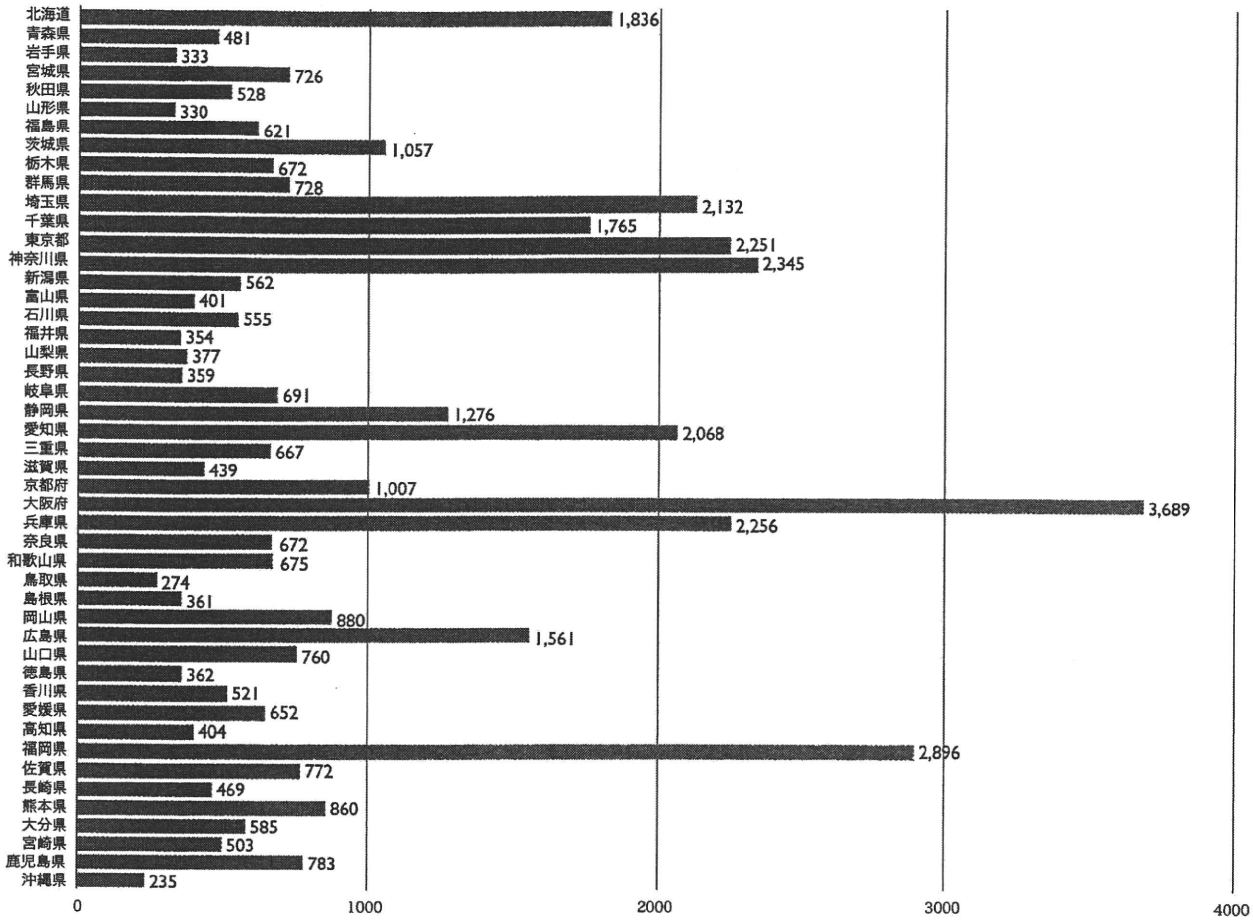

図1　平成20年度肝炎IFN治療受給者証交付申請件数 / 合計44,731名

なお悪性新生物の死亡率は増え続けている．なかでも肝癌は，他の悪性新生物と異なりハイリスクグループを絞り込むことができる唯一の癌である．しかし，肝炎ウイルス感染者の多くは自覚症状がない．そこで，感染者を発見するために，厚生労働省は平成14年度から肝炎等緊急総合対策の一環として，地域住民を対象とした肝炎ウイルス検診(節目・節目外検診)を開始した．本事業は5年間実施されたが，肝炎ウイルス感染者が発見されても，そのすべてが治療に結びつくわけではないという問題点が浮き彫りになった．また2008年4月よりIFN治療に関する医療費助成をはじめとした総合対策が推し進められているものの，IFN治療の申請者数は伸び悩んでいるのが現状である(図1)．

4　IFN治療の普及をめざして～患者と医師双方のアンケート調査から得られたもの

C型慢性肝炎に対するIFN治療は，本邦では1992年に認可された．その後，IFN自体の改善や治療法の進歩によって，C型肝炎ウイルス(HCV)の駆除率も飛躍的に向上した．現在，IFN治療はウイルスの駆除に留まらず，肝線維化の改善，肝癌の発生阻止，肝外病変への治療効果，さらに生命予後の改善が明らかにされるなどの効果をあげている[2,3]．わが国の肝癌の約8割がHCVに起因しているため，肝癌の撲滅にはC型肝炎に対する治療戦略が重要な意義を持つ．IFN治療は，さま

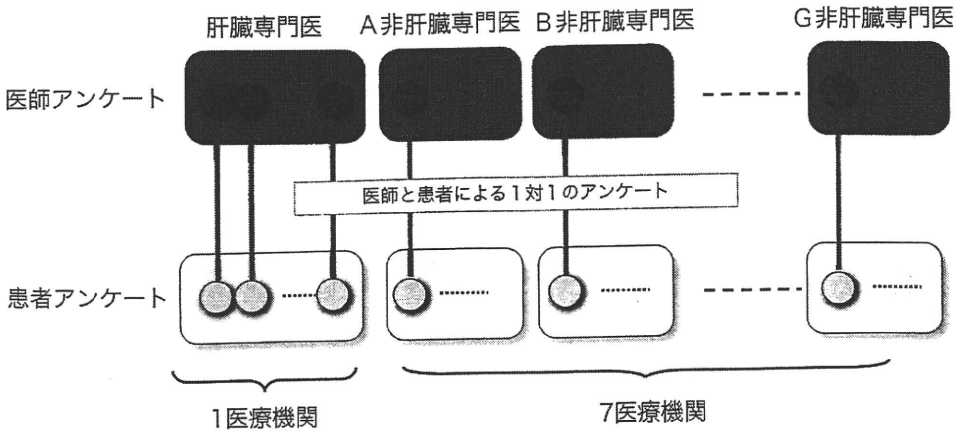

図2　方　法

アンケート実施期間2005.10.1～2006.2.28
(1)長尾，佐田他，リサーチペーパー・シリーズ32, 2006,
2) Nagao Y, Sata M et al : Med Sci Monit 14, 2008より)

ざまな副作用はあるものの治療効果の向上はめざましく，C型肝炎の第一選択薬として高く評価されている．ただし，肝炎ウイルス感染者に対するIFN治療の導入とその効果を上げるためには，医療連携システムの構築が重要であり，患者の視点に立った医療が求められる．

なぜ，日本ではHCV感染者に対して想定よりもIFN治療の普及率が低いのだろうか？

そこで私どもは，2005年10月より全国に先駆けて，なぜIFN治療が普及しないのかについて，その問題点の解析と解決策の確立を求めて検討した．九州X町におけるHCV感染患者およびその担当医師の双方へアンケートによる調査を実施することにより，IFN治療の普及に向けた医療の在り方を報告した[4]．その結果，IFN治療の受療率は通院先の医療機関により大きな違いを認め，肝臓専門医でのIFN治療の実施率は対象者の78.2％であったのに対し，非肝臓専門医でのIFN治療の実施率は15.7％であった．

