understanding of body iron metabolism at a molecular level enables us to elucidate the mechanism of iron toxicity more precisely. Improvement of patients' outcomes is becoming promising if a correct early diagnosis is made, and suitable management of these intractable conditions using iron chelation with high compliance is conducted.

References

1. Andrews NC. Disorders of iron metabolism. N Engl J Med. 1999;341:1986–95.
2. McKie AT, Latunde-Dada GO, Miret S, et al. Molecular evidence for the role of a ferric reductase in iron transport. Biochem Soc Trans. 2002;30:722–4.
3. Trinder D, Fox C, Vautier G, Olynyk JK. Molecular pathogenesis of iron overload. Gut. 2002;51:290–5.
4. Sargent PJ, Farnaud S, Evans RW. Structure/function overview of proteins involved in iron storage and transport. Curr Med Chem. 2005;12:2683–93.
5. Cabantchik ZI, Breuer W, Zanninelli G, Cianciulli P. LPI-labile plasma iron in iron overload. Best Pract Res Clin Haematol. 2005;18:277–87.
6. Olivieri NF, Brittenham GM. Iron-chelating therapy and the treatment of thalassemia. Blood. 1997;89:739–61.
7. Angelucci E, Brittenham GM, McLaren CE, et al. Hepatic iron concentration and total body iron stores in thalassemia major. N Engl J Med. 2000;343:327–31.
8. Ikuta K, Zak O, Aisen P. Recycling, degradation and sensitivity to the synergistic anion of transferrin in the receptor-independent route of iron uptake by human hepatoma (HuH-7) cells. Int J Biochem Cell Biol. 2004;36:340–52.
9. Liuzzi JP, Aydemir F, Nam H, Knutson MD, Cousins RJ. Zip14 (Slc39a14) mediates non-transferrin-bound iron uptake into cells. Proc Natl Acad Sci USA. 2006;103:13612–7.
10. Schranzhofer M, Schifrer M, Cabrera JA, et al. Remodeling the regulation of iron metabolism during erythroid differentiation to ensure efficient heme biosynthesis. Blood. 2006;107:4159–67.
11. Fleming MD. The genetics of inherited sideroblastic anemias. Semin Hematol. 2002;39:270–81.
12. Park CH, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem. 2001;276:7806–10.
13. Inamura J, Ikuta K, Jimbo J, et al. Upregulation of hepcidin by interleukin-1beta in human hepatoma cell lines. Hepatol Res. 2005;33:198–205.
14. Ganz T. Hepcidin in iron metabolism. Curr Opin Hematol. 2004;11:251–4.
15. Bridle KR, Frazer DM, Wilkins SJ, et al. Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homoeostasis. Lancet. 2003;361:669–73.
16. Pietrangelo A. Hemochromatosis: an endocrine liver disease. Hepatology. 2007;46:1291–301.
17. Gardenghi S, Marongiu MF, Ramos P, et al. Ineffective erythropoiesis in β-thalassemia is characterized by increased iron absorption mediated by down-regulation of hepcidin and up-regulation of ferroportin. Blood. 2007;109:5027–35.
18. Cazzola M, Huebers HA, Sayers MH, MacPhail AP, Eng M, Finch CA. Transferrin saturation, plasma iron turnover, and transferrin uptake in normal humans. Blood. 1985;66:935–9.
19. Breuer W, Hershko C, Cabantchik ZI. The importance of non-transferrin bound iron in disorders of iron metabolism. Transfus Sci. 2000;23:185–92.
20. Koorts AM, Viljoen M. Ferritin and ferritin isoforms I: structure-function relationships, synthesis, degradation and secretion. Arch Physiol Biochem. 2007;113:30–54.
21. Harrison PM, Arosio P. The ferritins: molecular properties, iron storage function and cellular regulation. Biochim Biophys Acta. 1996;1275:161–203.
22. Jacobs A, Beamish MR, Allison M. The measurement of circulating ferritin. J Clin Pathol. 1972;25:1003.
23. Jacobs A, Miller F, Worwood M, Beamish MR, Wardrop CA. Ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. Br Med J. 1972;4:206–8.
24. Piperno A. Classification and diagnosis of iron overload. Haematologica. 1998;83:447–55.
25. Saito H, Hayashi D, Ohya T, Ohya F, Yamada H. Clinical evaluation on serum ferritin (author's transl). Rinsho Ketsueki. 1979;20:1317–25.
26. Galanello R, Piga A, Forni GL, et al. Phase II clinical evaluation of deferasirox, a once-daily oral chelating agent, in paediatric patients with β-thalassaemia major. Haematologica. 2006;91:1343–51.
27. Cappellini MD, Cohen A, Piga A, et al. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with beta-thalassemia. Blood. 2006;107:3455–62.
28. Jensen PD, Jensen FT, Christensen T, Eiskjaer H, Baandrup U, Nielsen JL. Evaluation of myocardial iron by magnetic resonance imaging during iron chelation therapy with deferrioxamine: indication of close relation between myocardial iron content and chelatable iron pool. Blood. 2003;101:4632–9.
29. Takatoku M, Uchiyama T, Okamoto S, et al. Retrospective nationwide survey of Japanese patients with transfusion-dependent MDS and aplastic anemia highlights the negative impact of iron overload on morbidity/mortality. Eur J Haematol. 2007;78:487–94.
30. Gattermann N. Guidelines on iron chelation therapy in patients with myelodysplastic syndromes and transfusional iron overload. Leuk Res. 2007;31(Suppl 3):S10–5.
31. Olivieri NF, Brittenham GM, Matsui D, et al. Iron-chelation therapy with oral deferiprone in patients with thalassemia major. N Engl J Med. 1995;332:918–22.
32. Long JA Jr, Doppman JL, Nienhus AW, Mills SR. Computed tomographic analysis of beta-thalassemic syndromes with hemochromatosis: pathologic findings with clinical and laboratory correlations. J Comput Assist Tomogr. 1980;4:159–65.
33. Brittenham GM, Farrell DE, Harris JW, et al. Magnetic-susceptibility measurement of human iron stores. N Engl J Med. 1982;307:1671–5.
34. Anderson LJ, Westwood MA, Holden S, et al. Myocardial iron clearance during reversal of siderotic cardiomyopathy with intravenous desferrioxamine: a prospective study using T2* cardiovascular magnetic resonance. Br J Haematol. 2004;127:348–55.
35. Crichton RR, Wilmet S, Legssyer R, Ward RJ. Molecular and cellular mechanisms of iron homeostasis and toxicity in mammalian cells. J Inorg Biochem. 2002;91:9–18.
36. Yen AW, Fancher TL, Bowlus CL. Revisiting hereditary hemochromatosis: current concepts and progress. Am J Med. 2006;119:391–9.
37. Pietrangelo A. Hereditary hemochromatosis—a new look at an old disease. N Engl J Med. 2004;350:2383–97.
38. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet. 1996;13:399–408.
39. Franchini M. Hereditary iron overload: update on pathophysiology, diagnosis, and treatment. Am J Hematol. 2006;81:202–9.

40. Bonkovsky HL, Lambrecht RW, Shan Y. Iron as a co-morbid factor in nonhemochromatotic liver disease. Alcohol. 2003;30:137–44.
41. Zurlo MG, De Stefano P, Borgna-Pignatti C, et al. Survival and causes of death in thalassaemia major. Lancet. 1989;2:27–30.
42. McGowan JH, Cleland JG. Reliability of reporting left ventricular systolic function by echocardiography: a systematic review of 3 methods. Am Heart J. 2003;146:388–97.
43. Anderson LJ, Holden S, Davis B, et al. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. Eur Heart J. 2001;22:2171–9.
44. Telfer PT, Prestcott E, Holden S, Walker M, Hoffbrand AV, Wonke B. Hepatic iron concentration combined with long-term monitoring of serum ferritin to predict complications of iron overload in thalassaemia major. Br J Haematol. 2000;110:971–7.
45. Olivieri NF. The β-thalassemias. N Engl J Med. 1999;341:99–109.
46. Fung EB, Harmatz PR, Lee PD, et al. Increased prevalence of iron-overload associated endocrinopathy in thalassaemia versus sickle-cell disease. Br J Haematol. 2006;135:574–82.

