者に多い老年症候群

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プライマリ・ケアにおけるポイント

・認知症は「生活機能障害」が診断基準．生活の変化を察知することが入り口である．
・治る認知症を見逃さないため，treatble dementiaを知ることが大切である．
・主要な認知症の特徴を身につける．
・正常加齢，軽度認知障害，軽症認知症，重症認知症をおおまかに区別する．
・生活習慣病と認知症での予防に関するライフスタイルの共通点と相違を知る．
・治療開始のポイント，中断・終了の目安をどうつけるか考える．
・周辺症状に対する薬物療法・家族指導の基本を身につける．
・専門医への紹介のタイミング，連携の方法をどうするか？

Ⅰ 認知症の診断・鑑別診断

① 早期診断

認知症の診断はDSM-Ⅳにしたがって行われるが，その具体的例示が乏しいため，一般医家にわかりにくさが否めない．したがって，特異度が低くても感度のよいスクリーニング機能を有する簡易な検査の必要がある．このための簡便な早期発見シートを用いている(表1)．

日常生活では，複雑な料理ができない，薬の飲み忘れ，同じものを買ってきてしまう，入浴が嫌いになるなどが早期に起きる変化である．

② 鑑別診断

アルツハイマー型認知症は，海馬－視床－帯状回後部，側頭葉など記憶に関する回路の症状が早期に出現する．脳血管性認知症では，共通に前頭葉血流の低下から，うつ・悲哀など感情面の変化が見られる．部位によって，頻尿・歩行障害・嚥下障害など身体的愁訴が主体のことがあり，見逃さないようにする．

レビー小体型認知症では，パーキンソン症状が明確でない場合は，先行する睡眠時の行動異常(レム睡眠行動障害)で，突然起き上がったり，ベッドパートナーをこづいたりすることも注意する．また，精神病薬を新しく処方したときに幻覚・妄想などが出現すれば強く疑う．人物や動物などの幻視はとくに診断価値が高い．

前頭側頭型認知症では，共通に意欲の低下と傍若無人的な性格の増強や，同じ時間に同じことをする性向が強まったときに疑う(表2)．

表1　早期発見：私の記憶は大丈夫？

❶ 物の名前が出ないことがある
人の名前が出ないことがある
⇒思い当たれば❷へ
❷ 昨日の夕食をいえない
今日が何曜日なのかいえない
孫の名前を全員いえない
(男)メモがないと買い物ができない
(女)材料から料理ができない
⇒1つでも当てはまれば❸へ
❸ 同じことを何度も尋ねる
以前あった興味や関心の低下
物をなくす，物のしまい忘れ

表2　早期に現れる周辺症状から見た認知症の特徴

アルツハイマー	• 記憶の低下 同じ話を繰り返す，物をなくす，さがす
脳血管性認知症	• 感情鈍麻 表情が平板，悲哀，とじこもり • う　つ
レビー小体型認知症	• 幻　覚 幻視，夢見が悪い，薬で錯乱 • う　つ
前頭側頭型認知症	• 意欲の低下 無関心，自発性低下，ものぐさ，無気力 • 常同行動 同じ椅子に座る，同じものを食べる • 情動変化 にこにこ，不機嫌

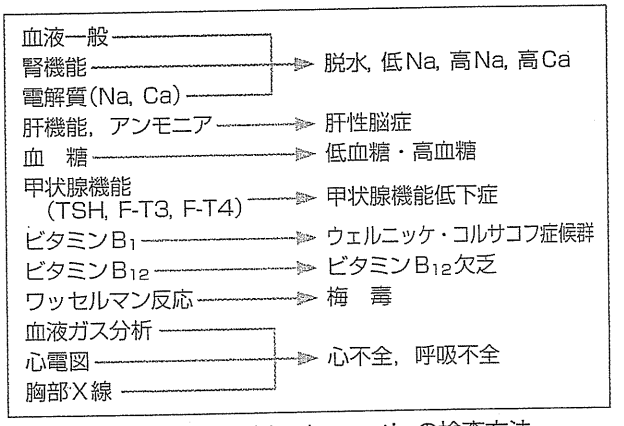

図1　鑑別診断 treatable dementiaの検査方法

③ 治る認知症を見逃さない

多くは身体疾患であるが，隠れたうつ状態でも，身体的愁訴が前面に出ている場合があり，「自分は無力(あるいは役に立たない人間)だと思いますか」といった，うつの簡便な質問を行うことは有用である．うつ以外の治る認知症のスクリーニング方法を図1に示す．

II 認知症の重症度・進行度を知る

認知症の重症度は，主として日常生活活動度(ADL)と意思疎通によって判断される．

中等度では，料理，買物などが自立不能となり，促し入浴が始まる．高度になると着衣介助，尿失禁，入浴での洗身介助となる．新聞やテレビへの関心は中等度で阻害され，高度ではレクリエーションなどへの参加も消極的になる．口数が減ってきて，限られた言葉しか出なくなれば最高度に近づき，「あー，うー」などという言語に限られ，周囲の働きかけに無反応になると最高度である．

III 生活習慣病と認知症での予防に関するライフスタイルの共通点と相違

認知症の診療では，認知症の発症を遅らすにはどうすればいいですか？　少しでも進行を防ぐには日常生活でどのような注意が必要ですか？　と毎回訊かれる．患者は高血圧，糖尿病などの合併疾患をもつ者も多く，生活指導での共通項と相違を知ることは有用である(表3)．

表3　認知症の予防治療のための生活習慣

	認知症	高血圧	糖尿病	骨粗鬆症
運　動	○	○	◎	○
体重を減らす	×	○	◎	×
油を少なく	飽和脂肪酸	○	◎	
魚を多く	◎	○		
野菜を多く	○	○	○	
Caを多く		○		◎
食品添加物	×		×	×
大酒を飲まない	○	◎	◎	○
禁　煙		○	○	○

	認知症	高血圧	糖尿病	骨粗鬆症
ゲームで興奮	○	×		
昔　話	◎			
音楽で悠々	○	○		
散　歩		○	○	○
自転車	×	○	○	○
釣り・パチンコ	×			
麻　雀	○			
昼寝(30分くらい)	○	○		