さらに，私どもはIFN治療を受けるべき患者がIFN治療を受けない要因を分析し，IFN治療の導入を妨げる要因を明らかにするため

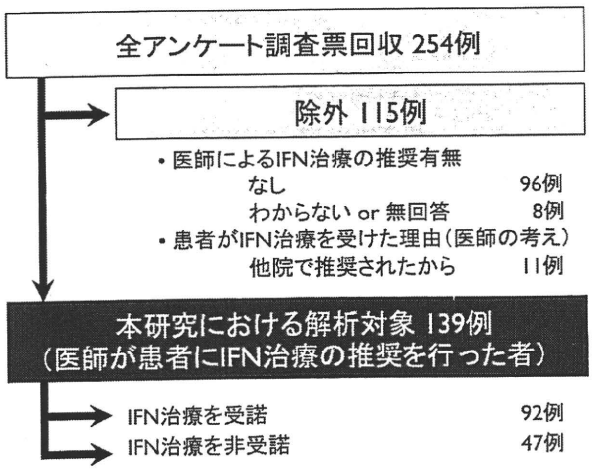

図3　本研究の検討対象群

(Nagao Y, Sata M et al : Med Sci Monit 14, 2008)

の解析を行った[5]．調査を行った地域は，私どもが1990年より肝疾患ならびに肝外病変の経時的な疫学調査を行ってきた地域である[6-15]．

1．対象と方法

九州X町で開業しているすべての8医療機関(肝臓専門医ではない内科，外科，脳神経外科などの7医療機関，肝臓専門医が常勤する1医療機関)と，そこに通院しているHCV慢性肝疾患患者らに同意を取得し，担当医師と患者に1対1のアンケート調査を実施した

表1　IFN治療の諾否に影響する因子(多変量解析)

1	通院先	非専門医にかかっている患者は，専門医に比べ，治療を拒否する倍率が18.06倍高い
2	性　別	女性は，男性より治療を拒否する倍率が3.65倍高い
3	合併症	合併症を持つ患者は，持たない患者に比べ，治療を拒否する倍率が3.63倍高い

(Nagao Y, Sata M et al : Med Sci Monit 14, 2008)

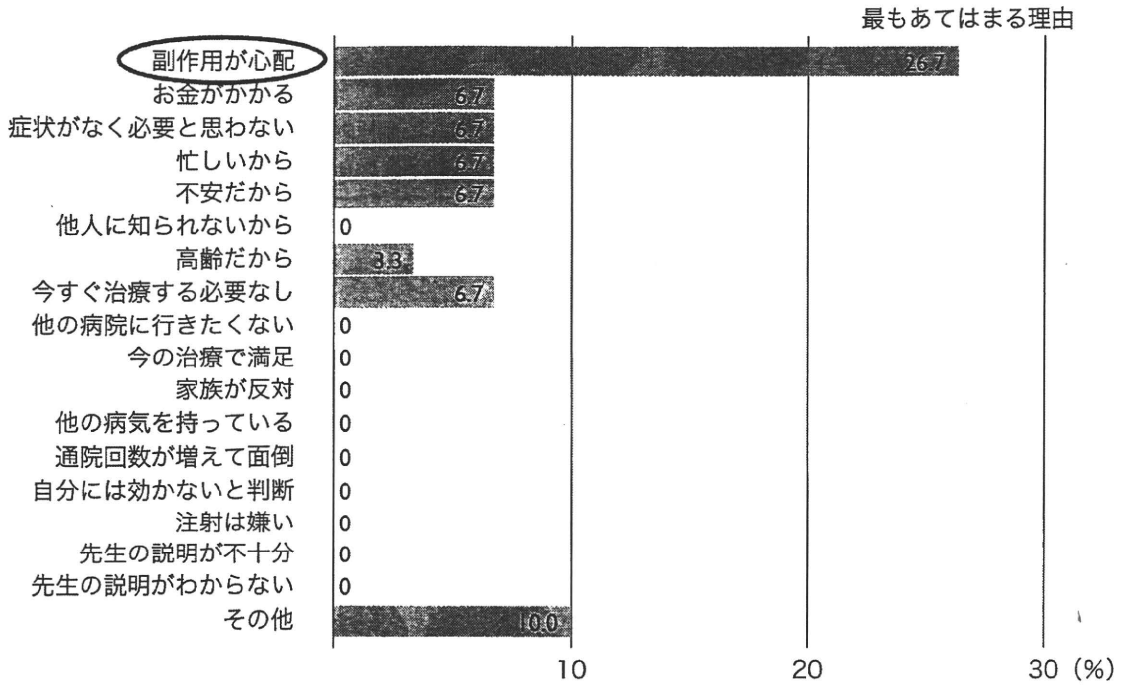

図4　患者がIFN治療を受諾しなかった理由
(Nagao Y, Sata M et al : Med Sci Monit 14, 2008)

(図2)．これにより254組の回答を回収した．医師が患者にIFN治療の推奨を行ったと回答した139例(IFN治療の適応患者とみなす)の患者群を本研究の検討対象群とし(図3)，患者のIFN治療の諾否に影響を与える要因を分析した[5]．

2. 結果と考察

医師が患者にIFN治療を推奨した139例のうち92例(66.2％)が治療を受諾した．肝臓専門医の病院では86例中74例(86.0％)が，非肝臓専門医の診療所では53例中18例(34.0％)がIFN治療を受諾した．またロジスティック回帰分析の結果，通院先，性別，および合併症が患者のIFN治療の諾否に影響を与える因子であり，調整オッズ比はおのおの18.06，3.65，3.63であった(表1)．医師がIFN治療を推奨しない要因に挙げている年齢(高齢者)や肝病態の進展度は，患者のIFN治療の諾否には影響していなかった．

患者がIFN治療を断る最大の理由は，「副作用の心配」であった(図4)．副作用を心配するのは，男性よりも女性の方が多かった(男性18.2％，女性33.3％)．医師は女性の方がIFN治療に対する不安感が強い傾向にあるこ

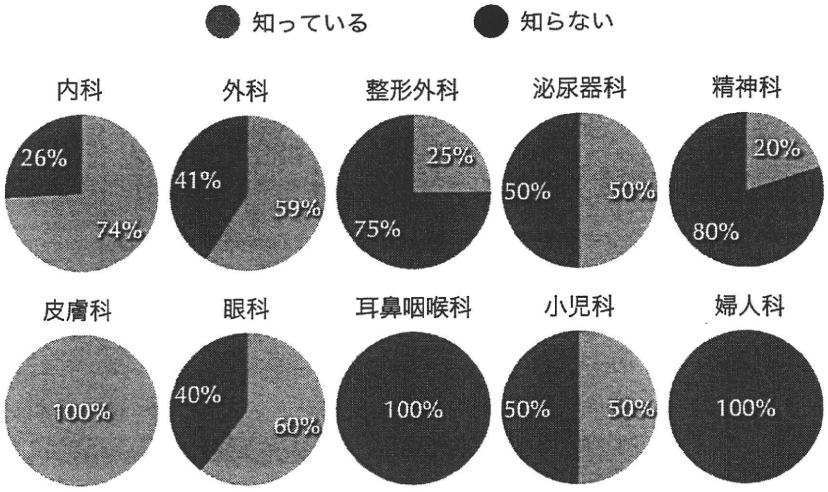

図5　某医師会で実施した診療科別の医師による肝外病変の認識
(2003.10.1～11.30実施(回答数82))

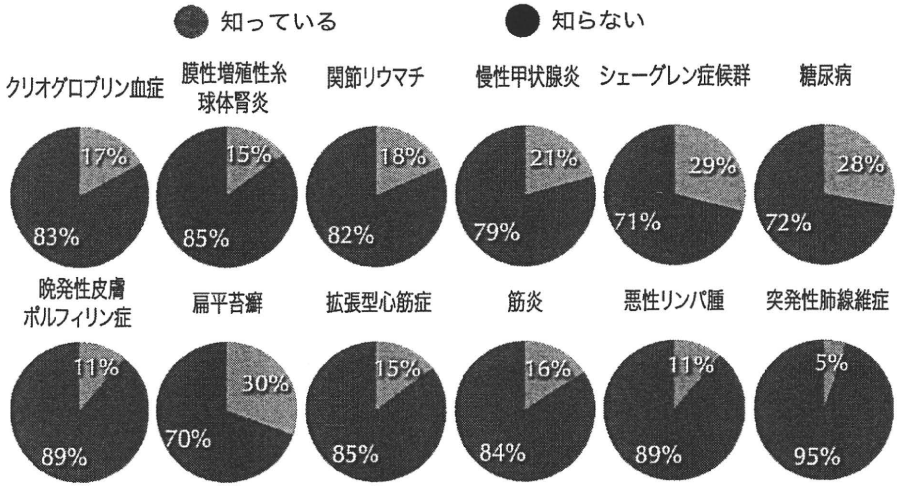

図6　某医師会で実施した各肝外病変の医師の認知度
(2003.10.1～11.30実施(回答数82))