特集・鉄代謝と鉄過剰症

2．生体内不安定鉄と鉄毒性と鉄キレート療法

生田 克哉*・佐々木勝則**
Ikuta Katsuya Sasaki Katsunori
鳥本 悦宏***・高後 裕*1)
Torimoto Yoshihiro Kohgo Yutaka

*旭川医科大学内科学講座 消化器・血液腫瘍制御内科学分野 1)教授
**旭川医科大学消化管再生修復医学講座
***旭川医科大学病院腫瘍センター

Summary 鉄は生体にとって必須の金属であるが，過剰に存在すると容易にラジカル産生を引き起こすため有害となる。そのため，血液中ではトランスフェリン，細胞内ではフェリチンといった鉄結合蛋白によって鉄はfreeであることを回避されている。しかし，これらのcapacityを超えると，血液中では非トランスフェリン結合鉄が，また，細胞内ではlabile iron poolと呼ばれる「不安定な鉄」が出現し，生体に障害をもたらす。鉄キレート療法はこれらの有害な鉄を除去する治療法であり，最近，新規経口鉄キレート剤が本邦でも使用可能になり注目されている。

1．生体内鉄代謝の概要

鉄は，ヘモグロビンの構成要素として赤血球造血に必須なだけではなく，DNA合成など全身の細胞の様々な代謝に必要であり，生体にとって必要不可欠な金属元素である[1]。

生体内における鉄の動き，すなわち生体内鉄代謝の概要を生体に鉄が取り込まれるところからみてみると，食事に含まれる鉄は消化管にて吸収されて血液中に入り，トランスフェリン（transferrin：Tf）と呼ばれる蛋白に結合した形で運搬される[2]。一部の鉄は肝臓での貯蔵や筋肉などの全身の組織での利用にまわされるが，大部分の鉄は骨髄での赤血球造血に利用されている。一方，生体は鉄を積極的に体外に放出する機構を有しておらず，通常では消化管粘膜上皮細胞の脱落などに伴うわずかな損失しかなく，それを補う分だけ消化管から吸収している。生体内のほとんどの鉄は各臓器を行き来しながら生体内にとどまり，半閉鎖的な回路を構築している。生体で利用される大部分の鉄は，網内系で寿命となった赤血球のヘモグロビンから鉄が取り出され，再びTfと結合して全身を循環し再利用されることによってまかなわれている（**図1**）[3]。

Tf（transferrin；トランスフェリン）

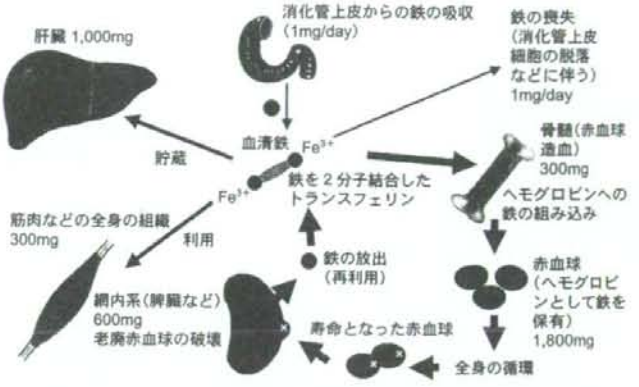

図1　生体内鉄代謝の概要

生体内で多くの鉄は赤血球造血に利用され，赤血球中のヘモグロビンに含まれ全身を循環しているが，一部は肝臓に貯蔵されたり，筋肉などの全身の細胞によって利用されている。生体は鉄を積極的に体外に排出する機構を有しておらず，通常はこれに見合った少量しか消化管から吸収しておらず，大部分の鉄は老廃赤血球を網内系で破壊して再利用してまかなっている。そのため，生体内鉄代謝は半閉鎖系を構築していると言える。

2．鉄の毒性

鉄代謝は多くの分子が複雑に関与して調節されていることが最近の研究で明らかになってきている[3]。これは，鉄が生体内に必要不可欠である一方で，過剰に存在するようになると逆に毒性を呈してくるためである。そこで次に，鉄が生体に対して及ぼす毒性について見ていく。

鉄は遷移金属であり，細胞内環境において，electron donorである2価鉄とelectron acceptorである3価鉄の間で容易に反応するため，生体内での様々な生化学的反応に利用されている。しかしながら，こうした特性は逆に有害なラジカルを産生するよう働き得るため，過剰状態では生体に対して毒性を示すこととなる。これは主にFenton反応によるもので，最終的にスーパーオキシド（O_2^-）や過酸化水素（H_2O_2）から，ヒドロキシラジカル（OHラジカル：$OH^\cdot$）が産生される（図2）。これらは活性酸素種reactive oxygen species（ROS）と呼ばれるが，OHラジカルはその中でも最も強力なもので，多糖類，蛋白質，核酸，脂質などを標的とし，細胞成分の酸化によって細胞に障害を与え細胞死を引き起こしたり，変性疾患の発症や発癌とも関連すると考えられている[4]。

また，鉄過剰状態ではOHラジカルのみではなく，peroxyl（$ROO^\cdot$），alkoxyl（$RO^\cdot$），thiyl（$RS^\cdot$），thiyl-peroxyl（$RSOO^\cdot$）などの各種のラジカルが産生される。

ROSにより生体内の様々な分子が修飾されるが，その一部は酸化ストレスのマーカーとして利用されている。主に脂質過酸化の指標としては

ROS (reactive oxygen species)

$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^- + OH^{\cdot}$
(Fenton 反応)

$Fe(III) + O_2^{-} \rightarrow Fe(II) + O_2$

(net reaction)

$H_2O_2 + O_2^{-} \rightarrow OH^- + OH^{\cdot} + O_2$
(Haber - Weiss 反応)

図2 Fenton 反応および Harber-Weiss 反応
生体内に鉄が過剰になると，これらの反応を介してラジカルが産生され，生体に対して障害をもたらす。

4-hydroxy-2-nonenal (**HNE**) が，また，DNA 障害の指標としては 8-hydroxy-2'-deoxyguanosine (**8-OHdG**) が知られている。アルコール性肝障害における肝細胞での HNE と鉄の局在が一致するという報告[5]や，C 型慢性肝炎において瀉血と鉄制限食により 8-OHdG の低下が認められるという報告[6]は，ともに鉄が ROS を介して肝障害をもたらすことを示唆するものと考えられる。

こうした ROS 産生は，特に「free (自由)」な鉄が関与するため，生体内では鉄はヘム鉄（ヘモグロビンやミオグロビンなど），フェリチン，ヘモジデリン，トランスフェリンなどといった，鉄結合蛋白に結合する形となり，free な状態で存在することは回避されている[7]。しかしながら，生体が何らかの原因によって鉄過剰状態になると，鉄結合蛋白に結合しえなくなった free な鉄が出現してくることになる。Free な鉄は種々の形態をとっているが，その結合は弱いものであり，容易に ROS 産生などに関与することから，いわゆる「不安定鉄」と呼ばれる。これには，循環血液中における非トランスフェリン結合鉄 (non-transferrin bound iron：**NTBI**) や，細胞内における不安定鉄プール (labile iron pool：**LIP**) といったものがあげられる[8~11]。

3．循環血液中での不安定鉄：非トランスフェリン結合鉄

まず，循環血液中における不安定鉄について見てみる。血液中では鉄は主に Tf に結合した状態である。この Tf は肝臓で産生され全身の血液中を循環している分子量約８万の糖蛋白であり，Tf1 分子で２分子の鉄を結合することができる[2]。Tf の鉄に対する親和性は非常に高く，正常な状態においては消化管から流入した鉄や網内系から再利用に回された鉄はすぐに Tf に結合することができ，そのため，鉄は血液中で free の状態にならずにすむ[7]。鉄の中でも，特に free の状態で存在する鉄は，redox cycling を介した ROS を産生する反応に容易に関与して生体に毒性をもたらすが，Tf と結合することで，こうした鉄の毒性が回避されている。血液中において，Tf の鉄を結合する capacity は，total iron binding capacity (**TIBC**) という形で表わされるが，通常は大きくて十分な予備力を有しており，正常な場合では 30%程度の Tf しか鉄で飽和されていない。しかし，厳密に制御されているはずの生体内鉄代謝調節が，何らかの原因によって崩れてしまい鉄が体内に過剰に存在する方向に傾くと，まず血液中で Tf が過剰な鉄を結合していくが，次第に Tf の鉄を結合できる capacity が飽和されてくる。Tf 飽和度が 100%もしくはそれに近い状態になると，Tf に結合しきれなかった鉄が free の形で血液中に出現してくることになり，これを総称して NTBI と呼ぶ[8]。生体内鉄代謝が崩れ鉄過剰に傾き得る原因として代表的なものは，サラセミア，

HNE (4-hydroxy-2-nonenal)　8-OHdG (8-hydroxy-2'-deoxyguanosine)
NTBI (non-transferrin bound iron；非トランスフェリン結合鉄)　LIP (labile iron pool；不安定鉄プール)
TIBC (total iron binding capacity)

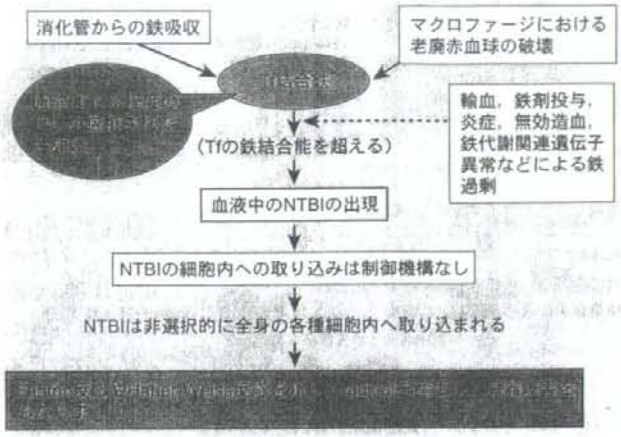

図3 NTBIの出現と臓器障害

消化管から吸収された鉄や，マクロファージにおける老廃赤血球の破壊により得られた鉄は，血液中ではトランスフェリン (transferrin：Tf) と結合して存在する。通常は30%のTfしか飽和されていないが，輸血，鉄剤投与，炎症などに伴う鉄過剰が加わると，次第にTfの鉄の結合能力を超えてしまい，血液中に非トランスフェリン結合鉄 (non-transferrin bound iron：NTBI) が出現してくる。NTBIの細胞内への取り込みには制御機構がなく，全身の細胞に非選択的に取り込まれてしまい，最終的にFenton反応などを介してラジカル産生を引き起こし，細胞・臓器障害をもたらす。