Ⅳ 治療開始のポイント，中断・終了の目安をどうつけるか考える

治療開始のポイントは，認知症と診断されればできるだけ早期に開始する．

軽度認知障害では，アルツハイマーへの移行の可能性が脳血流シンチや脳ブドウ糖代謝などの詳しい検査である程度判別できる．迷ったら専門医療機関へ紹介するべきである．

認知症への移行は，MMSEやHDSRという30点満点の検査で，早いものでは1年間に3点低下し，MMSE26点が1年後に認知症レベルになる．したがって，ドネペジルを投与しない場合，6ヵ月に1回認知機能検査を行い，点数が低下していれば，治療を開始する．

消化器症状や興奮などでドネペジルを中断せざるを得ないケースも少なくない．軽度の症状であれば，2週間の休薬後に，少量から再開し副作用を見ながら慎重に増量する．

高血圧などあれば，アセチルコリンの合成を促進する釣藤散などへの変更も有力である．

Ⅴ 周辺症状に対する薬物療法・家族指導の基本を身につける

暴言，興奮，易刺激性などには「抑肝散」が副作用が少なく使いやすい．ADLを落とすことなく，過鎮静もきわめて少ない．レビー小体型認知症の幻視にも効果が高い．2ヵ月程使用すれば，1ヵ月の休薬後も効果は持続しており，いったん休薬が可能であるので低カリウム血症などの副作用予防も容易である．

陽性症状が強い場合は，適応外であるが，リスペリドンやオランザピンなどの薬剤が必要になる．この場合，使用経験がなければ，もの忘れ外来や精神科などにコンサルトが望ましい．投与量は通常の1/4や1/2で開始し，軽度の効果があれば，あまり増量しないことがコツである．

周辺症状は反応性であるので，家族の対応はきわめて重要である．起き得る症状への理解，無視しないが過敏にならずに上手に受け入れる指導を行う．これによって入所が1年間伸びる成績が出されている．

Ⅵ 専門医との連携

前述のように，鑑別診断に画像検査が必要な場合，薬物投与開始を迷う場合，周辺症状に対する薬物療法に自信がない場合，家族指導に不慣れな場合，定期的な心理検査を行う場合などが専門医との連携が必要なケースである．

認知症の専門医療機関はどこもパンク状態である．「かかりつけ医の講習」のレベルを格段に高め，実地医家が認知症診療の大部分を分担しないかぎり，認知症セーフティーネットの構築は難しい．

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Nutrition 27 (2011) 471–478

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Time course of vitamin C distribution and absorption after oral administration in SMP30/GNL knockout mice

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ARTICLE INFO

Article history:
Received 12 December 2009
Accepted 30 April 2010

Keywords:
Gluconolactonase
Knockout
Plasma
Senescence marker protein-30
Urine

ABSTRACT

Objective: Because vitamin C (VC) has multiple metabolic and antioxidant functions, we investigated the movement of VC throughout the tissues of senescence marker protein-30 (SMP30)/gluconolactonase (GNL) knockout (KO) mice.
Methods: SMP30/GNL KO mice, which cannot synthesize VC in vivo, were divided into two groups: VC sufficient and VC deficient. Starting at 2 mo of age, both groups had free access to water containing 1.5 and 0.0375 g/L of VC for 1 mo.
Results: The average rate of VC retention in 20 tissues of VC-deficient SMP30/GNL KO mice was only 13.7% of that in VC-sufficient mice. Tissues that retained over 20% of VC were the cerebellum, white fat, testes, eyeballs, and pancreas, and those with less than 5% VC were the kidneys and heart. These results clearly indicate the different VC retention capacities among tissues. Next, we examined the time course of VC distribution and absorption in VC-deficient SMP30/GNL KO mice. After oral VC administration, VC content in the liver and kidney peaked at 3 h and then decreased. VC content in the lungs, adrenal glands, skin, white fat, and pancreas peaked at 6 h and in the cerebellum, cerebrum, skeletal muscles, eyeballs, thyroid gland, and testes at 12 h.
Conclusion: In this study, we found that exogenous VC administered orally in VC-deficient SMP30/GNL KO mice was distributed at distinctly different rates within individual tissues. The SMP30/GNL KO mice used in this study are a useful animal model that provides unique opportunities for investigating VC movement and metabolism in the entire body.

Introduction

Many animals can synthesize vitamin C (VC) in vivo; however, others such as humans and guinea pigs have lost the ability to make VC because of mutations in the L-gulono-γ-lactone oxidase gene, which is essential for VC synthesis in vivo [1]. Therefore, animals without enzyme activity of L-gulono-γ-lactone oxidase must obtain VC from dietary sources.

Vitamin C has numerous metabolic functions that are largely dependent on its potent reducing properties [2]. VC acts as a cofactor in reactions catalyzed by several metal-dependent oxygenases, e.g., Cu^{+}-dependent mono-oxygenases including peptidylglycine α-amidating mono-oxygenase involved in peptide hormone synthesis [3,4], dopamine β-hydroxylase involved in norepinephrine synthesis [5,6], and Fe^{2+}/α-ketoglutarate-dependent dioxygenases including prolyl and lysyl hydroxylases involved in collagen synthesis [7], 6-N-trimethyllysine dioxygenase and γ-butyrobetaine dioxygenase involved in carnitine synthesis [8], and asparaginyl hydroxylase, which modifies hypoxia-inducible factor-1 [9]. Moreover, VC has non-enzymatic reductive activity in chemical reactions. That is, VC has a strong antioxidant function evident in its ability to scavenge superoxide radicals in intracellular and extracellular reactions [10]. VC decreases oxidative DNA and protein damage, low-density lipoprotein oxidation, lipid peroxidation, oxidants and nitrosamines in gastric juice, and extracellular oxidants from neutrophils [10]. VC enhancement is evident in its ability to increase endothelium-dependent vasodilation [11].