とを念頭に置いて，治療の説明や推奨を推進する必要がある．

IFN治療をはじめとする薬物療法が適切に普及するためには，専門医と非専門医間で医療の在り方を協議し，医療連携の仕組みを整備すること，そして一方では患者と医師のコミュニケーションの向上を図るための施策を考えることが不可欠である．地域の専門医と非専門医が協議し，病院と診療所が連携できる仕組みづくりが求められている．

5　肝外病変と医療連携の重要性

HCVは，肝障害のみならず種々の臓器障害を引き起こす．いわゆる肝外病変として，クリオグロブリン血症，膜性増殖性糸球体腎炎，晩発性皮膚ポルフィリン症，シェーグレン症候群，悪性リンパ腫，筋炎，心筋障害，扁平苔癬，口腔癌，糖尿病，間質性肺炎，モーレン角膜潰瘍，関節リウマチ，慢性甲状腺炎などが報告されている．口腔領域に高頻度に出現する扁平苔癬は，慢性肝疾患患者の

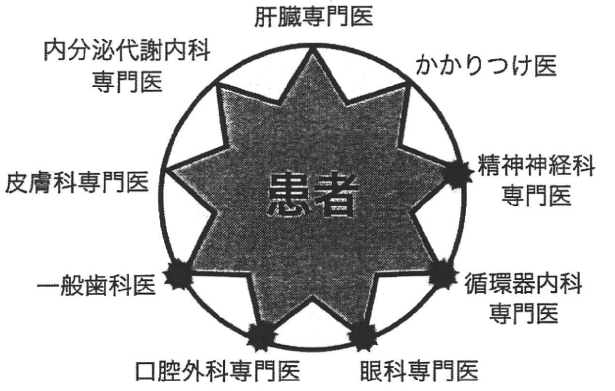

図7　IFN治療には、医療連携とチーム医療が大切
－IFN治療前に受診する診療科（✸久留米大学）－

表2　福岡県におけるIFN初期導入医療機関の指定要件
－福岡県内60施設が指定（県庁HPで公開中）－

1	施設内に肝臓専門医（社団法人日本肝臓学会認定）が１名以上常勤
2	C型慢性肝炎に対する年間おおむね10例以上のIFN初期導入実績がある
3	B型慢性肝炎に対するIFN治療あるいは経口抗ウイルス剤の投与実績がある
4	IFN治療導入時に必要となる内科，精神科，眼科，皮膚科など複数の診療科との連携システムを構築できる
5	救急対応が可能
6	過去5年間にウイルス性疾患に関する研究事業報告が1つ以上ある
7	医療機関における肝炎ウイルス無料検査の陽性者について県へ必ず報告する
8	ウイルス性肝炎の治療において，かかりつけ医との紹介・逆紹介へ努めている
9	講習会を受講すること

（福岡県肝炎対策協議会委員長　佐田通夫）

QOLの低下につながるだけでなく，IFN治療により増悪することがあるため注意が必要な疾患である．しかし，このような肝外病変についての知識は，医療従事者でさえ必ずしも認知しているわけではない．某医師会に所属する医師を対象に，HCVが引き起こす肝外病変の存在を認知しているかどうかを検討したところ，内科医であっても4人のうち１人は肝外病変の知識がない（図5）．各肝外病変の疾患についても認知度は低い（図6）．

引き起こされる病態が多岐に及ぶという特徴が肝外病変の早期発見を遅らせる．臓器別診療体制の傾向が強い医療施設では，各診療科間の十分な連携のもとで，HCV感染者の経過観察が必要となる．

久留米大学では，IFN治療を受ける患者は，循環器内科医，眼科医，精神科医，一般歯科医，さらに口腔外科医による精査を受けることがクリニカルパスになっている（図7）．事前に検査を受けることで，IFN治療中の副作用発現時にも診療科間の連携がスムーズに行うことができる長所を持ち，このことは結果的にIFN治療の完遂率を上げると推測している．肝外病変からみた医療連携も今後さらに

第1回	2005	10.15	福岡県	久留米市
第2回	2006	6.17	福岡県	久留米市
第3回		10.21	福岡県	朝倉市
第4回	2007	2.10	大分県	大分市
第5回		5.26	福岡県	久留米市
第6回		12.09	福岡県	遠賀郡
第7回	2008	5.11	福岡県	久留米市
第8回		7.26	佐賀県	鹿島市
第9回		11.16	福岡県	久留米市
第10回	2009	5.24	福岡県	久留米市
第11回		7.25	大分県	大分市
第12回		9.26	福岡県	遠賀郡
第13回		11.14	福岡県	久留米市
第14回	2010	5.22	福岡県	久留米市
第15回		7.10	大分県	大分市

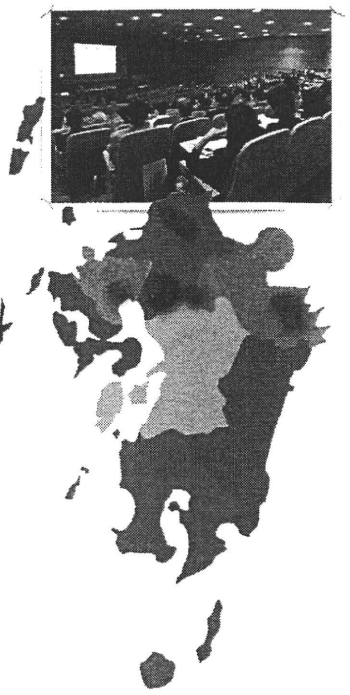

図8　患者と医療従事者への情報提供のために消化器病教室を開催

重要になるはずである．平成21年度より福岡県では，IFN初期導入医療機関には8つの指定要件があり，現在福岡県庁ホームページ上で公開されている(平成21年度は60施設)(表2)．

6 消化器病教室の活動を通してIFN治療の普及と医療連携の効率化をめざす

おのおのの専門医と専門医ではない医師の間で，情報を共有する環境作りや適切な治療を患者に行うためには，地域の特徴に沿った仕組みを考える必要がある．医療連携の環境整備によって，患者が受ける医療の質を向上させられるはずである．

医療消費者である患者が，治療の意思決定に主体的に関わりたいと望むなら，患者自身も積極的に医師とコミュケーションを図る必要がある．そのために私どもでは，2005年より患者と家族そして医療従事者を対象に，肝臓を中心とした消化器の病気について理解を深めていただくための教育として「消化器病教室」を開催している(図8)(http://www.med.kurume-u.ac.jp/med/joho/)．この教育が，地域の医療連携のシステム作りや医師と患者のコミュニケーションに関する質の向上につながればと考えている．

私どもが消化器病教室を開催する背景には，次のような根拠に基づいている．①1990年より長期的に実施してきたHCV高浸淫地区のさまざまな疫学調査を通じて，十数年前から医療連携の重要性を訴えてきたこと[6-15]，②医師と患者を対象にIFN治療の実態を把握し，薬物療法のさらなる普及に向けた医療の在り方を考察したこと(http://www.jpma.or.jp/opir/research/article32.html にてダウンロード可)[4]，③IFN治療の適応患者が医師から治療を推奨されても治療を拒絶する因子は，通院先と性別と合併症の有無に規定されること[5]．

7 おわりに

優れた治療法や医薬品が普及するためには，かかりつけ医と専門医間の医療連携が不可欠である．無駄のない効率的な医療体制を構築するためには，診療所と病院の具体的な役割分担を明確化するとともに，行政のサポートも必要である．患者の視点に立って考える医療の進展には，患者が治療を受ける際に，診療のアクセスを改善するための情報公開や相談窓口の設置なども必要であろう．

さらに，医療従事者が患者の説明に使う医療用語が患者の理解と判断の障害にならないように，わかりやすく話すことも非常に重要である．独立行政法人国立国語研究所の「病院の言葉」委員会は，2009年3月に患者にとって難しい医療用語をわかりやすく説明するための最終報告を発表した．書籍としても発刊された「『病院の言葉を分かりやすく－工夫の提案－(勁草書房)』」．患者中心の医療の実現には，医療従事者がわかりやすく説明するという基本的な姿勢を忘れてはならない．