骨髄異形成症候群，再生不良性貧血などの輸血依存性を呈する血液疾患，遺伝性ヘモクロマトーシス，鉄剤の投与を受けている透析患者などが知られている[12]。さらに最近では，全身感染症，C型慢性肝炎，アルコール性肝障害[13]，各種の化学療法中[14]，cardiopulmonary bypass[15]などの際にも，NTBIが出現してくることが明らかとなっている。最近特に注目されているのは輸血による鉄過剰症で，輸血は短期間に急激に生体内に鉄を負荷する状態をつくるため，頻回で長期間にわたる輸血は容易に鉄過剰症をきたす。以前は，Tfに結合しきれない状態になって，すなわちTf飽和度が100%になって初めて血中に出現すると考えられていたが，最近では必ずしもそうではなく，Tfの結合能がある程度残っていても血液中にfreeな鉄を認める場合が確認されてきており，Tf飽和度が80%程度から血液中でNTBIが検出されると考えられるようになってきた。

Tfに結合しているTf結合鉄は，Tfと非常に高い親和性で結合する細胞膜表面のtransferrin receptor 1 (**TfR1**) を介して，骨髄中の赤芽球のように鉄を大量に必要とする細胞に選択的に取り込まれ利用されている[16]。このようにTf結合鉄に関しては行き先の方向性が明確であるが，NTBIにはこうした方向性がないと考えられており，そのため様々な細胞に無秩序に入り込み，最終的には細胞内鉄過剰状態をもたらし，ROS産生などを介して細胞障害を引き起こす(**図3**)。例

TfR1 (transferrin receptor 1)

えば，無トランスフェリン血症と呼ばれる稀な疾患では，血清中のTf量が非常に少ないためTfと結合する鉄量は極めて少なく，その一方でNTBIが非常に増加し肝臓などに鉄の蓄積を引き起こす。

NTBIについては，1978年にHershkoらが提唱してからすでに30年ほど経過するが，詳しいことは実は未だによくわかっていない[17]。これまでの情報から，NTBIは単一の形態で存在するものではなく，heterogeneousなものであると考えられている。まず，Tfと結合できなかった鉄は，血液中で様々な物質と結合する形になるが，例えばcitrateやalbuminがその代表として考えられている。また，NTBIは様々なキレート剤でキレートしてみることで，heterogeneousなものであるということがわかる。Desferrioxamine（**DFO**）でキレートされる分画や，oxalateでmobilizeされなければdetectできない分画などがあり，疾患それぞれで認められるNTBIが異なっていると考えられている。

最近，labile plasma iron（**LPI**）という用語が使用されるようになってきているが，これはNTBIのうち，特にredox cyclingに影響を及ぼすactiveな分画で鉄キレート剤によってキレートされ，全身の細胞に対して極めて細胞障害をもたらしやすい分画を示している[18]。

臨床的にはNTBIが測定できると，特に鉄過剰状態における鉄代謝の状態を詳細に把握することができるため非常に有用と考えられるが，これが極めて難しく，現在まで様々な定量法の報告はあるが，まだ信頼性・普遍性・簡便性のどれをも満たすような方法はないのが実情である。現在までに報告されている方法には以下のようなものがある。① 最初にNTBIをshuttle molecule（ethylene diamine tetraacetic acid（**EDTA**），nitrilotriacetic acid（**NTA**）など）でmobilizeさせ，その後血清蛋白をMW 30,000でcut-offされるmicrofilterで分離し，低分子量の分画をatomic absorptionもしくはcolorimetry，またはcolorimetryを組み合わせたHPLCで解析する方法は，現時点では最も信頼性はあるとされている。一方，ごく限られた施設でしか施行し得ない方法である[19]。② 上記方法に対して，NTBIのmobilizationと検出を血清蛋白の分離なしで行う方法がある。はじめに抗生物質であるbleomycinの鉄キレート能を利用してNTBIを捕捉し，次にbleomycinと鉄の複合体をascorbateなどの強力な還元剤の存在下でラジカル産生を誘導する。ここにDNAなどの酸化を受けうる物質を加えることで，酸化量とbleomycin-鉄複合体との関係が得られ，すなわちNTBIがどれだけ存在したか定量できる。ここで使用するascorbateは，還元剤としての作用とmobilizerとしての作用の両方を担うことになる。利点は比較的簡便に測定でき得ることであるが，原理的に間接的測定法であるため信頼性にやや劣ると考えられている[20]。③ 次に，蛍光標識したDFO（fluorescein tagged DFO：**Fl-DFO**）もしくはTf（fluorescein tagged Tf：**Fl-Tf**）を用いる方法があげられる。まず，血清に対しこれらをdirectに加えることで，NTBIの中でもDFO-chelatableなNTBI分画が結合し，その結果，蛍光が減弱するので，この変化を検出することでDFO-chelatable-NTBI量を定量することができる。加えて，oxalateやNTAでmobilize処理することでnon-DFO-chelatableなNTBI分画もFl-DFOに結合するため，これを検出できるというものである。Fl-Tfはoxalateと結合した鉄とは結合するが，Tfからの鉄を奪わないというものとして開発されており，特にoxalateでmobilizeされるNTBI分画を定量する[21]。利点としては感

DFO (Desferrioxamine)　LPI (labile plasma iron)　EDTA (ethylene diamine tetraacetic acid)
NTA (nitrilotriacetic acid)　Fl-DFO (fluorescein tagged DFO)　Fl-Tf (fluorescein tagged Tf)

度が高いとされ，かなり簡便であるが，蛍光強度のquentingで検出する方法のため，HPLC測定法に比較すると正確性に欠ける恐れがある。

4．細胞内での鉄代謝と細胞内不安定鉄

循環血液中に認められ得る不安定鉄としてNTBIがあげられたが，細胞内においても不安定な鉄は存在する。

血液中に存在する鉄は，ヘモグロビン合成やDNA合成をはじめとした各種の代謝に必須であるため全身の細胞に取り込まれるが，取り込まれた後の細胞内においても鉄は厳密に制御される必要がある。各種代謝に利用されず，細胞にとって余分となった鉄は，主に細胞質内のフェリチンと呼ばれる蛋白に格納される。フェリチンは，H-subunitとL-subunitという異なる2種類の分子が合計24個集合して構成されており，卵の殻のような形状で，内部に最大で4,500分子の鉄を貯蔵することができる[22]。このフェリチンのおかげで，細胞内では多くの鉄はfreeの形では存在しないですみ，過剰鉄が細胞に毒性を示すことが防がれている。しかし，細胞内の一部の鉄はLIPと呼ばれるfreeな形で存在すると考えられている。LIPは，細胞内各小器官と細胞質内での鉄の受け渡しなどを，種々の不安定な形態で速やかに行っていると考えられている鉄の「プール」である。フェリチンは十分な鉄貯蔵能力を有している上，細胞内鉄濃度が上昇すると，細胞はフェリチン蛋白の合成を亢進させてさらに格納能力を増やすように機能している。しかしながら，これらを超えるほど細胞内に多量の鉄が流入してくるようになると，フェリチンに格納しきれない分がLIPの増加につながる。LIPはfreeの鉄であるため容易にROS産生に働き，細胞障害をもたらすことになる。

LIPの存在形態に関しては詳細は不明な部分も多いが，Fe^{2+}とFe^{3+}両方の鉄イオン形態で存在し，鉄との親和性がそれほど強くない低分子物質と結合している。そのような低分子のキレート物質としては，アデニンやグアニンのような核酸，システインやチロシンのようなアミノ酸，あるいはクエン酸，アスコルビン酸，リン酸，リン脂質，ポリペプチドといった低分子化合物などが知られている。LIPは細胞内鉄総量の3～5%程度とその割合は極めて少ないとされるが，理論的には細胞内に取り込まれた鉄は，必ず一度はLIPを通過して目的となる鉄結合蛋白に至ることとなる。

LIPの動態には細胞内の種々の小器官が関与しており[23]，平衡状態でのLIPレベルは，細胞内への鉄の供給，細胞内における鉄の需要，細胞外への排出のバランスによって調整されている。組織によってその様式は大きく異なるものの，細胞外から細胞内への鉄の取り込みに関しては，例えばTfR1を介したTf結合鉄の取り込み経路や，Fe^{2+}のトランスポーターであるdivalent metal transporter 1（**DMT1**）[24, 25]やZIP14[26]などを介したNTBIの取り込み経路が知られている（**図4**）。TfR1を介した鉄取り込みであっても，細胞内エンドソーム内ではTfからはずれたFe^{3+}がFe^{2+}に還元された後，エンドソーム上のDMT1によって細胞内に移動するため，いずれの経路によっても，細胞質に移動した直後の鉄はFe^{2+}と考えられている。細胞にTf結合鉄やNTBIを負荷することにより細胞内のLIPレベルが増加することが知られており，細胞内への鉄供給がLIPレベルを規定する因子であると言えるが，これに加えて，細胞内からのLIPへの鉄供給も認められる。例えばフェリチンは，鉄を貯蔵し有害な鉄を隔離する機