This study is supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan (to A. I.), and a Grant-in-Aid from the Smoking Research Foundation, Japan (to A. I.).
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0899-9007/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.nut.2010.04.010

Recommendations for humans' dietary VC intake derive from several sources: steady-state concentrations in plasma and excretion in urine relative to dose after VC intake, saturation of the body with VC and estimates of the urinary response, and amount of VC intake that prevents scorbutic symptoms [10]. In 1979, the metabolism, half-life, turnover rates, elimination rates, and size of body pool of VC for humans were calculated by using radiolabeled VC [12]. Others reported the time course of VC levels in human plasma and urine after oral administration of non-radiolabeled VC [13–18]. Padayatty et al. [15] then documented peaks of VC levels in plasma approximately 3 h after a single administration of VC and a return to the steady-state level at 24 h. Levine et al. [13] considered the rate of actual VC usage for metabolism in the body as most effective after administration of a single 200-mg dose of VC and noted that no VC was excreted in urine after an intake of up to 100 mg of VC. Moreover, administering a single dose of VC higher than 500 mg of VC resulted in lowering the rate of VC absorption, and the large amount of VC absorbed was excreted immediately in urine [13]. Thus, the most recent recommended amount and interval of VC intake for humans have been determined mainly from analyzing plasma and urine, because investigating VC uptake and distribution directly in human tissues is an ethically difficult problem.

Recently, we established senescence marker protein-30 (SMP30)/gluconolactonase (GNL) knockout (KO) mice [19,20], which are incapable of synthesizing VC in vivo, because they lack SMP30/GNL, a necessary component of the VC biosynthetic pathway [21]. By providing VC in the food and drinking water of SMP30/GNL KO mice, we can control their bodily content of VC. Thus, SMP30/GNL KO mice are a useful animal model for investigating the retention capacities of VC in various tissues and its internal movements after oral administration in vivo. In the present study, we investigated VC distribution, absorption, and retention in a tissue-by-tissue study of VC-deficient SMP30/GNL KO mice.

Materials and methods

Animals

The SMP30/GNL KO mice were previously generated by the gene targeting technique [19]. Female KO mice (*SMP30/GNL*$^{-/-}$) were mated with male KO mice (*SMP30/GNL*$^{Y/-}$) to produce the KO mice used in this study, and only males were included. After weaning at 30 d of age, SMP30/GNL KO mice were fed a VC-deprived diet (CL-2, CLEA Japan, Tokyo, Japan) and had free access to water containing sufficient VC (1.5 g/L) and 10 μM ethylenediaminetetra-acetic acid (EDTA) until 2 mo of age. Then, SMP30/GNL KO mice were divided into VC-sufficient and VC-deficient groups. Both groups had free access to water containing 1.5 and 0.0375 g/L of VC containing 10 μM EDTA until 3 mo of age, respectively. Water bottles were changed every 3 or 4 d until the experiment ended. Male wild-type (WT; *SMP30/GNL*$^{Y/+}$) mice at 4 wk old were purchased from Japan SLC (Shizuoka, Japan). WT mice were fed a VC-deprived diet and had free access to water without VC. Throughout the experiments, animals were maintained on a 12-h light/dark cycle in a controlled environment. All experimental procedures using laboratory animals were approved by the animal care and use committee of Toho University (Chiba, Japan) and the Tokyo Metropolitan Institute of Gerontology (Tokyo, Japan).

Preparation of plasma, urine, and tissues

Urine was collected in microtubes by pushing the hypogastrium from both groups of SMP30/GNL KO mice at 3 mo of age. Then, these mice were sacrificed, and their blood was collected from the inferior vena cava. Blood was gently mixed with EDTA and centrifuged at 880 × *g* for 15 min at 4 °C. The resulting supernatants were used as plasma for further analysis. Afterward, mice were systemically perfused with ice-cold phosphate buffered saline through the left ventricle to wash out remaining blood cells, and tissues of interest were collected and stored at −80 °C until use.

VC distribution and absorption

For the study of VC distribution and absorption after oral administration, VC-deficient groups of 3-mo-old SMP30/GNL KO mice were fasted overnight, after which various amounts (0, 0.5, 1.0, 3.5, and 7.0 mg) of VC dissolved in 250 μL of water were orally administered by using stomach probes (Natsume Seisakusho, Tokyo, Japan). Urine samples were collected immediately or 3, 6, 12, and 24 h later, and then mice were sacrificed. Their urine, plasma, and tissues were stored at −80 °C until use.

Measurement of VC

Vitamin C in urine, plasma and tissues was measured by a high-performance liquid chromatographic electrochemical detection method [22]. In this study we measured only the reduced form of VC because it has numerous metabolic functions and strong antioxidant functions [2,10]. Tissues were homogenized in 14 vol of 5.4% metaphosphate containing 1 mM EDTA and centrifuged at 21 000 × *g* for 10 min at 4 °C. Plasma and urine were mixed with equal volumes of 10% metaphosphate containing 1 mM EDTA and centrifuged at 21 000 × *g* for 10 min at 4 °C. The supernatants obtained were rapidly frozen to prevent oxidation of VC and were kept at −80 °C until use. Samples were analyzed by high-performance liquid chromatography using an Atlantis dC18 5-μm column (4.6 × 150 mm; Nihon Waters, Tokyo, Japan). The mobile phase was 50 mM phosphate buffer (pH 2.8), 0.2 g/L of EDTA, 2% methanol at a flow rate of 1.3 mL/min, and electrical signals were recorded by using an electrochemical detector (2465, Nihon Waters) with a glassy carbon electrode at +0.6 V [23,24]. Creatinine levels in urine were measured with a Wako Creatinine Test kit (Wako Pure Chemical, Osaka, Japan) according to the manufacturer's instructions, and VC levels in urine were normalized by creatinine value. VC retention in tissues was calculated as VC retention (%) = (VC content of tissue from VC-deficient SMP30/GNL KO mice)/(VC content of tissue from WT or VC-sufficient SMP30/GNL KO mice) × 100.

Statistical analysis

Results are expressed as means ± standard errors of the mean. The probability of statistical differences between experimental groups was determined by unpaired *t* test using KaleidaGraph software (Synergy Software, Reading, PA, USA). Statistical differences were considered significant at $P < 0.05$.