文　献

1) 川上　裕：日米の医薬品市場における製品年齢．策研ニュース16：8–10, 2005
2) Yoshida H, Shiratori Y, Moriyama M et al : Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. Ann Intern Med 131 : 174–181, 1999
3) Yoshida H, Arakawa Y, Sata M et al : Interferon therapy prolonged life expectancy among chronic hepatitis C patients. Gastroenterology 123 : 483–491, 2002
4) 長尾由実子，佐田通夫，鈴木史雄，他：優れた薬物療法のさらなる普及をめざして－C型肝炎ウイルス感染者におけるインターフェロン療法受療の現状と考察－．リサーチペーパーシリーズ32：1–81, 2006（http://www.jpma.or.jp/opir/research/ article32.html）
5) Nagao Y, Kawakami Y, Yoshiyama T et al : Analysis of factors interfering with the acceptance of interferon therapy by HCV-infected patients. Med Sci Mont 14 : 45–51, 2008
6) Sata M, Nakano H, Suzuki H et al : Sero-epidemiologic study of hepatitis C virus infection in Fukuoka, Japan. J Gastroenterol 33 : 218–222, 1998
7) Fukuizumi K, Sata M, Suzuki H et al : Hepatitis C virus seroconversion rate in a hyperendemic area of HCV in Japan: a prospective study. Scand J Infect Dis 29 : 345–347, 1997
8) Fukuizumi K, Sata M, Suzuki H et al : Natural disappearance of serum HCV RNA; prospective study in a hyperendemic area. Hepatol Res 9 : 144–151, 1997
9) Noguchi S, Sata M, Suzuki H et al : Routes of transmission of hepatitis C virus in an endemic rural area of Japan. Molecular epidemiologic study of hepatitis C virus infection. Scand J Infect Dis 29 : 23–28, 1997
10) Nagao Y, Sata M, Fukuizumi K et al : High incidence of oral precancerous lesions in a hyperendemic area of hepatitis C virus infection. Hepatol Res 8 : 173–177, 1997
11) Nagao Y, Sata M, Fukuizumi K et al : High incidence of oral lichen planus in an HCV hyperendemic area. Gastroenterology 119 : 882–883, 2000
12) Nagao Y, Fukuizumi K, Tanaka K et al : The prognosis for life in an HCV hyperendemic area. Gastroenterology 125 : 628–629, 2003
13) Nagao Y, Tanaka K, Kobayashi K et al : A cohort study of chronic liver disease in an HCV hyperendemic area of Japan: a prospective analysis for 12 years. Int J Mol Med 13 : 257–265, 2004
14) Nagao Y, Tanaka K, Kobayashi K et al : Analysis of approach to therapy for chronic liver disease in an HCV hyperendemic area of Japan. Hepatol Res 28 : 30–35, 2004
15) Tanaka K, Nagao Y, Ide T et al : Antibody to hepatitis B core antigen is associated with the development of hepatocellular carcinoma in hepatitis C virus-infected persons: A 12-year prospective study. Int J Mol Med 17 : 827–832, 2006

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Original Article

Oxidized albumin is associated with water retention and severity of disease in patients with chronic liver diseases

Masahiro Sakata [a,*], Takumi Kawaguchi [a,b], Eitaro Taniguchi [a], Akira Nakayama [c], Sonoko Ishizaki [c], Ichiro Sonaka [c], Toru Nakamura [a], Minoru Itou [a], Tetsuharu Oriishi [a], Mitsuhiko Abe [a], Chikatoshi Yanagimoto [a], Hironori Koga [a], Michio Sata [a,b]

[a] Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan
[b] Department of Digestive Disease Information & Research, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan
[c] Pharmaceutical Research Laboratories, Ajinomoto Co. Inc., Japan

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Bioelectrical impedance analyzer

SUMMARY

Background & aims: Serum albumin exists in oxidized and reduced forms. Although oxidation of albumin affects some functions of albumin, the involvement of oxidized albumin in disease progression and water retention in patients with chronic liver disease remains unclear. The aim of this study was to determine whether there is an association between oxidized albumin and water retention in patients with chronic liver disease.

Methods: Seventy-nine patients with chronic viral liver diseases and 31 cirrhotic patients with hypoalbuminemia were enrolled. The oxidized albumin percentage was determined by high-performance liquid chromatography. Water retention was assessed by the extra cellular fluid/total body fluid ratio (ECF/TBF) using a bioelectrical impedance analyzer.

Results: The oxidized albumin percentage was significantly increased according to disease progression (chronic hepatitis; 28.3 ± 1.1, Child A; 33.5 ± 1.2, Child B and C; 37.8 ± 1.3, $P < 0.05$). Moreover, the ECF/TBF showed a significant positive correlation with the oxidized albumin percentage ($P = 0.010$, $R^2 = 0.161$), but no correlation with serum albumin levels. A low proportion of oxidized albumin was an independent predictor of reduction in body weight (OR 10.6, 95%CI 1.304–86.307, $P = 0.0272$).

Conclusions: Oxidized albumin was related to disease progression and water retention in patients with chronic liver disease.

1. Introduction

Human serum albumin (HSA) is synthesized only by hepatocytes and is the most abundant protein in plasma. HSA is widely measured in daily clinical examination in order to evaluate liver function, severity of disease or hypermetabolic states.[1,2] Roles of HSA include maintenance of colloid osmotic pressure and transport of endogenous substances (e.g. bilirubin, hormones or unesterified free fatty acids) and exogenous substances.[3,4]

HSA is divided into two fractions, reduced albumin (human mercaptalbumin) and oxidized albumin (human nonmercaptalbumin), by high-performance liquid chromatographic (HPLC) analysis.[5,6] Given the presence of a free sulfhydryl group in position 34 (Cys-34), HSA reacts with radical oxygen species as an antioxidant.[7] In healthy adults, about 75% of the Cys-34 in albumin contain a free sulfhydryl group (reduced albumin), while about 25% form a disulfide with small sulfhydryl compounds such as another cysteine, homocysteine or glutathione (oxidized albumin).[8–10] The fraction of oxidized albumin increases in some physical conditions such as aging and during intensive exercise.[11,12] Oxidized albumin also increases in various diseases such as liver diseases,[13,14] diabetes,[8,15] and coronary artery disease.[16] Moreover, oxidized albumin is negatively correlated with creatinine clearance in patients with renal dysfunction.[5,17] In cirrhosis, in particular, the percentage of oxidized albumin within total serum albumin increases with the progression of liver disease.[14] Recently, not only the quantity but also the quality of albumin has been discussed.[18,19] Oxidized albumin clearance in the body is more rapid compared with reduced albumin.[20] In addition, ligand binding and antioxidant potential are lower in oxidized albumin

Abbreviations: BIA, bioelectrical impedance analyzer; BMI, body mass index; ECF/TBF, extra cellular fluid/total body fluid; HPLC, high-performance liquid chromatography; HSA, human serum albumin; MELD, model for end-stage liver disease.

* Corresponding author. Tel.: +81 942 35 3311; fax: +81 942 34 2623.
E-mail address: sakata_masahiro@med.kurume-u.ac.jp (M. Sakata).

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than in reduced albumin.[21,22] Thus, oxidation of HSA is associated with structural and functional changes.

We focused on water retention, a function of albumin. HSA accounts for 80% of plasma colloid osmotic pressure, and it is known that 1.0 g of HSA has a water maintenance ability of 20 mL.[23] When the serum albumin level decreases to 50% or less of a reference value, it causes decreased colloid osmotic pressure, resulting in edema.[24] However, some cases of edema cannot always be explained by decreases in albumin level. In fact, there are patients who have no edema or ascites even though they are in a state of hypoalbuminemia. Although Ballmer et al. and Tessari et al. reported that the potential to synthesize albumin decreases with disease progression, leading to water retention in cirrhotic patients,[25,26] Rothschild et al. reported that albumin synthesis in cirrhotic patients with ascites is accentuated or normal.[27] In contrast, Weinbren et al. reported that one of the causes of hypoalbuminemia in cirrhotic patients originates in the abnormal distribution of albumin within the body.[28] Furthermore, edema disappears with a low protein diet (0.6/kg/weight) in patients with hypoalbuminemia, although the serum albumin level does not change.[29,30]Although oxidized albumin correlates with the function of albumin, it remains unclear whether oxidized albumin correlates with water retention in patients with chronic liver disease. The aim of this study was to determine whether there is an association between oxidized albumin and water retention.