DMT1（divalent metal transporter 1）

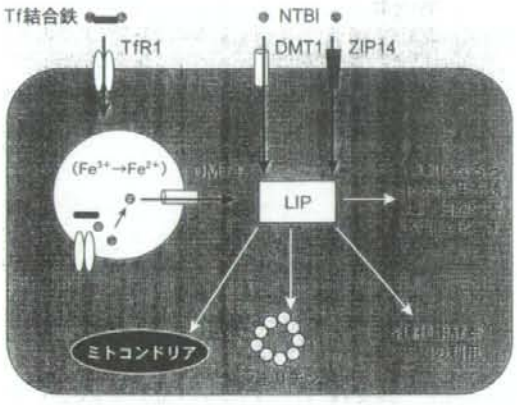

図4 細胞内のLIP

細胞は，トランスフェリン結合鉄(Tf結合鉄)をトランスフェリン受容体1 (transferrin receptor 1：TfR1)で取り込んだり，NTBIをDMT1やZIP14などのtransporterによって取り込む。これらはいずれにしても一度細胞質内においてLIPとなり，それからミトコンドリアで利用されたり，フェリチンに格納されたり，その他の各種代謝に利用されたりする。細胞内でのバランスが崩れLIPが過剰になると，細胞に対して障害をもたらす。

能を有しLIPレベルを減少させる蛋白であるが，逆にLIPレベルが減少した際には蓄えた鉄分子を放出しそのレベルを維持している。また，主たる赤血球の処理細胞である網内系細胞においては，ヘム蛋白がheme oxygenase-1により分解されて生じる鉄は，LIP形成に重要な因子となる。

LIPレベルは臓器の種類によって異なっている[27]。例えば，赤血球産生を行う赤芽球では，Tfからの鉄はミトコンドリアのferrochelataseに非常に効率的に渡されヘム合成に使用されるため，細胞質にはほとんどLIPは検出できないと報告されている[28]。一方，マクロファージなどの網内系細胞では，赤血球を貪食しヘモグロビンを分解し，heme oxygenase-1によるヘム鉄の放出が行われているため，LIPの増加が予測されている。また，鉄の貯蔵を主に担う肝細胞においてもLIPの存在が確認されている[11]。

各細胞内小器官におけるLIPレベルも，細胞の種類によって異なると考えられている。ミトコンドリアは細胞内の主要な鉄消費器官であるが，同時にスーパーオキシドの産生部位でもある。ミトコンドリアでは，ヘムや鉄-硫黄クラスター蛋白へ取り込まれる鉄と，ミトコンドリアに入る鉄量は厳密にリンクしていることから，ミトコンドリア内のLIPレベルは非常に少ないと考えられている。しかし，キレート可能な鉄の存在が培養肝細胞や心筋細胞のミトコンドリア内に認められ，キレート物質がミトコンドリア内でのROS産生を抑制することも報告されている[29]。また，鉄-硫黄クラスター蛋白合成に必須のfrataxin蛋白異常に起因するFriedreich's ataxiaや，ミトコンドリアの鉄トランスポーターであるABC7遺伝子の異常であるX連鎖性鉄芽球性貧血などのようなミトコンドリア内に鉄蓄積を来す疾患においては，ミトコンドリア内のLIPが増加し，病態形成に寄与している可能性も考えられている[30]。

5. 鉄キレート療法

これまで見てきたように，循環血液中のNTBIや細胞内におけるLIPは，フリーの形で存在する「不安定鉄」であり，細胞障害を引き起こし，各種の臓器障害にも強く関連していることが明らかとなってきた。こうした「不安定鉄」に対する治療として，単純ではあるがそれらの不安定鉄を除去する方法がまず考えられ，鉄キレート剤を用いた鉄キレート療法が期待される（**表1**）。

わが国では鉄過剰症に対して使用可能な唯一の

表1 鉄キレート剤の比較

	Desferrioxamine	Deferiprone	Deferasirox
投与経路	静注，皮下注	経口	経口
血中半減期	短い（5～20分程度）	やや短い（< 2 hours）	長い（8～16 hours）
投与方法	反復投与や持続投与が望ましい	1日2～3回の分割投与	1日1回投与
Molar chelating efficiency	High (hexadentate)	Low (bidentate)	Moderate (tridentate)
副作用	聴覚障害，骨や成長への影響，注射部位への局所皮膚反応など	重篤な顆粒球減少など	消化器症状，軽度の発疹，軽度の血清クレアチニン上昇（腎障害）など

現在まで臨床応用されている3つの鉄キレート剤の比較を示す。

治療薬がDFOであった。DFOの登場はサラセミア・メジャー患者の生存率を向上させたが，血中半減期は約5～20分と短く，経口バイオアベイラビリティが低いのが欠点で，静脈内または皮下投与が行われるが，さらに十分な血中濃度を保つために反復投与や持続ポンプを使用しての皮下注射などの工夫が必要であった。そのため，コンプライアンスも当然のことながら不良であり，十分な臨床効果をあげられる症例は多くなかった。

欧州で使用されているdeferiproneは低分子量で2座配位の経口鉄キレート剤であり，心臓に蓄積した鉄を効果的に除去するとされるが，血中半減期が約1.5時間程度と短いため1日2～3回の分割投与が必要であり，さらに顆粒球減少症の副作用が出やすいという問題があった。

一方，最近本邦でも認可になったdeferasiroxは新規トリデント鉄キレート剤であり，鉄に高い選択性を示す3座キレート剤である。血中半減期が8～16時間と長いため，1日1回水に懸濁して服用することで，定期的なDFO投与と同等の有効性が認められる。有害事象も他剤にくらべ少なく，コンプライアンスを高めることができ，鉄過剰症の予防および治療に非常に期待が持たれている。Deferasiroxの投与で，サラセミアの患者においてLPIが実際に低下するという報告もある。さらに，deferasiroxは細胞内に入ることから細胞内LIPもキレートする可能性が考えられ，鉄キレート療法が生体内不安定鉄を取り除くことで，将来的に鉄過剰によってもたらされ得る臓器障害を軽減，もしくは回避する可能性が示唆されている。

文 献

1) Aisen P, Enns C, Wessling-Resnick M: Chemistry and biology of eukaryotic metabolism. Int J Biochem Cell Biol **33**: 940-959, 2001
2) Gomme PT, McCann KB, Bertolini J: Transferrin: structure, function and potential therapeutic actions. Drug Discov Today **10**: 267-273, 2005
3) Andrews NC: Forging a field: the golden age of iron biology. Blood **112**: 219-230, 2008
4) Halliwell B, Gutteridge JMC: Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol **186**: 1-85, 1990
5) Ohhira M, Ohtake T, Matsumoto A, et al: Immunohistochemical detection of 4-hydroxy-2 nonenal-modified-protein adducts in human alcoholic liver diseases. Alcohol Cin Exp Res **22**: 145S-149S, 1998
6) Kato J, Kobune M, Nakamura T, et al: Normalization of elevated hepatic 8-hydroxy-2'-deoxyguanosine levels in chronic hepatitis C patients by phlebotomy and low iron diet. Cancer Res **61**: 8697-8702, 2001
7) Sahlstedt L, von Bonsdorff L, Ebeling F, et al: Effective binding of free iron by a single intra-

venous dose of human apotransferrin in haematological stem cell transplant patients. Br J Haematol **119** : 547-553, 2002

8) Breuer W, Hershko C, Cabantchik ZI : The importance of non-transferrin bound iron in disorders of iron metabolism. Transfus Sci **23** : 185-192, 2000

9) Greenberg GR apd Wintrobe MW : A labile iron pool. J Biol Chem **165** : 397-398, 1946

10) Jacobs A : An intracellular transit iron pool. Ciba Found Symp **51** : 91-106, 1976

11) Kakhlon O, Cabantchik ZI : The labile iron pool : characterization, measurement, and participation in cellular processes (1) . Free Radic Biol Med **33** : 1037-1046, 2002

12) Scheiber-Mojdehkar B, Lutzky B, Schaufler R, et al : Non-transferrin-bound iron in the serum of hemodialysis patients who receive ferric saccharate : no correlation to peroxide generation. J Am Soc Nephrol **15** : 1648-1655, 2004

13) De Feo TM, Fargion S, Duca L, et al : Non-transferrin-bound iron in alcohol abusers. Alcohol Clin Exp Res **25** : 1494-1499, 2001

14) Bradley SJ, Gosriwitana I, Srichairatanakool S, et al : Non-transferrin-bound iron induced by myeloablative chemotherapy. Br J Haematol **99** : 337-343, 1997

15) Pepper JR, Mumby S, Gutteridge JM : Blood cardioplegia increases plasma iron overload and thiol levels during cardiopulmonary bypass. Ann Thorac Surg **60** : 1735-1740, 1995

16) Aisen P : Transferrin receptor 1. Int J Biochem Cell Biol **36** : 2137-2143, 2004

17) Hershko C, Graham G, Bates GW, Rachmilewitz EA : Non-specific serum iron in thalassaemia : an abnormal serum iron fraction of potential toxicity. Br J Haematol **40** : 255-263, 1978

18) Cabantchik ZI, Breuer W, Zanninelli G, Cianciulli P : LPI-labile plasma iron in iron overload. Best Pract Res Clin Haematol **18** : 277-287, 2005

19) Singh S, Hider RC, Porter JB : A direct method for quantification of non-transferrin-bound iron. Anal Biochem **186** : 320-323, 1990

20) von Bonsdorff L, Lindeberg E, Sahlstedt L, Lehto J, Parkkinen J : Bleomycin-detectable iron assay for non-transferrin-bound iron in hematologic malignancies. Clin Chem **48** : 307-314, 2002

21) Esposito BP, Breuer W, Sirankapracha P, et al : Labile plasma iron in iron overload : redox activity and susceptibility to chelation. Blood **102** : 2670-2677, 2003