Results

VC retention capacity of tissues

The VC content was measured in 20 tissue sites and plasma of WT mice at 3 mo of age and, as Figure 1 shows, amounts of VC per tissue weight varied considerably. Listed in order of the highest to lowest VC content, these were the adrenal gland, cerebellum, cerebrum, spleen, thyroid gland, small intestine, lung, submaxillary gland, testes, large intestine, stomach, kidney, liver, eyeballs, pancreas, brown fat, heart, skin, skeletal muscle, and white fat. The largest amount of VC was in the adrenal glands, which had 8.3 ± 0.8 μmol/g of tissue, and white fat had the lowest VC content at 19.0 ± 3.0 nmol/g of tissue. In addition, the concentration of VC in plasma was 38.0 ± 5.1 μM. We next measured the VC content in 20 tissues and plasma of VC-sufficient SMP30/GNL KO mice at 3 mo of age; after weaning, these mice had free access to water containing 1.5 g/L of VC. Mean water consumption during the experiment was 4.6 ± 0.3 mL/d per mouse (6.9 ± 0.4 mg VC/d per mouse). The amount of VC in tissues and the concentration of VC in plasma from WT mice were almost the same as in VC-sufficient SMP30/GNL KO mice except for the thyroid gland and white fat (Fig. 1). That is, VC contents in thyroid glands from WT and VC-sufficient SMP30/GNL KO mice were 1.9 ± 0.3 and 0.8 ± 0.1 μmol/g of tissue, respectively, and VC contents in white fat from WT and VC-sufficient SMP30/GNL KO mice were 19.0 ± 3.0 and 42.0 ± 9.0 nmol/g of tissue, respectively. In contrast, the amounts of VC in tissues of VC-deficient SMP30/GNL KO mice, which had free access to water containing 0.0375 g/L of VC for 1 mo, were all significantly lower than those of WT and VC-sufficient SMP30/

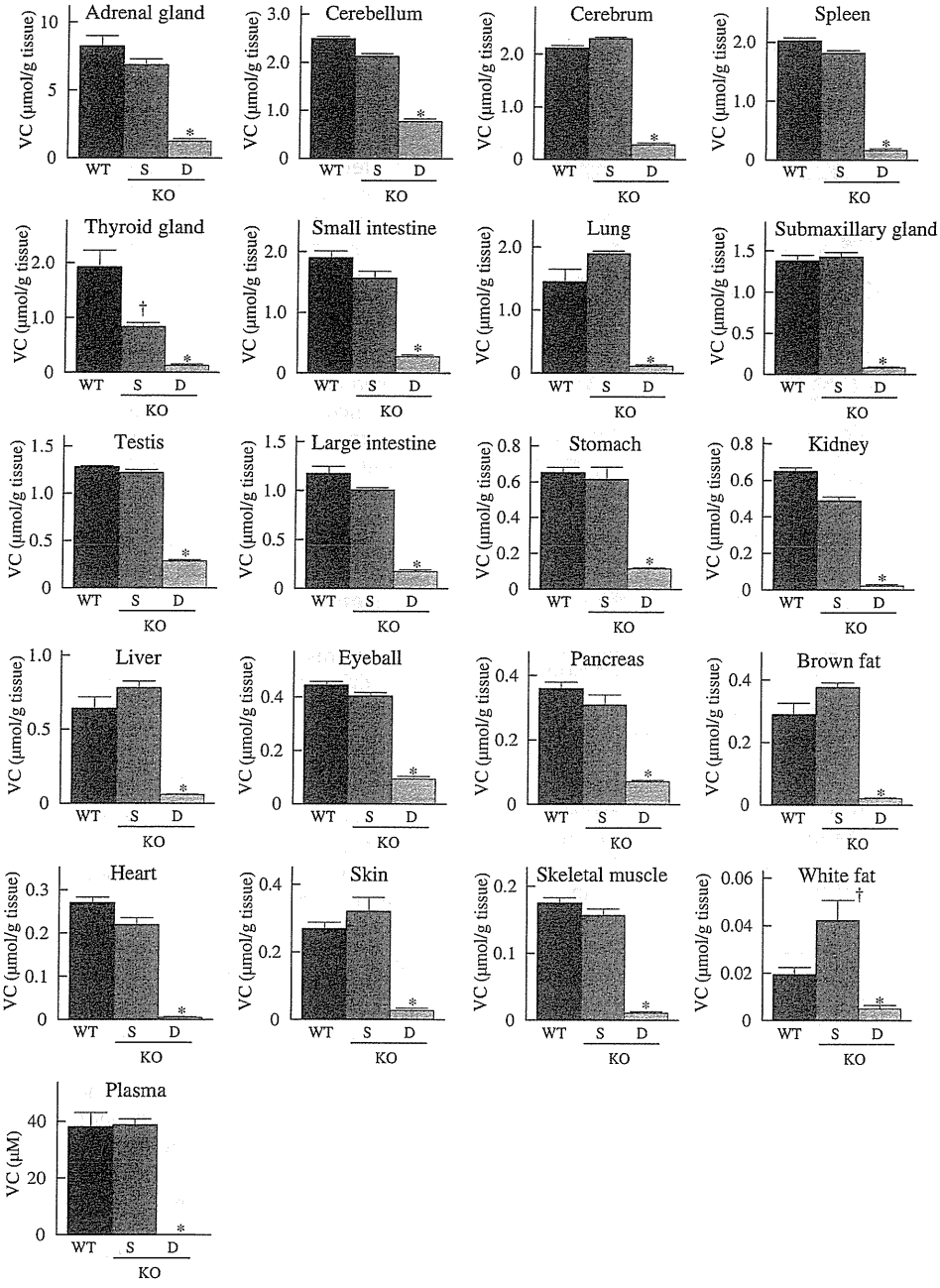

Fig. 1. VC content in 20 tissues and plasma of WT and VC-sufficient and VC-deficient senescence marker protein-30/gluconolactonase KO mice at 3 mo of age. The S and D senescence marker protein-30/gluconolactonase KO mice had free access to water containing 1.5 and 0.0375 g/L of VC containing 10 μM ethylenediaminetetra-acetic acid from 2 to 3 mo of age, respectively. WT mice had free access to water without VC. Tissues were homogenized in 14 vol of 5.4% metaphosphate containing 1 mM ethylenediaminetetra-acetic acid and centrifuged at 21 000 × *g* for 10 min at 4 °C. Plasma were mixed with equal volumes of 10% metaphosphate containing 1 mM ethylenediaminetetra-acetic acid and centrifuged at 21 000 × *g* for 10 min at 4 °C. VC contents of centrifugal supernatants of tissues and plasma were analyzed by high-performance liquid chromatographic electrochemical detection as described in MATERIALS AND METHODS. Values are expressed as mean ± SEM of five animals. * $P < 0.01$ compared with WT and S mice. † $P < 0.01$ compared with WT and D mice. D, vitamin C deficient; KO, knockout; S, vitamin C sufficient; VC, vitamin C; WT, wild-type.