2. Materials and methods

2.1. Patients

We examined relationships between oxidized albumin and water retention using a cross-sectional analysis and a retrospective analysis. In the cross-sectional analysis, we enrolled a total of 79 consecutive patients with chronic viral liver diseases for whom the percentage of oxidized albumin and water retention had been measured at the same time. We did liver biopsies or used aspartate aminotransferase (AST)-to-platelet ratio index (APRI)[31] to differentiate between chronic viral hepatitis with and without cirrhosis. Patients who met any of the following criteria were excluded from this study: 1) alcoholic liver disease, 2) renal failure, 3) heart failure or 4) hypothyroidism. For experiments using a multifrequency-bioelectrical impedance analyzer (BIA) (InBody®, BIOSPACE Co., Ltd, Tokyo, Japan), regardless of liver function, 24 patients with edema or ascites were excluded because BIA has a possibility to show in accurately evaluated body water in cirrhotic patients with fluid overload.[32–34] In the retrospective analysis, we enrolled 31 patients with hypoalbuminemia (hepatitis C virus-related, $n = 20$; alcoholic, $n = 6$; primary biliary cirrhosis, $n = 1$; cryptogenic, $n = 4$) for whom water retention had been evaluated before and after HSA infusion. All patients were treated with an intravenous infusion of HSA for supportive therapy against hepatoma, varices or intractable ascites. The percentage of oxidized albumin is different in each commercially available brand of HSA.[35] Therefore, we measured the oxidized albumin percentage of commercial HSA purchased from four different manufacturers and divided them into two groups by percentage of oxidized albumin. One group was given low oxidized albumin following HSA (oxidized albumin%: Company A, 49.2%, Company B, 50.7%; average oxidized albumin% = 49.95%, $n = 19$). A second group was given high oxidized albumin following HSA (oxidized albumin%: Company C, 61.5%; Company D, 62.7%, average oxidized albumin% = 62.1%, $n = 12$). We investigated whether there was a difference in improvement in water retention in these two groups. For precise evaluation, in this study, exclusion criteria were BMI < 18.5, BMI ≥ 30, renal failure, no increase in serum albumin levels after administration of HSA, addition or withdrawal of diuretic drugs. Moreover, there was no significant group difference between the number of days from HSA infusion and evaluation of water retention (Table 2).

None of the subjects were institutionalized. Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by our Institutional Review Board.

2.2. Laboratory determinations

Blood samples were taken from the peripheral vein at 6 AM after 8 h of fasting. All serum samples for measurement of albumin level were tested at Kurume University Hospital immediately using an improved bromocresol purple method. Its normal range is 4.0–5.0 g/dL.[36]

2.3. Determination of oxidized and reduced albumin

All serum samples for determination of oxidized and reduced albumin percentages were immediately frozen after they were drawn, and were stored at −80 °C until assayed by HPLC. Briefly, HPLC was performed using 25 μL aliquots of each serum sample and a Shodex Asahipak ES-502N column (Showa Denko, Tokyo, Japan; column temperature: 35 ± 0.5 °C). The HPLC system consisted of a Model SCL-10Avp system controller, a Model LC-10ATvp double-plunger pump with a Model FCV-10ALvp gradient and a Model SCL-10ADvp autosampler, a Model RF-10AXL fluorescence detector (excitation wavelength 280 nm; emission wavelength 340 nm). All instruments were purchased from SHIMADZU Co. (Tokyo, Japan). Elution was carried out with a linear gradient of increasing ethanol concentrations from 0 to 5%, in 0.05 M sodium acetate–0.40 M sodium sulfate buffer (pH 4.85) (acetate–sulfate buffer) at a flow rate of 1.0 ml/min. Serum samples were injected by means of an auto-sampler at a fixed volume of 25 μL.

2.4. Evaluation of water retention

The degree of water retention was assessed by physical examination and classified into 2 groups: with edema or with no edema. Water retention was more accurately assessed by the extra cellular fluid/total body fluid ratio (ECF/TBF), measured by BIA. The normal range of ECF/TBF is less than 0.35.

2.5. Assessment of severity of cirrhosis

The severity of cirrhosis was assessed by the model for end-stage liver disease (MELD) score[37] and Child-Pugh classification.[38]

2.6. Statistical analysis

All data are expressed as mean ± SD. Differences between two groups were analyzed using the Mann-Whitney U test. Comparisons among three groups were done by analysis of variance followed by post hoc tests (Fisher's PLSD). Spearman rank correlation coefficients were used to test the relationship between albumin levels or oxidized albumin percentage and ECF/TBF. In order to identify independent variables for reduction of body weight, logistic regression was used. P values < 0.05 were considered significant.

Table 1
Characteristics of patients.

	Reference value	Value
Number		79
HCV/HBV		65/14
Age (yr)		62.5 ± 1.4
Sex (M/F)		55/24
Body weight (kg)		60.2 ± 1.4
Height (cm)		162.4 ± 1.1
BMI	22–25	22.7 ± 0.4
Hemoglobin (g/dL)	14.0–18.0	12.6 ± 0.3
Platelet ($\times 10^4/\mu L$)	13.0–36.0	12.1 ± 0.6
AST (IU/L)	13–33	61.6 ± 3.8
ALT (IU/L)	6–30	55.5 ± 5.4
GGT (U/L)	10–47	78.4 ± 11.0
Total cholesterol (mg/dL)	128–219	151.7 ± 4.0
Total protein (g/dL)	6.70–8.30	7.35 ± 0.06
Albumin (g/dL)	4.00–5.00	3.40 ± 0.09
Total bilirubin (mg/dL)	0.30–1.20	1.35 ± 0.10
Prothrombin time (%)	70–130	82.0 ± 1.8
BUN (mg/dL)	8.0–22.0	15.9 ± 0.6
Creatinine (mg/dL)	0.60–1.10	0.76 ± 0.03
CH/Child-Pugh A/B/C		25/21/23/10

Note: Values are given as number or mean ± SD. Abbreviations: HCV, hepatitis C virus; HBV, hepatitis B virus; BMI, body mass index; AST, aspartate aminotransaminase; ALT, alanine aminotransaminase; GGT, gamma-glutamyl transpeptidase; BUN, blood urea nitrogen; CH, chronic hepatitis.

3. Results

3.1. Characteristics of patients in the cross-sectional analysis

Clinical and laboratory data for these subjects are summarized in Table 1. Laboratory data showed that platelet counts and serum albumin levels were lower, and that serum aspartate aminotransferase and alanine aminotransferase levels were higher than normal values. Renal function, cardiac function and thyroid function were within normal limits (data not shown).

3.2. The association between disease progression and oxidized albumin percentage

The oxidized albumin percentage was significantly increased by disease progression (Fig. 1(a), CH vs. Child A, $P = 0.006$; Child A vs. Child B and C, $P = 0.014$; CH vs. Child B and C, $P < 0.001$). Furthermore, the oxidized albumin percentage was significantly correlated with the MELD score (Fig. 1(b), $R^2 = 0.336$, $P < 0.001$).

3.3. Serum albumin levels and oxidized albumin percentages in patients with edema

Serum albumin levels were significantly higher in patients with no edema than in patients with edema (Fig. 2(a), $P = 0.0021$). Similarly, the oxidized albumin percentage was significantly higher in patients with edema than in patients with no edema (Fig. 2(b), $P < 0.001$).

3.4. Correlation of (a) ECF/TBF and (b) serum albumin levels or oxidized albumin percentage

Serum albumin levels were negatively correlated and oxidized albumin percentages showed positively correlated with ECF/TBF in patients with hypoalbuminemia (Fig. 3(a) and (b)). However, in patients with albumin levels < 3.66 g/dL (median value of serum albumin levels), we found that 63.0% (17/27) did not show water retention. Since, in patients with albumin levels ≥ 3.66 g/dL, 3.7% (1/28) of patients showed water retention, these findings show heterogeneity in distribution of serum albumin plots.