22) Harrison PM, Arosio P : The ferritins : molecular properties, iron storage function and cellular regulation. Biochim Biophys Acta **1275** : 161-203, 1996

23) Breuer W, Shvartsman M, Cabantchik ZI : Intracellular labile iron. Int J Biochem Cell Biol **40** : 350-354, 2008

24) Gunshin H, Mackenzie B, Berger UV, et al : Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature **388** : 482-488, 1997

25) Shindo M, Torimoto Y, Saito H, et al : Functional role of DMT1 in transferrin-independent iron uptake by human hepatocyte and hepatocellular carcinoma cell, HLF. Hepatol Res **35** : 152-162, 2006

26) Liuzzi JP, Aydemir F, Nam H, Knutson MD, Cousins RJ : Zip14 (Slc39a14) mediates non-transferrin-bound iron uptake into cells. Proc Natl Acad Sci U S A **103** : 13612-13617, 2006

27) Kruszewski M : Labile iron pool : the main determinant of cellular response to oxidative stress. Mutat Res **531** : 81-92, 2003

28) Richardson DR, Ponka P, Vyoral D : Distribution of iron in reticulocytes after inhibition of heme synthesis with succinylacetone : examination of the intermediates involved in iron metabolism. Blood **87** : 3477-3488, 1996

29) Glickstein H, El RB, Shvartsman M, Cabantchik ZI : Intracellular labile iron pools as direct targets of iron chelators : a fluorescence study of chelator action in living cells. Blood **106** : 3242-3250, 2005

30) Napier I, Ponka P, Richardson DR : Iron trafficking in the mitochondrion : novel pathways revealed by disease. Blood **105** : 1867-1874, 2005

Hepatology Research 2007; 37: 615–619 doi: 10.1111/j.1872-034X.2007.00095.x

Original Article

Evaluation of the effects of combination therapy with branched-chain amino acid and zinc supplements on nitrogen metabolism in liver cirrhosis

Miho Hayashi,[1] Kenji Ikezawa,[1] Akiko Ono,[1] Sachiyo Okabayashi,[1] Yoshito Hayashi,[1] Satoshi Shimizu,[1] Tatsuyoshi Mizuno,[1] Kosaku Maeda,[1] Tomofumi Akasaka,[1] Masafumi Naito,[1] Tomoki Michida,[1] Dan Ueshima,[2] Takayuki Nada,[2] Kiyotaka Kawaguchi,[2] Tekefumi Nakamura[2] and Kazuhiro Katayama[1]

[1]*Department of Internal Medicine, Osaka Koseinenkin Hospital and* [2]*Department of Internal Medicine, Kitano Hospital, Osaka, Japan*

Aim: Disorders of protein metabolism in liver cirrhosis can affect prognosis or cause complications. Treatment with branched-chain amino acid (BCAA) and zinc supplements has been shown to be effective against abnormal nitrogen metabolism in liver cirrhosis. There are, however, few studies on the effects of combining these supplements. In this study, the effect of combining BCAA and zinc treatment in cirrhosis was investigated.

Methods: Forty patients with liver cirrhosis who had blood albumin levels of 3.5 g/dL or less and blood zinc levels of 70 μg/dL or less were randomized to receive either BCAA alone or a combination of BCAA and zinc supplements. Blood albumin, the Fischer ratio, and ammonia levels were compared over 5–6 months of treatment.

Results: In the combination group, the post/pre treatment change ratio in blood ammonia levels decreased significantly (0.87 ± 0.26 vs. 1.22 ± 0.38, $P = 0.0033$), and the change ratio in the Fischer ratio increased significantly (1.22 ± 0.29 vs. 1.08 ± 0.16, $P = 0.0165$) in comparison with the BCAA monotherapy group. The change ratio in blood albumin levels showed no significant difference between the groups (1.01 ± 0.07 vs. 1.03 ± 0.08, $P = 0.4646$).

Conclusions: More improvement in disorders of nitrogen metabolism in liver cirrhosis occurred after administration of BCAA with zinc than after BCAA alone over 5–6 months of treatment. Further investigation is necessary to determine mechanisms of the action and longer-term clinical efficacy.

Key words: albumin, ammonia, branched-chain amino acid, Fischer ratio, urea-cycle

INTRODUCTION

LIVER CIRRHOSIS IS associated with various metabolic disorders, among which protein and energy malnutrition plays a role in the reduction of prognosis and the occurrence of complications such as hepatic encephalopathy.[1–5] Treatment using branched-chain amino acid (BCAA) has been demonstrated to contribute to improvement of the nitrogen metabolism, decrease in the incidence of complications, and improvement of prognosis.[6–8] In liver cirrhosis, BCAA is consumed in the skeletal muscle when ammonia that is no longer processed by the liver is detoxified via glutamine synthesis, resulting in BCAA deficiency.[9] Administration of BCAA corrects the amino acid imbalance and improves the protein metabolism, but BCAA itself imposes a nitrogen load and does not alleviate hyperammonemia.[6]

Liver cirrhosis is also associated with a high incidence of zinc deficiency, which contributes to nitrogen metabolism disorder. In particular, the urea-cycle enzyme ornithine-transcarbamylase, which plays a major role in ammonia metabolism in the liver, is a zinc enzyme. The activity of the urea-cycle enzymes is reduced by zinc deficiency. Several factors such as poor dietary intake, impaired intestinal absorption, and excessive urinary losses may be responsible for reduced whole-body zinc content.[10–13] Supplementation of zinc mainly improves nitrogen and ammonia metabolism in the liver.[10–14]

Correspondence: Dr Kazuhiro Katayama, Department of Internal Medicine, Osaka Koseinenkin Hospital, Osaka 553-0003, Japan
Email: kk8233@okn.gr.jp
Received 21 September 2006; final revision 20 February 2007; accepted 26 February 2007.

Because of their different mechanisms of action for improving nitrogen metabolism, BCAA and zinc may further enhance their therapeutic effects when administered concomitantly. Our preliminary data showed more improvement of the ammonia metabolism when these two treatments were combined.[12] In this study, we investigated the effect of combining these supplements using more indices, in addition to serum ammonia levels, and increased the number of patients examined.

METHODS

THIS STUDY ENROLLED 40 cirrhotic patients with the inclusion criteria of diagnosis of cirrhosis based on clinical and laboratory data, serum albumin levels of 3.5 g/dL or less, and serum zinc levels of 70 μg/dL or less. The patients were randomized into two groups to receive either BCAA granules alone or BCAA granules plus zinc sulfate. The BCAA was administered as Livact Granules (Ajinomoto, Tokyo, Japan). One sachet containing 4 g of BCAA, 952 mg L-isoleucine, 1904 mg L-leucine, and 1144 mg L-valine was taken orally after each meal. The zinc sulfate dose was 600 mg/day after each meal for those with blood zinc levels of <50 μg/dL, and 200 mg/day after breakfast for those with blood zinc levels of 50–70 μg/dL.

The patients were followed in the outpatient clinic about every 10 weeks, and the data before the therapy were compared with those of the subsequent visits. During the follow-up period of 5–6 months, changes in blood albumin, Fischer ratio, and ammonia levels were compared between the groups. There were no significant differences between the groups in age, sex, cause of liver disease, liver function test, blood zinc, ammonia, and Fischer ratio at baseline (Table 1).

This clinical investigation was approved by the institutional review board, and oral or written informed consent was obtained from all patients.

Statistical analysis

Continuous variables for patient characteristics of the groups were expressed as mean ± SD and the groups were compared using the Mann–Whitney *U*-test. Non-continuous variables such as sex and cause of liver disease were compared between the groups using the chi-squared test. For therapeutic effects between the groups, the difference between pre- and post-treatment values for each variable (change ratio) was calculated, and the mean ± SD values of the change ratio were compared using the Mann–Whitney *U*-test. Values measured before and after treatment were compared using the Wilcoxon signed rank sum test. The correlations between variables were calculated using Spearman rank correlations. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

THE BLOOD AMMONIA levels (Fig. 1) increased significantly in the BCAA group ($P = 0.016$), whereas the levels showed a tendency to decrease in the BCAA + zinc group ($P = 0.0707$), with a significant difference in the post/pre treatment change ratio (1.22 ± 0.38 vs. 0.87 ± 0.26, $P = 0.0033$).

The Fischer ratio (Fig. 2) did not show significant difference in the BCAA group ($P = 0.1984$), but increased

Table 1 Characteristics at baseline of cirrhotic patients treated with branched-chain amino acid (BCAA) alone or with BCAA and zinc (BCAA + Zn)

Variable	BCAA ($n = 21$)	BCAA + Zn ($n = 19$)	*P*-value†
Age (years)	65.1 ± 11.3	66.0 ± 9.9	0.745
Sex (male/female)	13/8	10/9	0.553
Etiology (HBV/HBC/other)	3/16/2	1/18/0	0.220
T.Bil (mg/dL)	1.3 ± 0.4	1.2 ± 0.7	0.233
Albumin (g/dL)	3.3 ± 0.2	3.3 ± 0.2	0.525
PT (%)	66.4 ± 12.6	69.1 ± 11.6	0.551
Fischer ratio	1.67 ± 0.48	1.45 ± 0.50	0.093
Ammonia (μg/dL)	146.3 ± 23.0	60.1 ± 36.5	0.194
Zn (μg/dL)	60.2 ± 9.0	58.4 ± 9.2	0.616

†Analysis of continuous variables performed using Mann–Whitney *U*-test; analysis of non-continuous variables performed using chi-squared test. Values are expressed as *n* or as mean ± SD.
HBV, hepatitis B virus carrier; HBC, hepatitis C virus carrier; PT, prothrombin time; T.Bil, total bilirubin.