GNL KO mice (Fig. 1). Moreover, VC in plasma from VC-deficient SMP30/GNL KO mice was nearly undetectable. Mean water consumption during the experiment was 4.0 ± 0.3 mL/d per mouse (0.15 ± 0.01 mg VC/d per mouse), and mean amounts of food consumed by VC-deficient and VC-sufficient SMP30/GNL KO mice were 3.9 and 4.0 g/d per mouse, respectively. Body weight of VC-deficient and VC-sufficient SMP30/GNL KO mice at 2 mo of age were 26.1 ± 1.0 and 25.2 ± 0.7 g, respectively, and at 3 months of age were 30.9 ± 1.1 and 29.3 ± 1.2 g, respectively. There were no differences in the appearance or behavior of VC-deficient and VC-sufficient SMP30/GNL KO mice during the experiment. The percentages of VC retention in 20 tissues of SMP30/GNL KO mice deprived of VC for 1 mo are listed in Table 1. These values varied from 1.9% to 36.4% compared with WT and VC-sufficient SMP30/GNL KO mice, and the percentage of VC retention in plasma was 0.4%. The average VC retentions in the same 20 tissues were 13.2% and 13.7% compared with WT and VC-sufficient mice, respectively. Tissues with greater than 20%

Table 1
Percentage of VC retention in 20 tissues and plasma of VC-deficient SMP30/GNL KO mice deprived of VC for 1 mo compared with WT and VC-sufficient SMP30/GNL KO mice

Tissues	VC retention (%)*	
	Comparison with WT mice	Comparison with VC-sufficient KO mice
Cerebellum	31.1	36.4
White fat	25.9	11.9
Testis	22.1	23.2
Eyeball	21.2	23.3
Pancreas	20.0	23.3
Stomach	17.6	18.7
Large intestine	14.9	17.4
Adrenal grand	14.7	17.7
Small intestine	14.5	17.7
Cerebrum	13.4	12.3
Skin	10.6	8.9
Liver	9.6	7.9
Spleen	8.1	9.1
Lung	7.6	5.8
Brown fat	7.3	5.6
Thyroid gland	6.6	15.2
Skeletal muscle	6.5	7.3
Submaxillary gland	5.8	5.6
Kidney	3.5	4.7
Heart	1.9	2.3
Plasma	0.4	0.4

KO, knockout; SMP30/GNL, senescence marker protein-30/gluconolactonase; VC, vitamin C; WT, wild-type

* VC retention (%) = (VC content of tissue from VC-deficient SMP30/GNL KO mice)/(VC content of tissue from WT or VC-sufficient SMP30/GNL KO mice) × 100.

VC retention were the cerebellum, white fat, testes, eyeballs, and pancreas. Notably, the cerebellum had the highest retention of 31.1% and 36.4% among all tissues examined in the VC-deficient group; however, the cerebral tissue contained 13.4% and 12.3% compared with WT and VC-sufficient SMP30/GNL KO mice, respectively. Tissues of the VC-deficient SMP30/GNL KO mice with less than 5% of comparative VC values were the kidneys and heart, i.e., only 3.5% and 1.9%, compared with WT mice, respectively. Thus, SMP30/GNL KO mice deprived of VC for 1 mo showed a significantly decreased VC content in multiple tissues and plasma.

Distribution and absorption of VC

To clarify VC distribution in and absorption by various tissues and plasma after oral administration, we administered one-time doses of 0 to 7 mg of VC to VC-deficient SMP30/GNL KO mice. Subsequent measurement of VC content in 10 tissues and plasma 24 h later showed that the amount of VC in all tissues examined increased significantly in a dose-dependent manner (Fig. 2). Most prominently, spleens of VC-deficient mice given 7 mg of VC contained almost as much as in the VC-sufficient SMP30/GNL KO mice. Moreover, VC content in the hearts of VC-deficient recipients of 7 mg of VC actually exceeded that amount in VC-sufficient SMP30/GNL KO mice. However, the VC concentration in plasma did not increase significantly even when the dose of VC administered was 7 mg.

Time course of VC distribution and absorption

To investigate the time course of VC distribution and absorption throughout the body, we administered 3.5 mg of VC orally once to VC-deficient SMP30/GNL KO mice and measured VC content in 15 tissues, plasma, and urine at 3, 6, 12, and 24 h afterward. As Figure 3 illustrates, VC content in the liver and kidneys peaked at 3 h after VC administration and then decreased. The liver VC content 3 h after exposure was almost the same in VC-deficient and VC-sufficient SMP30/GNL KO mice. In the lungs, adrenal gland, skin, white fat, and pancreas, VC levels topped at 6 h after administration and then decreased. The VC content in white fat at 6 h was almost equal in the VC-deficient and VC-sufficient SMP30/GNL KO mice. However, that in the cerebellum, cerebrum, skeletal muscle, eyeballs, thyroid gland, and testes, which reached a peak at 12 h after VC administration before decreasing, varied. At the 12-h high point, the cerebellum and thyroid gland had almost as much in VC-deficient as in VC-sufficient SMP30/GNL KO mice. VC content in the heart increased until 6 h after VC administration and then remained almost at the same level until 24 h. VC content in the spleen increased until 24 h after VC administration, but in the plasma peaked at 3 h, decreased until 12 h, and remained at the same level from 12 to 24 h. Urine contained the most VC 3 h after administration before decreasing. Thus, the time course of VC distribution and absorption after oral administration differed markedly in each tissue tested.

Discussion

In the present study, we clearly demonstrate that individual tissues differ substantially in their usage of VC by tracking its retention, distribution, and absorption in SMP30/GNL KO mice made deficient in VC. Because these mice are unable to synthesize VC in vivo, depriving them of VC supplementation for 1 mo results in an effective VC deficiency. In this animal model, the time course of VC distribution to and absorption by each tissue after oral administration of a single dose of VC were also quite different. Our findings strongly suggest that each tissue's VC content is tightly controlled to express VC-specific functions. These functions include but are not limited to acting as a cofactor in reactions catalyzed by a number of oxygenases and dioxygenase [3–9] and as an antioxidant to scavenge superoxide radicals in intracellular and extracellular reactions [10].