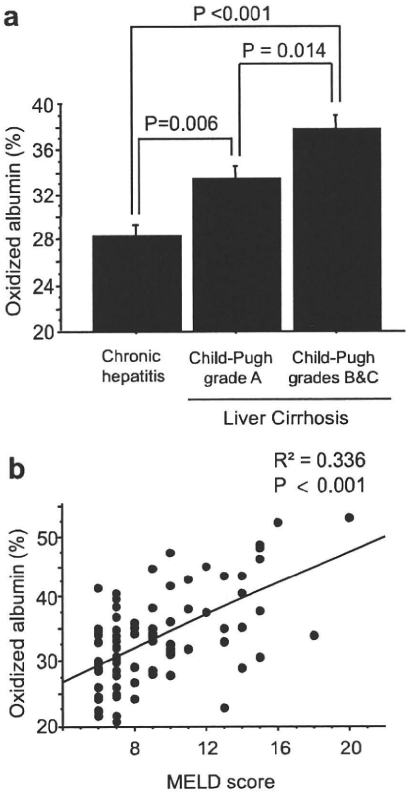

Fig. 1. Oxidized albumin percentage and disease progression in patients with chronic viral liver diseases. (a) Disease progression and oxidized albumin percentage. Values are expressed as mean ± SD. Comparisons among groups were made using analysis of variance followed by post hoc tests. (b) MELD score and oxidized albumin percentage. The correlation between MELD score and the oxidized albumin percentage was done using Spearman rank correlation.

We examined the impact of oxidized albumin percentage in patients with hypoalbuminemia. In patients with albumin levels ≥ 3.66 g/dL, both serum albumin levels and oxidized albumin percentage were significantly correlated with ECF/TBF. In patients with albumin levels < 3.66 g/dL, no significant correlation was seen between serum albumin levels and ECF/TBF (Fig. 4(a), $P = 0.101$, $R^2 = 0.141$). On the other hand, the oxidized albumin percentage showed a significant positive correlation with ECF/TBF (Fig. 4(b), $P = 0.010$, $R^2 = 0.161$).

3.5. Effect of HSA infusion on patients with hypoalbuminemia

Characteristics of patients are summarized in Table 2. There were no significant differences in clinical and laboratory data between the two groups. Moreover, there was no significant difference in follow-up period, the number of days from albumin infusion to evaluation of water retention. In addition, there was no significant difference in the total doses of infused HSA between two groups (Table 2).

Effects of low and high oxidized albumin infusion on changes of water retention were evaluated by changes of body weight. Logistic regression analysis showed that changes in serum albumin levels from before to after albumin infusion, total doses of albumin, and the follow-up period, were not associated with a reduction in body weight. On the other hand, consumption of a low oxidized albumin

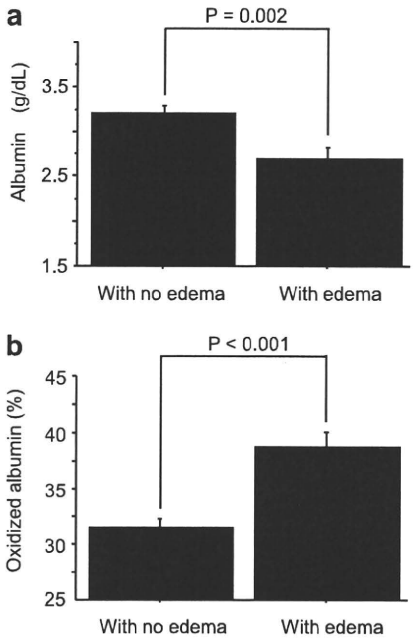

Fig. 2. Serum albumin levels and the oxidized albumin percentage in patients with edema or no edema. (a) Serum albumin levels and physical findings (with edema or no edema) in patients with chronic viral liver diseases. Values are expressed as mean ± SD. Comparisons between two groups were made using the Mann-Whitney *U* test ($P = 0.002$). (b) The oxidized albumin percentage and physical findings (with edema or no edema) in patients with chronic viral liver diseases. Values are expressed as mean ± SD. Comparisons between two groups were made using the Mann-Whitney *U* test ($P < 0.001$).

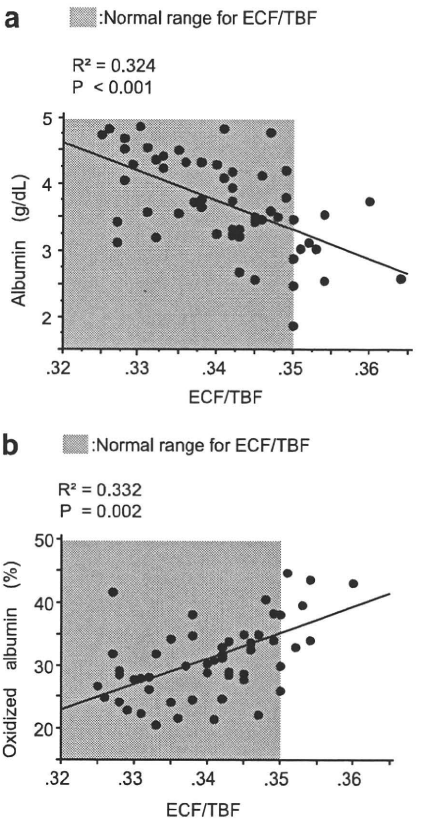

Fig. 3. Serum albumin levels and the oxidized albumin percentage in patients excluded due to edema or ascites on physical examination ($n = 55$). (a) The correlation between serum albumin levels and ECF/TBF. Values are expressed as mean ± SD. Correlations between two groups were made using Spearman rank correlation. (b) The oxidized albumin percentage and water retention (ECF/TBF). Values are expressed as mean ± SD. Correlations between two groups were made using Spearman rank correlation coefficient. Abbreviation: ECF/TBF, extra cellular fluid/total body fluid.

preparation was an independent predictor of reduction in body weight (OR 10.6, 95%CI 1.304–86.307, $P = 0.0272$) (Table 3).

4. Discussion

We showed that the oxidized albumin percentage was significantly increased according to disease progression in patients with chronic viral liver diseases and significantly correlated with MELD scores. Serum albumin levels were negatively correlated and oxidized albumin percentages were positively correlated with ECF/TBF and low oxidized albumin preparation was an independent factor for reduction of body weight. These findings suggest that oxidized albumin is associated with the severity of chronic viral liver disease. In addition, oxidation of albumin might also be associated with water retention in patients with chronic liver diseases.

The oxidized albumin percentage was increased according to the Child-Pugh classification. This finding is in good agreement with previous reports.[14] In addition, we demonstrated a significantly positive correlation between oxidized albumin percentage and MELD score. Although the mechanisms for oxidation of albumin remain unclear, two possibilities exist: (1) because oxidative stress assessed by 8-hydroxy-2′-deoxy-guanosine, 8-isoprostane, malondialdehyde, and lipid peroxide increased with disease progression and caused structural changes in HSA,[39–43] an increased percentage of oxidized albumin may reflect increasing oxidative stress in chronic liver diseases; (2) the albumin half-life is prolonged in cirrhosis.[44] Prolonged albumin half-life results in longer exposures to high oxidative stress and the oxidized albumin percentage consequently increased. In support of this latter mechanism, Fukushima et al. reported that the recovery from prolonged half-life of albumin by branched-chain amino acid supplementation results in decreased oxidized albumin percentages.[45]

We investigated water retention, a key function of albumin. Although it is well known that hypoalbuminemia is associated with edema,[24] we demonstrated that the oxidized albumin percentage is also associated with edema in patients with chronic viral liver diseases. Recently, even in cirrhotic patients with no clinical signs of fluid overload, BIA enables us to assess body composition including ECF/TBF, a marker of water retention.[46] In this study, we first demonstrated that both serum albumin levels and oxidized albumin percentages were significantly correlated with ECF/TBF in patients with no edema or ascites. On the other hand, we sometimes work with patients who have no edema or ascites despite hypoalbuminemia, and the serum albumin level does not always reflect edema or ascites in severe cirrhosis.[47] In addition, the distribution of serum albumin plots displayed heterogeneity; although only 3.7% (1/28) of patients showed water retention in patients with albumin levels ≥ 3.66 g/dL (median value of serum albumin levels), 63.0% (17/27) of patients did not show water retention in patients with albumin levels < 3.66 g/dL. Therefore, we focused on the patients with lower albumin levels and examined the correlation between ECF/TBF and oxidized albumin percentage.