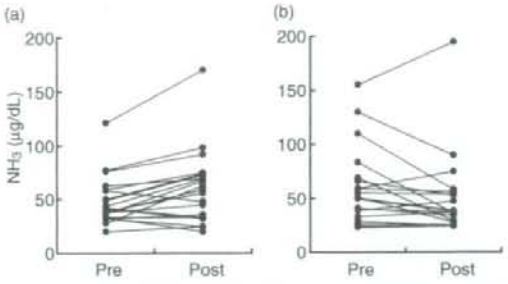

Figure 1 Changes and change ratio in blood ammonia levels after treatment with (a) branched-chain amino acid (BCAA) or (b) BCAA + zinc. Blood ammonia levels increased significantly in the BCAA group ($P = 0.016$), whereas the levels showed a tendency to decrease in the BCAA + zinc group ($P = 0.0707$). There was a significant difference in the post/pre treatment change ratio between the groups (BCAA 1.22 ± 0.38 vs. BCAA + zinc 0.87 ± 0.26, $P = 0.0033$).

significantly in the combined group ($P = 0.0007$). The change ratio also increased more significantly in the combined group (1.08 ± 0.16 vs. 1.22 ± 0.29, $P = 0.0165$).

In contrast, the blood albumin levels (Fig. 3) showed no significant difference between pre- and post-treatment in both groups, and the change ratio also showed no significant difference between the groups (1.03 ± 0.08 vs. 1.01 ± 0.07, $P = 0.4646$).

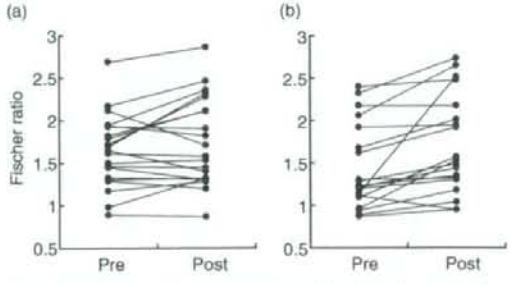

Figure 2 Changes and change ratio in blood Fischer ratio after treatment with (a) branched-chain amino acid (BCAA) or (b) BCAA + zinc. The blood Fischer ratio did not show significant difference in the BCAA group ($P = 0.1984$), but increased significantly in the BCAA + zinc group ($P = 0.0007$). There was a significant difference in the post/pre treatment change ratio between the groups (BCAA 1.08 ± 0.16 vs. BCAA + zinc 1.22 ± 0.29, $P = 0.0165$).

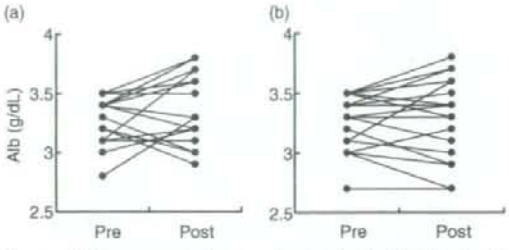

Figure 3 Changes and change ratio in blood albumin levels after treatment with (a) branched-chain amino acid (BCAA) or (b) BCAA + zinc. No significant change was observed before and after treatment both in the BCAA ($P = 0.2227$) and the BCAA + zinc groups ($P = 0.6701$). There was no significant difference in the post/pre treatment change ratio between the groups (BCAA 1.03 ± 0.08 vs. BCAA + zinc 1.01 ± 0.07, $P = 0.4646$).

There was a significant change in the blood zinc levels (Fig. 4) in the BCAA group ($P = 0.0352$), and the levels increased significantly ($P = 0.0005$) in the combined group. The change ratio was also significantly higher in the combined group (1.08 ± 0.15 vs. 1.33 ± 0.27, $P = 0.0018$).

We examined the relationships among ammonia, Fischer ratio, and zinc concentration. Before and after the treatment, there were significant correlations between the Fischer ratio and ammonia (before treatment, $r = -0.646$, $P = 0.0061$; after treatment, $r = -0.529$, $P = 0.0248$). However, there was no significant correlation between zinc and ammonia (before treatment, $r = 0.001$, $P = 0.9970$; after treatment,

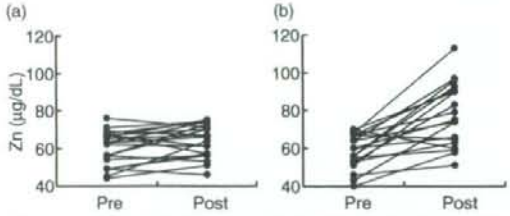

Figure 4 Changes and change ratio in blood zinc levels after treatment with (a) branched-chain amino acid (BCAA) or (b) BCAA + zinc. The blood zinc levels increased significantly in the BCAA group ($P = 0.0352$) and in the BCAA + zinc group ($P = 0.0005$). There was a significant difference in the post/pre treatment change ratio between the groups (BCAA 1.08 ± 0.15 vs. BCAA + zinc 1.33 ± 0.27, $P = 0.0018$).

$r = -0.124$, $P = 0.5985$), and was no significant correlation between zinc and the Fischer ratio (before treatment, $r = 0.121$, $P = 0.6062$; after treatment, $r = 0.346$, $P = 0.1421$). In this study, the albumin concentration before the treatment did not correlate with the change ratio of the Fischer ratio, albumin or ammonia in either group (data not shown).

DISCUSSION

SUPPLEMENTATION OF BCAA has been indicated to improve prognosis and reduce the incidence of complications.[6–8] This study demonstrated that combination treatment with BCAA and zinc supplements in cirrhotic liver patients with hypoalbuminemia or hypozincemia showed significantly higher efficacy in correcting amino acid imbalance and significantly greater ability to metabolize ammonia than when BCAA was given alone during the 6 months of the study period. Because zinc deficiency is a common disorder in patients with liver cirrhosis,[10–14] the addition of zinc supplementation to conventional therapy can be important for treatment of liver cirrhosis.

The amino acid imbalance (i.e. decreased BCAA levels) in liver cirrhosis may be partly caused by the fact that a decreased ability to process ammonia in the liver increases the proportion of unprocessed ammonia, and BCAA is consumed when the unprocessed ammonia is processed via glutamine synthesis in the skeletal muscle.[9] Supplementation of BCAA alone in this disease state does not eliminate the cause, namely decreased ability to process ammonia in the liver, although it contributes to the correction of amino acid imbalance. Therefore, its efficacy is limited in terms of ammonia metabolism. In fact, Horst *et al.*[6] reported that when proteins mainly composed of BCAA are administered to patients with liver cirrhosis, the blood ammonia levels increase to the same degree as when normal protein is administered.

The correction of zinc deficiency in liver cirrhosis leads to further improvement of the ability of the liver to metabolize nitrogen (mainly ammonia).[11–14] In addition, in the present study, the blood ammonia levels significantly increased in the BCAA group but showed a tendency to decrease in the combined group, with a significant difference in the change ratio between the groups. This is inferred to be due to an increased ability to metabolize ammonia in the liver with zinc administration as compared to the nitrogen load from BCAA supplementation. The accelerated processing of ammonia via glutamine synthetase in the skeletal muscle cannot, of course, be excluded.[15] Also in the study of Marchesini *et al.*,[14] zinc supplementation greatly improved the nitrogen metabolism in the liver, and the ammonia levels decreased more due to an increase in metabolic function than to metabolism in the skeletal muscle. Further investigation is necessary to reach a definite conclusion.

In this study, the Fischer ratio improved more significantly in the combined group. In view of the fact that in liver cirrhosis, BCAA is consumed mainly during ammonia metabolism in the skeletal muscle,[9,12] the improvement of ammonia detoxification in the liver by zinc administration in the combined group may have consequently decreased the amount of ammonia that had to be processed in the skeletal muscle, resulting in a decrease of BCAA consumption. In this case, the administered BCAA that was not consumed in the skeletal muscle was likely to have contributed to an improvement of the blood amino acid balance. The mechanism remains to be elucidated in future work.

Despite the alleviation of the amino acid imbalance in the combined group, the blood albumin levels were similar between the two groups in this study. BCAA, particularly leucine, has been shown to act as a signal for stimulating protein synthesis via m-TOR,[16] and improvement of the Fischer ratio probably influences protein synthesis, or albumin synthesis. The relatively short period of the present investigation may have prevented recognition of a difference in the ability to synthesize albumin. To more accurately examine the clinical efficacy with respect to prognosis and the incidence of other complications, a longer-term study is needed.

In this study, before and after the treatment, there were significant correlations between the Fischer ratio and ammonia. However, there was no significant correlation between zinc and ammonia, and between zinc and the Fischer ratio. In our previous study,[17] we found a significant correlation between zinc and ammonia, and between zinc and the Fischer ratio. These differences may have arisen from a difference in the range of liver diseases examined. In the present study, we enrolled patients with liver cirrhosis only, while in our previous study, subjects consisted of those with chronic hepatitis and liver cirrhosis.

In conclusion, more improvement in disorders of nitrogen metabolism in patients with liver cirrhosis was observed when BCAA was administered together with zinc than when given alone. Further investigation is necessary to determine the detailed mechanisms of the action and longer-term clinical efficacy.