The range of VC retention by each tissue from SMP30/GNL KO mice deprived of VC for 1 mo varied from 1.9% to 36.4% compared with retention by WT and VC-sufficient SMP30/GNL KO mice (Table 1). This differential VC retention capacity manifested the highest rate in the cerebellum of VC-deficient SMP30/GNL KO mice, which was 31.1% and 36.4% compared with WT and VC-sufficient SMP30/GNL KO mice, respectively, versus that in the cerebrum, which was 13.4% and 12.3%. These variations may be associated with different functional roles, different VC transport systems, and different oxidative stress–resistant abilities of the cerebellum and cerebrum, but the precise cause remains unclear. The VC retention percentage in plasma of SMP30/GNL KO mice after 1 mo of VC deficiency was the lowest of all test sites at only 0.4% compared with that in WT and VC-sufficient SMP30/GNL KO mice. Because VC was rapidly distributed to and absorbed by multiple tissues through the blood, its levels decreased first in the plasma.

The elevation of VC in tissues at 24 h after administration of VC was observed in VC-deficient SMP30/GNL KO mice in a dose-dependent manner (Fig. 2). Among 10 tissues evaluated, the spleen most effectively recovered normal VC levels at 24 h after administration of 7 mg of VC, and the heart even exceeded that criterion as indicated by the dotted line in Figure 2, which represents the VC content of VC-sufficient SMP30/GNL KO mice. These results denote the distinctly different VC transport and retention systems of each tissue.

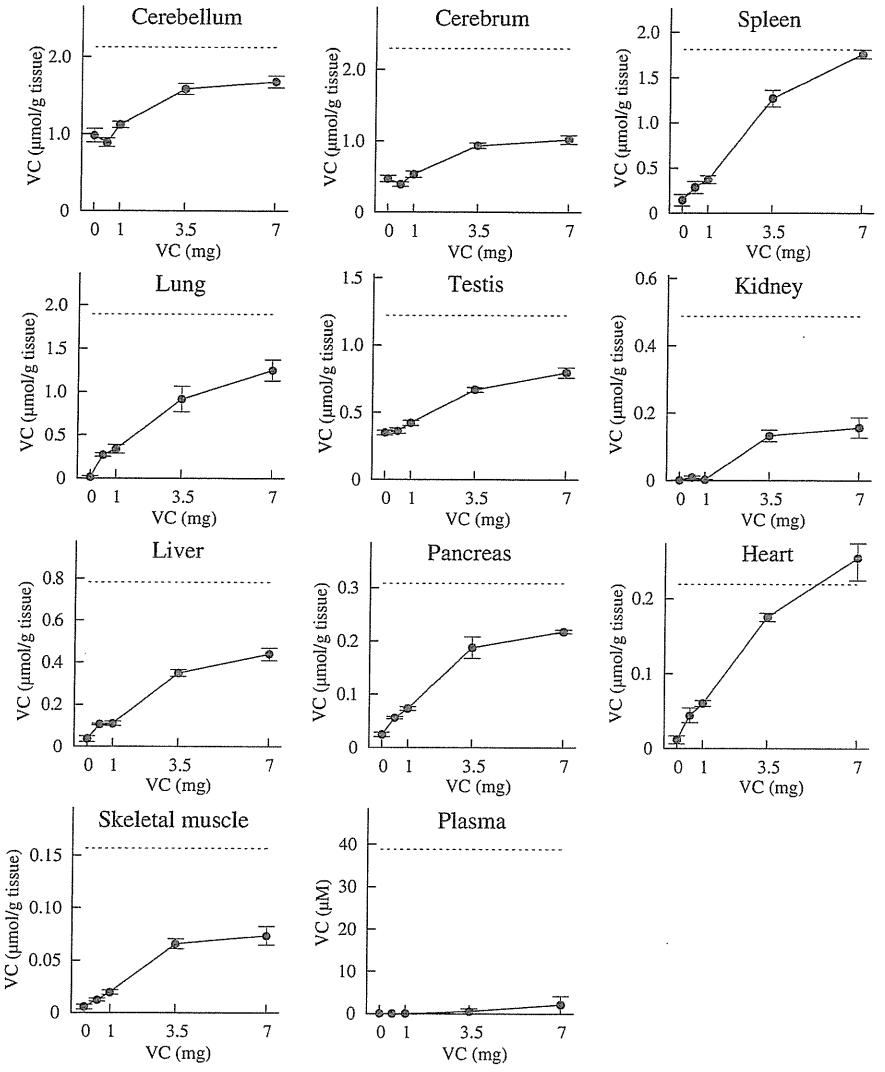

Fig. 2. Absorption of VC after oral administration and distribution to 10 tissues and plasma. Senescence marker protein-30/gluconolactonase knockout mice had free access to water containing 0.0375 g/L of VC containing 10 μM ethylenediaminetetra-acetic acid from 2 to 3 mo of age. The VC-deficient senescence marker protein-30/gluconolactonase knockout mice at 3 mo of age were fasted overnight and then given various amounts (0, 0.5, 1.0, 3.5, and 7.0 mg) of VC dissolved in 250 μL of water orally by using stomach probes. Mice were sacrificed 24 h later, and VC contents of tissues and plasma were analyzed by high-performance liquid chromatographic electrochemical detection. Values are expressed as mean ± SEM of four animals. Dotted lines represent the VC content of VC-sufficient senescence marker protein-30/gluconolactonase knockout mice. VC, vitamin C.

Vitamin C is conveyed from extracellular fluid into cytoplasm by the sodium-dependant VC transporters SVCT1 and SVCT2, which are 12-transmembrane proteins [25,26]. The homology of SVCT1 and SVCT2 transcripts among different species such as mouse, rat, guineapig, and human are about 86% to 95% and 89% to 95%, respectively [27–29]. A recent study indicated that SVCT1 is involved in the whole-body homeostasis of VC and exhibits a higher maximum velocity value than SVCT2 [30]. The Michaelis constant of SVCT1 ranges from 65 to 237 μM. Human SVCT1 is distributed in numerous tissues including the lung, liver, kidney, intestine, and skin [30]. In contrast, human SVCT2 occupies mainly the brain, eye, lung, heart, intestine, adrenal gland, bone, and skeletal muscle. SVCT2 has a lower Michaelis constant (8 to 115 μM) than SVCT1 and, as a high-affinity VC transporter, takes up lower concentrations of VC than does SVCT1 [27,30–36]. Dehydroascorbic acid, the oxidized form of VC, is also transported by glucose transporter isoforms: glucose transporter-1 (GLUT1), GLUT3, and GLUT4 [37,38].