Stratification of patients according to median values of albumin revealed that ECF/TBF had a significantly positive correlation with

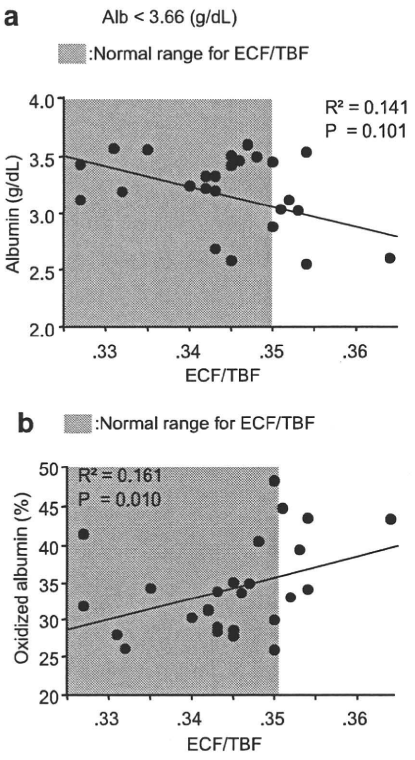

Fig. 4. Stratification of subjects according to median serum albumin levels (3.66 g/dL) in patients excluded due to edema or ascites on physical examination ($n = 55$). (a) The correlation between serum albumin levels and ECF/TBF. Values are expressed as mean ± SD. Correlations between two groups were made using Spearman rank correlation coefficient. (b) The oxidized albumin percentage and water retention (ECF/TBF). Values were expressed as mean ± SD. Correlations between two groups were made using Spearman rank correlation coefficient. Abbreviation: ECF/TBF, extra cellular fluid/total body fluid.

Table 2
Comparison of patient characteristics between low and high oxidized albumin groups.

	Oxidized albumin percentage		
	Low	High	*P*
Number	19	12	
Age (yr)	66.8 ± 2.1	65.0 ± 2.1	0.597
Sex (M/F)	12/7	7/5	0.792
Height (cm)	161.0 ± 2.2	155.3 ± 3.0	0.132
Body weight (kg)	61.0 ± 2.3	57.5 ± 3.2	0.394
BMI	23.3 ± 0.4	23.6 ± 0.6	0.951
BUN (mg/dL)	23.4 ± 3.0	20.6 ± 1.7	0.839
Creatinine (mg/dL)	1.11 ± 0.21	0.90 ± 0.05	0.543
Sodium (mEq/L)	137.0 ± 0.8	137.0 ± 1.2	0.807
Hemoglobin (g/dL)	11.1 ± 0.4	10.5 ± 0.3	0.291
Platelets ($\times 10^4/\mu L$)	8.6 ± 1.1	7.9 ± 0.9	0.792
Lymphocyte ($\times 10^2/mm^3$)	1206.3 ± 137.4	780.8 ± 115.2	0.316
Serum albumin (g/dL)	2.75 ± 0.12	2.53 ± 0.11	0.201
Prothrombin time (%)	70.3 ± 3.0	69.7 ± 3.6	0.792
Child-Pugh score	8.6 ± 0.4	8.4 ± 0.5	0.663
Salt intake (g/day)	7.58 ± 0.32	8.50 ± 0.58	0.264
Furosemide (mg/day)	38.4 ± 7.0	47.5 ± 15.9	0.951
Spironolactone (mg/day)	22.4 ± 4.2	32.9 ± 4.8	0.158
ECF/TBF ratio	0.351 ± 0.002	0.356 ± 0.006	0.968
Follow-up period (days)	5.90 ± 0.85	4.75 ± 1.28	0.268
Dose (g/dL)	62.5 ± 5.2	86.3 ± 12.5	0.146

Note: Values are given as number or mean ± SD. Abbreviations: BMI, body mass index; BUN, blood urea nitrogen; ECF/TBF, extra cellular fluid/total body fluid. Differences between two groups were analyzed using the Mann-Whitney *U* test.

Table 3
Logistic regression analysis for the reduction of body weight in cirrhotic patients.

	Logistic regression analysis		
	OR	95%CI	*P* value
Low oxidized albumin preparation	10.608	1.304–86.307	0.0272
ΔAlbumin	5.118	0.155–169.374	0.3604
Total doses of albumin	1.002	0.965–1.041	0.9029
Follow-up period	1.124	0.881–1.435	0.3476

Patients ($n = 31$).
Note: ΔAlbumin means change in serum albumin levels from before to after albumin infusion.

oxidized albumin percentage but not with serum albumin levels in patients with serum albumin levels < 3.66 g/dL. The reason for this discrepancy is not clear. However, the following possibilities exist. ECF consists of intravascular and extravascular fluid. Although albumin distribution is in equilibrium between intravascular and extravascular fluid in healthy subjects, an abnormal distribution of albumin is caused because of peripheral arterial vasodilation and an increased transcapillary escape rate of albumin in cirrhotic patients.[48–50] Since the serum albumin level does not predict the extravascular albumin pool, the serum albumin concentration may not be associated with ECF/TBF in cirrhotic patients. On the other hand, the oxidized albumin percentage predicts the state of albumin in both intravascular and extravascular fluid.[45] Therefore, the oxidized albumin percentage might be associated with ECF/TBF regardless of the serum albumin level. In addition, oxidative stress is known to up-regulate angiotensin II type 1 receptors, resulting in sodium retention.[51] Because oxidative stress also leads to oxidation of albumin, the oxidized albumin percentage might be correlated with ECF/TBF.

In commercial HSA preparations, oxidized albumin percentages vary.[35,52] We investigated changes in body weight in cirrhotic patients after administration of 2 types of commercial HSA preparations. We first demonstrated that low oxidized albumin preparation was an independent predictor of reduction of body weight. Although the reason for these discrepant results remains unclear, there are the following possibilities: (1) Drug binding, including binding of furosemide, is higher in reduced albumin than in oxidized albumin.[21,22,53] Furosemide bound to reduced albumin exerts a diuretic effect, leading to reduction of body weight. Although Chalasani et al. reported that albumin does not enhance the diuretic effects of furosemide in cirrhotic patients with ascites,[54] the reduced albumin percentage was not investigated. In addition, Inoue et al. and Gentilini et al. reported that furosemide does not exert a sufficient diuretic action unless it binds to albumin in analbuminemic rats and hypoalbuminemic patients,[55,56] supporting our hypothesis. (2) Reduced albumin is the main source of plasma thiols.[57] Low oxidized albumin preparations may increase plasma thiol levels compared to high oxidized albumin preparations, leading to reduction of body weight. Quinlan et al. reported a beneficial effect of albumin administration on plasma thiol repletion in patients with acute lung injury or patients with sepsis syndrome by improving antioxidant capacity in plasma.[58,59] Since the oxidized albumin percentage is associated with ECF/TBF, alleviation of oxidative stress might be more efficient in the low oxidized albumin group compared with the high oxidized albumin group and, consequently, body weight was significantly reduced.

A limitation of this study is to use BIA for body composition analysis in cirrhotic patients. Zillikens et al. reported that BIA can be used to predict total body water in cirrhotic patients without clinical signs of fluid overload, although accuracy is slightly lower than for healthy controls.[34] Recently, Hara et al. reported that the extracellular water ratio is a reliable tool for quantification of redistribution of body water by using 8-electrodes multifrequency

BIA.[46] However, Pirlich et al. and Kyle et al. reported that multi-frequency BIA is not a generally recommended method to assess water distribution and in particular extracellular water in cirrhotic patients.[33,60] Therefore, we have to be cautious when interpreting BIA values.

In conclusion, we demonstrated that the oxidized albumin percentage is significantly associated with Child-Pugh classification and MELD score in patients with chronic viral liver diseases. Moreover, the oxidized albumin percentage showed a significant positive correlation with ECF/TBF ratios in patients with hypoalbuminemia and treatment with a low oxidized albumin preparation was an independent predictor reduction in body weight. Thus, oxidized albumin appears to be associated with disease progression and water retention, and seems to influence the prognosis in patients with chronic viral liver diseases.

Conflict of Interest

The authors have no financial relationship to disclose relevant to this study.