REFERENCES

1 Christensen E, Schlichting P, Fauerholdt L *et al.* Prognostic value of Child-Turcotte criteria in medically treated cirrhosis. *Hepatology* 1984; **4**: 430–5.
2 Alberino F, Gatta A, Amodio P *et al.* Nutrition and survival in patients with liver cirrhosis. *Nutrition* 2001; **17**: 445–50.
3 Tajika M, Kato M, Mohri H *et al.* Prognostic value of energy metabolism in patients with liver cirrhosis. *Nutrition* 2002; **18**: 229–34.
4 Moriwaki H, Miwa Y, Tajika M *et al.* Branched-chain amino acids as a protein- and energy-source in liver cirrhosis. *Biochem Biophys Res Comm* 2004; **313**: 405–9.
5 Fischer JE, Baldessarini RI. False neurotransmitter and hepatic failure. *Lancet* 1971; **2**: 75–80.
6 Horst D, Grace ND, Conn HO *et al.* Comparison of dietary protein with an oral branched chain-enriched amino acid treatment in chronic hepatic encephalopathy: a randomized controlled trial. *Hepatology* 1984; **4**: 279–87.
7 Marchesini G, Bianchi G, Merli M *et al.* Nutritional supplementation with branched-chain amino acids in advanced cirrhosis: a double blind, randomized trial. *Gastroenterology* 2003; **124**: 1792–801.
8 Muto Y, Sato S, Watanabe A *et al.* Effects of oral branched-chain amino acid granules on event-free survival in patients with liver cirrhosis. *Clin Gastroenterol Hepatol* 2005; **3**: 705–13.
9 Hayashi M, Ohnishi H, Kawade Y, Muto Y, Takahashi Y. Augmented utilization of branched-chain amino acids by skeletal muscle in decompensated liver cirrhosis in special relation to ammonia detoxication. *Gastroenterol Jpn* 1981; **16**: 64–70.
10 Bode JC, Hanisch P, Henning H, Koenig W, Richter FW, Bode C. Hepatic zinc content in patients with various stages of alcoholic liver disease and in patients with chronic active and chronic persistent hepatitis. *Hepatology* 1988; **8**: 1605–9.
11 Reding P, Duchateau J, Bataille C. Oral zinc supplementation improves hepatic encephalopathy. Results of a randomized controlled trial. *Lancet* 1984; **ii**: 493–5.
12 Katayama K. Ammonia and hepatic encephalopathy. *Hepatol Res* 2004; **30S**: S71–8.
13 Riggio O, Merli M, Capocaccia L *et al.* Zinc supplementation reduces blood ammonia and increases liver ornithine transcarbamylase activity in experimental cirrhosis. *Hepatology* 1992; **16**: 785–9.
14 Marchesini G, Fabbri A, Bianchi G, Brizi M, Zoli M. Zinc supplementation and amino acid-nitrogen metabolism in patients with advanced cirrhosis. *Hepatology* 1996; **23**: 1084–92.
15 Lockwood AH, McDonald JM, Reiman RE *et al.* The dynamics of ammonia metabolism in man. Effects of liver disease and hyperammoninemia. *J Clin Invest* 1979; **63**: 449–60.
16 Hara K, Yonezawa K, Weng QR *et al.* Amino acid sufficiency and mTOR regulate p70S6 kinase and eIF-4E BP1 through a common effector mechanism. *J Biol Chem* 1998; **273**: 14484–94.
17 Katayama K, Ooka Y, Kikkawa S *et al.* The relationship between amino acid-nitrogen metabolism and plasma zinc in patients with chronic liver disease. *Kanzou* 2001; **42**: 120–5.

Hepatology Research 2008; **38**: 1087–1097 doi: 10.1111/j.1872-034X.2008.00391.x

Original Article

Effects of a late evening snack combined with α-glucosidase inhibitor on liver cirrhosis

Keiko Korenaga, Masaaki Korenaga, Koichi Uchida, Takahiro Yamasaki and Isao Sakaida

Department of Gastroenterology and Hepatology, Graduate School of Medicine, Yamaguchi University, Ube, Japan

Aim: A late evening snack (LES) is recommended for protein-energy malnutrition in patients with liver cirrhosis. However, many cases of liver cirrhosis have accompanying impaired glucose tolerance and there are concerns that LESs might aggravate glucose intolerance. In this study, we concomitantly used an α-glucosidase inhibitor with a LES and examined the effects on glucose tolerance. In addition, we examined whether or not there was an improvement in energy metabolism by slowing glucose absorption with the concomitant use of the α-glucosidase inhibitor.

Methods: The subjects were 11 patients with liver cirrhosis. From before the study, all the patients had been taking a LES supplementation with a branched-chain amino acid (BCAA)-enriched nutrient mixture. The patients were started on the concomitant use of α-glucosidase inhibitor (0.2 mg) taken just prior to the LES. The change of glucose tolerance and energy metabolism were examined using a 75-g oral glucose tolerance test and indirect calorimetry.

Results: One week and three months after the start of the concomitant use of the α-glucosidase inhibitor, the area under the concentration curve for plasma glucose was significantly decreased. Three months after the concomitant use, the non-protein respiratory quotient was significantly improved. There were no serious side effects during the follow-ups.

Conclusion: The concomitant use of the α-glucosidase inhibitor use with LES showed the possibility of improving glucose tolerance and energy metabolism. In patients with impaired glucose tolerance, the concomitant use of an α-glucosidase inhibitor with LES might be a useful measure for nutritional management.

Key words: α-glucosidase inhibitor, branched-chain amino acid, late evening snack, liver cirrhosis, nutritional therapy

INTRODUCTION

THE LIVER PLAYS a central role in the synthesis, metabolism and storage of nutrients. Liver cirrhosis is a condition in which there are impairments in these functions and leads to a variety of nutritional and metabolic disorders.

There is a high incidence of protein-energy malnutrition (PEM) in patients with liver cirrhosis.[1,2] When energy metabolism of liver cirrhosis patients is measured using an indirect calorimetry, their resting energy expenditure (REE) is increased, indicating a hypermetabolic state.[2] Meanwhile, the following also occur: a decrease in glycogen storage due to liver atrophy, insulin resistance (hyperinsulinemia), hyperglucagonemia and increases in serum concentrations of insulin antagonistic hormones such as catecholamines and cortisol. As a result, there is a marked decrease in use efficiency of carbohydrate as a physiological energy substrate. Body protein catabolism can accelerate and a liver cirrhosis patient can become hypercatabolic.[2] As shown by the decrease in the respiratory quotient (RQ) calculated by an indirect calorimetry, the efficiency of energy metabolism is markedly decreased in whole body.[2] In addition, the substrate oxidation rate of endogenous fat is increased more than that of carbohydrate.[2] The metabolic patterns of such patients after an overnight fast are similar to healthy individuals in a state of 2–3 days starvation.[3] This tendency becomes more marked as the severity of liver cirrhosis increases.[2] PEM is a factor that is significant in establishing the vital prognosis of liver cirrhosis

Correspondence: Dr Isao Sakaida, Departments of Gastroenterology and Hepatology, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan. Email: sakaida@yamaguchi-u.ac.jp

Received 27 February 2008; revision 21 April 2008; accepted 21 April 2008.

patients,[1,2] and thus, appropriate nutritional intervention is necessary.

A late evening snack (LES) is a superior nutritional therapy which improves the catabolic state during starvation in early morning fasting.[4–10] A late evening snack is recommended in the present guidelines of the American Society for Parenteral and Enteral Nutrition[11] and the European Society for Clinical Nutrition and Metabolism.[12]

Approximately 70% of the liver cirrhosis cases have concurrent glucose intolerance, and 40% have concurrent diabetes.[13] We previously reported the effect of the long-term use of LES (3 months) on glucose tolerance.[14] When patients had 2-hr glucose levels of < 200 mg/dL in a 75-g oral glucose tolerance test (OGTT) before they were started on LES, a nutritional improvement was observed without a significant effect of LES on glucose tolerance. In patients who had 2-hr OGTT glucose levels of ≥ 200 mg/dL, their diabetic pathology became aggravated. There has been only a small number of reports on efficiency of long-term LES, but a new approach might be necessary, including the control of glucose tolerance for continuous long-term efficiency of LES.[15]

An α-glucosidase inhibitor suppresses the final stage of glucose absorption, the hydrolysis of disaccharides to monosaccharides. Thus, it slows glucose absorption into the blood and ameliorates postprandial hyperglycemia.[16] The characteristic feature of diabetes which accompanies liver cirrhosis is postprandial hyperglycemia, and α-glucosidase inhibitor treatment is a sound pharmacotherapy for hepatic diabetes. The objective of our present study was to examine whether or not a combination of LES and α-glucosidase inhibitor could be a new adjuvant therapy. The examination was conducted by investigation of the effect of this nutritional therapy on glucose tolerance.

Liver cirrhosis patients in a state of starvation at fasting were reported to have accelerated postprandial glucose oxidation in the peripheral tissues.[17] Therefore, liver cirrhosis patients can properly oxidize the glucose load accompanying each meal intake, if the glucose load per meal is decreased by fractionating meals (frequent meals and LES). It is speculated that this process will improve not only postprandial hyperglycemia but also energy metabolism. Catabolism was reported to have improved by frequent meals in liver cirrhosis patients.[18] Therefore, our second objective was to examine whether or not energy metabolism efficiency would be improved by a combination of LES and α-glucosidase inhibitor.