Vitamin C from oral administration is first absorbed along the entire length of the small intestine, which contains polarized enterocytes [39]. VC absorbed from the small intestine is delivered to the liver, which receives 20% of blood as arterial flow and 80% of blood as portal flow from other tissues such as the intestine, spleen, and pancreas [40]. Because SVCT1 is known to be highly expressed in the liver [25], VC transported from the small intestine is rapidly absorbed into the liver with high-capacity transporter SVCT1. Therefore, in the present experiments, liver VC levels increased rapidly until reaching their maximal value and then quickly decreased owing to distribution among other tissues (Fig. 3). In the kidney, time-dependant changes of VC content resembled that in plasma. Proximal renal tubules are known to absorb VC from primitive urine and release it into plasma [41], and the VC level in primitive urine depends on that in plasma. Therefore, time-dependant changes of VC content in the kidney and the plasma must be parallel. Adrenal glands gained the highest VC content among the

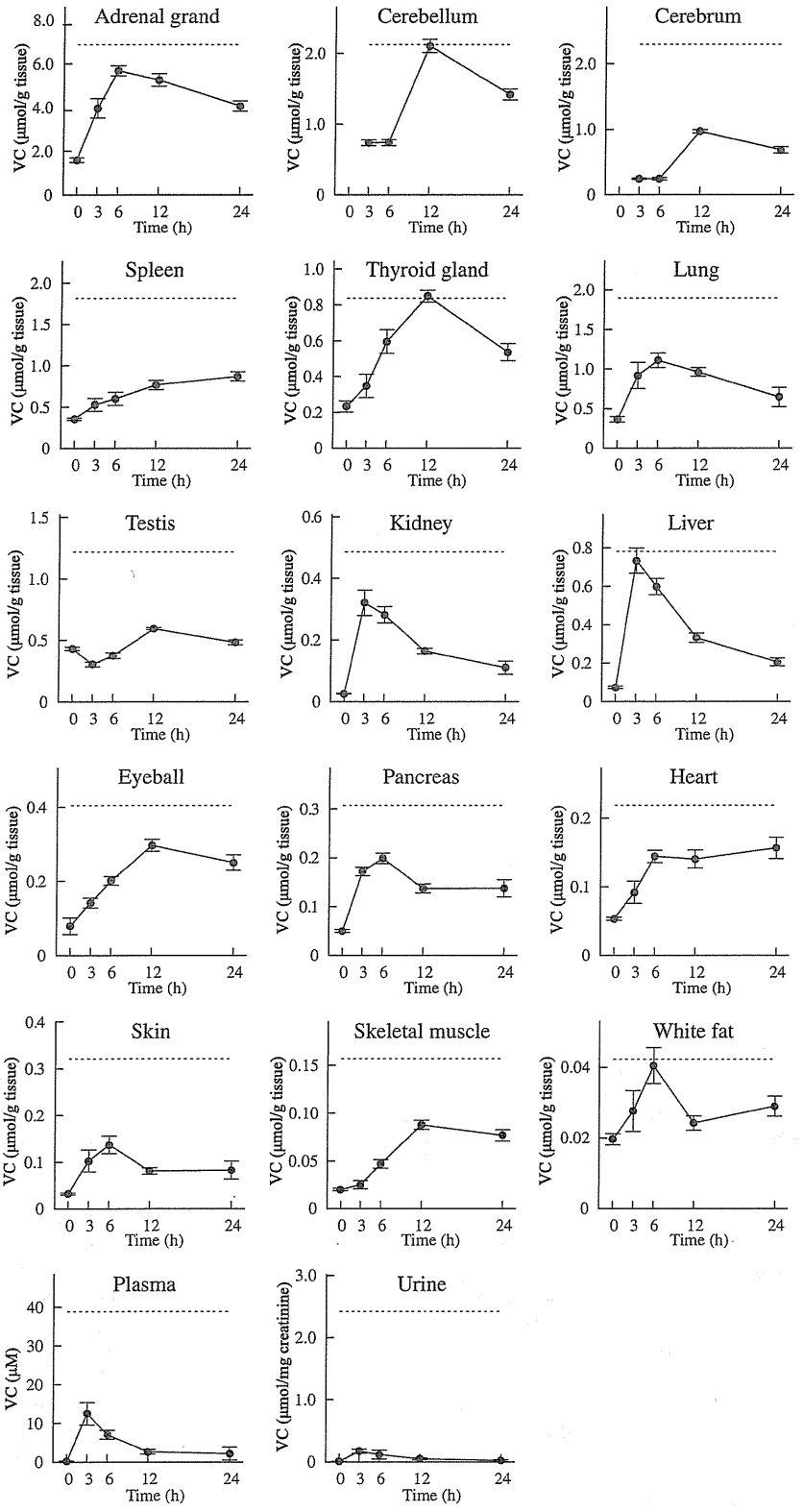

Fig. 3. Time course of VC distribution to and absorption by 15 tissues, plasma, and urine. The senescence marker protein-30/gluconolactonase knockout mice had free access to water containing 0.0375 g/L of VC containing 10 μM ethylenediaminetetra-acetic acid from 2 to 3 mo of age. The VC-deficient senescence marker protein-30/gluconolactonase knockout mice at 3 mo of age were fasted overnight, after which 3.5 mg of VC dissolved in 250 μL of water was administered orally through stomach probes. At the time of appraisal (0, 3, 6, 12, and 24 h), urine was collected, and mice were then sacrificed. VC contents of their tissues, plasma, and urine were analyzed by high-performance liquid chromatographic electrochemical detection. Values are expressed as mean ± SEM of five animals. Dotted lines represent the VC content of VC-sufficient senescence marker protein-30/gluconolactonase knockout mice. VC, vitamin C.

15 tissues tested at a peak of 6 h after VC-deficient SMP30/GNL KO mice received 3.5 mg of VC. Thereafter, the VC content decreased slightly but remained high for 24 h (Fig. 3). Because VC acts as a cofactor in reactions catalyzed by dopamine β-hydroxylase in norepinephrine synthesis by the adrenal gland [5,6], that gland must exhibit a strong ability to absorb and retain high concentrations of VC. The cerebellum and cerebrum of VC-deficient SMP30/GNL KO mice given 3.5 mg of VC showed similar time courses of VC absorption, which peaked at 12 h; however, the cerebellum absorbed approximately twice as much as the cerebrum (Fig. 3). This discrepancy may be associated with the different functional roles and different VC transport systems for the cerebellum and cerebrum. Although VC in plasma cannot pass the blood–brain barrier directly, VC and dehydroascorbic acid can travel in cerebrospinal fluid because SVCT2, GLUT1, and GLUT4 are expressed in the choroid plexus [25,42]. VC absorbed in cerebrospinal fluid must be taken up by neurons and glia cells; therefore, the peak time of VC acquisition in the cerebellum and cerebrum takes much longer than in other tissues.

In this study, we could determine time-dependant changes of VC concentrations in plasma, but not in leukocytes. It is important to know the relations between VC levels in plasma, leukocytes, and various tissues in animal models, because those data would enable us to estimate the status of VC in the same tissues of humans. Levine et al. [13] documented VC concentrations in plasma and leukocytes of healthy men who received a single dose of VC, but could not analyze VC levels in human tissues for ethical reasons. However, suitable animal models such as SMP30/GNL KO mice might provide valuable information to clarify the relations between VC levels in human plasma, leukocytes, and multiple tissues.

The inability of guinea pigs to make VC in their bodies because of mutations in the L-gulono-γ-lactone oxidase gene [1] is well-known. Because they share this incapacity with humans, guinea pigs have long been used for VC research. However, these laboratory animals present problems in terms of breeding, care, and studies because they are so much larger than mice. Moreover, guinea pigs oxidize VC all the way to carbon dioxide [43], whereas primates oxidize it only as far as 2- or 3-carbon carbohydrates including oxalic acid [43,44]. There are no reports about the way in which mice oxidize VC.

Recently, SVCT1 KO mice [45] and L-gulono-γ-lactone oxidase KO mice [46] were developed. Because SVCT1 is a sodium-dependant VC transporter conveyed from extracellular VC into cytoplasm, and SVCT1-deficient mice can synthesize VC in vivo, they are difficult to study for VC distribution and absorption after oral administration. L-gulono-γ-lactone oxidase KO mice may be comparable to SMP30/GNL KO mice because neither strain synthesizes VC in vivo.

Conclusion

In this study, we found that VC administered orally to SMP30/GNL KO mice without the capacity to synthesize their own VC was absorbed at distinctly different rates by individual tissues and that each of 15 tissues in addition to plasma and urine retained the VC for quite different periods.

Acknowledgments

The authors thank Ms. P. Minick for the excellent English editorial assistance. Vitamin C powder was kindly provided by DSM Nutrition, Japan.

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J Neuropathol Exp Neurol

Vol. 70, No. 2
February 2011
pp. 116–124

ORIGINAL ARTICLE

Subcellular Localization of Peptidylarginine Deiminase 2 and Citrullinated Proteins in Brains of Scrapie-Infected Mice: Nuclear Localization of PAD2 and Membrane Fraction-Enriched Citrullinated Proteins

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Abstract

Peptidylarginine deiminase (PAD) and citrullinated proteins have emerged as key molecules in various human diseases, but detailed subcellular localizations of PAD2 and citrullinated proteins are poorly mapped in brain under normal and pathologic conditions. We performed subcellular fractionation and electron microscopic analysis using brains of normal and scrapie-infected mice. Peptidylarginine deiminase 2 was abundantly present in cytosol and weakly in microsomal and mitochondrial fractions and expression in these fractions was higher in brains of scrapie-infected mice. Despite relatively low PAD2 expression, in microsomal and mitochondrial fractions, citrullinated proteins were present at high levels in these fractions in scrapie-infected brains. Surprisingly, increased PAD2 expression and accumulated citrullinated proteins were also found in nuclear fractions in scrapie-infected brains. By electron microscopy, PAD2 and citrullinated proteins in scrapie-infected brains were widely distributed in most cellular compartments including mitochondria, endoplasmic reticulum, glial filaments, nuclei, and Golgi apparatus in astrocytes and hippocampal neurons. Taken together, we report for the first time the nuclear localization of PAD2 and the detailed subcellular localization of PAD2 and of citrullinated proteins in scrapie-infected brains. Our findings suggest that different subcellular compartmentalization of PAD2 and citrullinated proteins may have different physiological roles in normal and neurodegenerative conditions.

Key Words: Citrullination, Electron microscopy, PAD2, Scrapie, Subcellular fraction.

INTRODUCTION

Recently, peptidylarginine deiminases (PADs) and PAD-mediated citrullinated proteins have emerged as key molecules in human diseases including rheumatoid arthritis, multiple sclerosis (MS), Alzheimer disease (AD), and prion diseases (1–5). Peptidylarginine deiminases are calcium (Ca^{2+})-dependent catalytic enzymes that are responsible for the irreversible posttranslational modification of guanidino groups to ureido groups on arginine residues in proteins, but they do not catalyze free arginine to citrulline (5). This process, termed *citrullination* or *deimination*, results in loss of the positive charge and an increase of ~1-d molecular mass for each citrullinated residue. Activated PADs alter some of the biochemical properties of various target proteins via modulation of the citrullination process both in vitro and in vivo. They change proteolytic susceptibility and cause dissociation of cellular structure and protein binding affinity to target molecules, such as filaggrin, myelin basic protein (MBP), CXC chemokine ligands, and prion protein (6–10). Peptidylarginine deiminase can also be involved in the induction of autoimmunity by citrullinated antigens (11) and in the regulation of gene expression via histone deimination and demethylimination (12, 13). These results suggest that PADs play crucial roles in protein functions under abnormal conditions.

In humans and rodents, PADs consist of 5 isotypes (PAD1–4 and 6) encoded by 5 separate genes that are located in a single gene cluster on chromosome 1 in humans, chromosome 4 in mice, and chromosome 5 in rats (4). These PAD enzymes are specifically associated with various mammalian tissues (3, 14). Among them, PAD types 2 and 4 are expressed in the central nervous system (CNS). Peptidylarginine deiminase 4, also known as PAD5, has a nuclear localization signal sequence: 56-PPAKKKST-63 (15). Hence, it is localized in the nucleus and citrullinates histone proteins in vitro and in vivo (12, 13, 16). Peptidylarginine deiminase 2 has been

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This work was supported by the Korea Research Foundation grant funded by Korean Government (MOEHRD, Basic Research Promotion Fund; KRF-2006-331-E00287) and a grant (code no. 20080401034016) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.