MATERIALS AND METHODS

Patients

OUR STUDY WAS conducted on 11 subjects with liver cirrhosis. All patients were receiving LES therapy involving the intake of 1 pack/day of a branched-chain amino acid (BCAA)-enriched nutrient mixture (Aminoleban EN; Otsuka, Japan). The period from the start of LES therapy to the concomitant use of voglibose, a α-glucosidase inhibitor, ranged from 4 weeks to 156 weeks.

Table 1 shows the subject profiles. There were 4 males and 7 females and their ages ranged from 44 to 78 years. The causes or types of liver cirrhosis were hepatitis C virus (HCV) in 7 patients, alcoholic cirrhosis in 1 patient, primary biliary cirrhosis (PBC) in 1 patient, and non-alcoholic steatohepatitis (NASH) in 2 patients. The severity of liver damage was grade A in 3 patients, grade B in 7 patients, and grade C in 1 patient according to the Child-Pugh classification. One patient (case 6) also had hepatocellular carcinoma (HCC). This patient had two lesions which were both < 3 cm. Two other patients had a history of HCC treatment, but they were confirmed to be relapse free in the 2-year period after therapy. From the results of the 75-g OGTT performed immediately before the start of concomitant voglibose use, 9 of 11 patients were determined to have diabetes mellitus (DM) and 2 patients were determined to have impaired glucose tolerance (IGT) according to the World Health Organization criteria.[19] Among the 9 patients who showed a diabetic pattern in the 75-g OGTT, 5 patients had fasting glucose levels of < 110 mg/dL and 6 patients had HbA1c levels of < 5.8%, indicating levels within normal limits. Only 1 patient (case 6) of 9 patients with a diabetic type OGTT result was receiving insulin administration as a treatment for diabetes. However, the dosage of insulin was not changed during this study. Other patients were not receiving any drugs for diabetes until the start of concomitant voglibose use.

Study protocol

Figure 1 shows the protocol used in our study. The subjects received nutritional guidance from dieticians prior to the start of the study. The daily nutritional intake for each subject was calculated to be 25–30 kcal with 1.2–1.3 g of protein per kilogram of ideal body weight per day.[8] The patients were instructed that the actual daily nutritional intake from meals was found by subtracting the calories of LES (210 kcal) and protein (13.5 g) from the aforementioned calculated nutritional intake. One pack of the BCAA-enriched mixture used as LES food has

Table 1 Characteristics of patients before voglibose administration

Case #	Age	Sex	Etiology of cirrhosis	Body mass index (kg/m^2)	Child–Pugh classification (score)	Period from the start of LES to concomitant use of volibose (weeks)	75-g OGTT	FPG (mg/dL)	HbA1c (%)
1	73	Female	HCV	23.3	B (7)	30	DM	129	5.6
2	44	Male	alcohol	22.1	A (6)	24	IGT	94	5.3
3	72	Female	HCV	23.0	A (6)	5	DM	96	5.2
4	64	Female	HCV	26.8	B (8)	135	DM	152	8.6
5	72	Male	NASH	28.7	A (6)	5	DM	129	6.4
6	64	Male	HCV	23.4	C (10)	44	DM	143	5.7
7	64	Male	HCV	30.5	B (9)	12	DM	114	5.1
8	69	Female	HCV	26.1	B (8)	4	DM	109	7.4
9	68	Female	PBC	19.0	B (8)	4	IGT	87	4.8
10	78	Female	HCV	24.0	B (7)	156	DM	109	4.9
11	62	Female	NASH	33.0	B (7)	84	DM	106	4.9

DM, diabetes mellitus; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HCV, hepatitis C virus; IGT, impaired glucose tolerance; LES, late evening snack; NASH, non alcoholic steatohepatitis; OGTT, oral glucose tolerance test; PBC, primary biliary cirrhosis.

210 kcal of energy, 31.05 g of carbohydrate, 13.5 g of protein, 3.5 g of fat, and trace amounts of minerals and vitamins.[20] During our study, the patients received nutritional guidance once a month from nutritional consultants, and considerations were given to prevent excessive intake of calories.

We examined the effectiveness of a combination of LES and α-glucosidase inhibitor. One 0.2-mg tablet of α-glucosidase inhibitor voglibose (Takeda, Japan) was orally administered immediately before LES supplementation. This voglibose dose was less than that used for non-insulin-dependent diabetes mellitus (NIDDM) (3 × 0.2 mg tabs/day, 0.6 mg). A 75-g oral glucose tolerance test (OGTT) was performed before the start of a combination treatment with voglibose, 1 week after the start, and 3 months after the start. A standard 75-g OGTT (TrelanG; Shimizu, Japan) was performed after LES intake the night before but after at least 10 hours of resting fasting state. The plasma glucose levels and insulin levels were measured before glucose load and 30, 60, 90, 120 min after glucose load. The area under the concentration curve for glucose (AUC glucose) and the area under the concentration curve for insulin (AUC insulin) were calculated, and these values were compared before and after the concomitant voglibose use.

Energy metabolism was analyzed with indirect calorimetry (Deltatrac II; Detex Ohmeda, Finland). Indirect calorimetry was performed on the same day and before 75-g OGTT. Indirect calorimetry was performed for 30 min on patients who were in a state of overnight bed rest and fasting after LES intake. We also measured oxygen consumption per minute (VO_2) and carbon dioxide production per minute (VCO_2) during early-morning fasting. The total urine nitrogen (TUN) levels were measured previously. These values were used to calculate the non-protein respiratory quotient (npRQ): (i) oxidation ratio of nutrients; (ii) substrate oxidation of carbohydrate (% CHO); (iii) fat (% FAT); (iv) protein (% PRO); and (v) resting energy expenditure (REE).

A physician provided a medical examination once a month to each patient. In the examination, the physician assessed physical and physiological findings and subjective symptoms and confirmed patient compliance. A multi frequency-bioelectrical impedance analysis method (InBody 3.2; Biospace, Japan) was used for the anthropometric measurements. The creatinine height index (CHI) was calculated by the following formula: CHI = (urinary creatinine excretion per day (mg))/(ideal body weight × A), where A is 23 for males

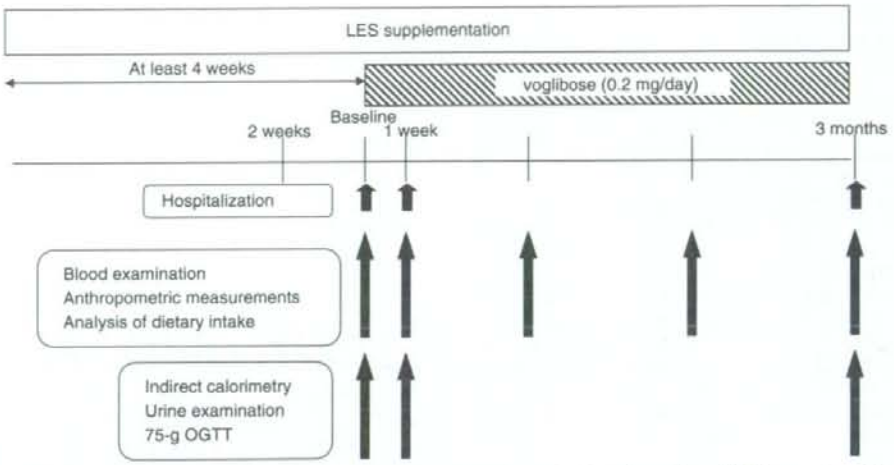

Figure 1 Study protocol to determine effects of a late evening snack combined with α-glucosidase inhibitor on liver cirrhosis. All patients, who had been taking late evening snack (LES) supplementation of a branched-chain amino acid-enriched nutrient mixture, were started on a concomitant dose of α-glucosidase inhibitor (voglibose, 1 tab, 0.2 mg) at baseline. OGTT, oral glucose tolerance test.

and 18 for females. Blood biochemical tests were performed following standard methods. The content of the study was approved by the Clinical Trial Review Committee, School of Medicine, Yamaguchi University, Japan, prior to the start of the study. The subjects participated in this study after being thoroughly informed on the content of the study and voluntarily providing consent.

Statistical analysis

Data were expressed as the mean ± SD. Comparisons between data were evaluated by two-tailed paired Student's *t*-test. The Pearson's coefficient of correlation was used. *P*-values of less than 0.05 were considered significant.

RESULTS

Anthropometry

ANALYSIS OF THE results of the 11 subjects indicated no significant changes in weight from the baseline immediately before voglibose was added to LES therapy and 3 months after this combination therapy started (61.9 ± 13.6 kg vs 62.1 ± 13.1 kg). There were also no significant differences before and after the start of the combination therapy in skeletal muscle mass, body fat mass, and percent body fat (data not shown).

Glucose tolerance

The AUC glucose values were significantly decreased in 75-g OGTT 1 week and 3 months after concomitant voglibose use compared with the values before its use

Table 2 Effects of α-glucosidase inhibitor administration combined with a late evening snack on the area under the concentration curve for 2-hour glucose (AUC glucose) and insulin (AUC insulin) during the 75-g oral glucose tolerance test in cirrhosis

	Baseline	1 week	3 months
AUC glucose (mg/dL × h)	622.70 ± 166.65	536.86 ± 140.42*	557.32 ± 141.53*
AUC insulin (mg/dL × h)	206.30 ± 105.72	213.20 ± 69.56	188.73 ± 83.14

Data expressed as mean ± SD. *$P < 0.05$ compared with baseline.