Acknowledgments

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References

1. Johnson A. Low levels of plasma proteins: malnutrition or inflammation? *Clin Chem Lab Med* 1999;**37**:91–6.
2. Anderson C, Wochos D. The utility of serum albumin values in the nutritional assessment of hospitalized patients. *Mayo Clin Proc* 1982;**57**:181–4.
3. Kragh-Hansen U. Structure and ligand binding properties of human serum albumin. *Dan Med Bull* 1990;**37**:57–84.
4. Peters TJ. Serum albumin. *Adv Protein Chem* 1985;**37**:161–245.
5. Mera K, Anraku M, Kitamura K, Nakajou K, Maruyama T, Otagiri M. The structure and function of oxidized albumin in hemodialysis patients: its role in elevated oxidative stress via neutrophil burst. *Biochem Biophys Res Commun* 2005;**334**:1322–8.
6. Sogami M, Era S, Nagaoka S, Kuwata K, Kida K, Miura K, et al. HPLC-studies on nonmercapt–mercapt conversion of human serum albumin. *Int J Pept Protein Res* 1985;**25**:398–402.
7. Carter D, Ho J. Structure of serum albumin. *Adv Protein Chem* 1994;**45**:153–203.
8. Beck J, Ambahera S, Yong S, Sheil M, de Jersey J, Ralph S. Direct observation of covalent adducts with Cys34 of human serum albumin using mass spectrometry. *Anal Biochem* 2004;**325**:326–36.
9. Isles T, Jocelyn P. The reaction of protein thiol groups with some disulphides. *Biochem J* 1963;**88**:84–8.
10. King T. On the sulfhydryl group of human plasma albumin. *J Biol Chem* 1961; **236**:PC5.
11. Era S, Kuwata K, Imai H, Nakamura K, Hayashi T, Sogami M. Age-related change in redox state of human serum albumin. *Biochim Biophys Acta* 1995;**1247**: 12–6.
12. Imai H, Hayashi T, Negawa T, Nakamura K, Tomida M, Koda K, et al. Strenuous exercise-induced change in redox state of human serum albumin during intensive kendo training. *Jpn J Physiol* 2002;**52**:135–40.
13. Oettl K, Stadlbauer V, Petter F, Greilberger J, Putz-Bankuti C, Hallström S, et al. Oxidative damage of albumin in advanced liver disease. *Biochim Biophys Acta* 2008;**1782**:469–73.
14. Watanabe A, Matsuzaki S, Moriwaki H, Suzuki K, Nishiguchi S. Problems in serum albumin measurement and clinical significance of albumin microheterogeneity in cirrhotics. *Nutrition* 2004;**20**:351–7.
15. Suzuki E, Yasuda K, Takeda N, Sakata S, Era S, Kuwata K, et al. Increased oxidized form of human serum albumin in patients with diabetes mellitus. *Diabetes Res Clin Pract* 1992;**18**:153–8.
16. Kadota K, Yui Y, Hattori R, Murohara Y, Kawai C. Decreased sulfhydryl groups of serum albumin in coronary artery disease. *Jpn Circ J* 1991;**55**:937–41.
17. Terawaki H, Yoshimura K, Hasegawa T, Matsuyama Y, Negawa T, Yamada K, et al. Oxidative stress is enhanced in correlation with renal dysfunction: examination with the redox state of albumin. *Kidney Int* 2004;**66**:1988–93.
18. Shaklai N, Garlick R, Bunn H. Nonenzymatic glycosylation of human serum albumin alters its conformation and function. *J Biol Chem* 1984;**259**:3812–7.
19. Yamada N, Nakayama A, Kubota K, Kawakami A, Suzuki E. Structure and function changes of oxidized human serum albumin: physiological significance of the biomarker and importance of sampling conditions for accurate measurement. *Rinsho Byori* 2008;**56**:409–15.
20. Bito R, Hino S, Baba A, Tanaka M, Watabe H, Kawabata H. Degradation of oxidative stress-induced denatured albumin in rat liver endothelial cells. *Am J Physiol Cell Physiol* 2005;**289**:C531–42.
21. Iwao Y, Anraku M, Hiraike M, Kawai K, Nakajou K, Kai T, et al. The structural and pharmacokinetic properties of oxidized human serum albumin, advanced oxidation protein products (AOPP). *Drug Metab Pharmacokinet* 2006;**21**:140–6.
22. Kawakami A, Kubota K, Yamada N, Tagami U, Takehana K, Sonaka I, et al. Identification and characterization of oxidized human serum albumin. A slight structural change impairs its ligand-binding and antioxidant functions. *FEBS J* 2006;**273**:3346–57.
23. Schwartzkopff W, Schwartzkopff B, Wurm W, Frisius H. Physiological aspects of the role of human albumin in the treatment of chronic and acute blood loss. *Dev Biol Stand* 1980;**48**:7–30.
24. Coward W. Serum colloidal osmotic pressure in the development of kwashiorkor and in recovery: its relationship to albumin and globulin concentrations and oedema. *Br J Nutr* 1975;**34**:459–67.
25. Tessari P. Protein metabolism in liver cirrhosis: from albumin to muscle myofibrils. *Curr Opin Clin Nutr Metab Care* 2003;**6**:79–85.
26. Ballmer PE, Walshe D, McNurlan MA, Watson H, Brunt PW, Garlick PJ. Albumin synthesis rates in cirrhosis: correlation with Child-Turcotte classification. *Hepatology* 1993;**18**:292–7.
27. Rothschild M, Oratz M, Zimmon D, Schreiber S, Weiner I, Van Caneghem A. Albumin synthesis in cirrhotic subjects with ascites studied with carbonate-14C. *J Clin Invest* 1969;**48**:344–50.
28. Weinbren I, Taggart P, Glass H. Oedema due to abnormal distribution of protein. *Lancet* 1965;**1**:512–3.
29. Kato M. Effect of branched-chain amino acid (BCAA) enriched-nutrient mixture on albumin metabolism in cirrhotic patients. *Acta Hepatol Jpn* 1991;**32**:692–9.
30. Golden M, Golden B, Jackson A. Albumin and nutritional oedema. *Lancet* 1980;**1**:114–6.
31. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003;**38**:518–26.
32. Pirlich M, Schütz T, Ockenga J, Biering H, Gerl H, Schmidt B, et al. Improved assessment of body cell mass by segmental bioimpedance analysis in malnourished subjects and acromegaly. *Clin Nutr* 2003;**22**:167–74.
33. Pirlich M, Schütz T, Spachos T, Ertl S, Weiss M, Lochs H, et al. Bioelectrical impedance analysis is a useful bedside technique to assess malnutrition in cirrhotic patients with and without ascites. *Hepatology* 2000;**32**:1208–15.
34. Zillikens MC, van den Berg JW, Wilson JH, Rietveld T, Swart GR. The validity of bioelectrical impedance analysis in estimating total body water in patients with cirrhosis. *J Hepatol* 1992;**16**:59–65.
35. Hayashi T, Imai H, Kuwata K, Sogami M, Era S. The importance of sample preservation temperature for analysis of the redox state of human serum albumin. *Clin Chim Acta* 2002;**316**:175–8.
36. Muramoto Y, Matsushita M, Irino T. Reduction of reaction differences between human mercaptalbumin and human nonmercaptalbumin measured by the bromocresol purple method. *Clin Chim Acta* 1999;**289**:69–78.
37. Kamath P, Wiesner R, Malinchoc M, Kremers W, Therneau T, Kosberg C, et al. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001;**33**:464–70.
38. Pugh R, Murray-Lyon I, Dawson J, Pietroni M, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973;**60**:646–9.
39. Pemberton PW, Smith A, Warnes TW. Non-invasive monitoring of oxidant stress in alcoholic liver disease. *Scand J Gastroenterol* 2005;**40**:1102–8.
40. Fan X. Determination of lymphocyte lipid peroxide in patients with hepatitis B and its immunological significance. *Zhonghua Yi Xue Za Zhi* 1990;**70**:608–10. 642.
41. Bhandari S, Agarwal M, Dwivedi S, Banerjee B. Monitoring oxidative stress across worsening child pugh class of cirrhosis. *Indian J Med Sci* 2008;**62**: 444–51.
42. Ichiba M, Yamada S, Ishii K, Gonda K, Murai R, Shimomura T, et al. Significance of urinary excretion of 8-hydroxy-2′-deoxyguanosine in healthy subjects and liver disease patients. *Hepatogastroenterology* 2007;**54**:1736–40.
43. Halliwell B, Gutteridge J. The antioxidants of human extracellular fluids. *Arch Biochem Biophys* 1990;**280**:1–8.
44. Wilkinson P, Mendenhall C. Serum albumin turnover in normal subjects and patients with cirrhosis measured by ^{131}I-labelled human albumin. *Clin Sci* 1963;**25**:281–92.
45. Fukushima H, Miwa Y, Shiraki M, Gomi I, Toda K, Kuriyama S, et al. Oral branched-chain amino acid supplementation improves the oxidized/reduced albumin ratio in patients with liver cirrhosis. *Hepatol Res* 2007;**37**:765–70.
46. Hara N, Iwasa M, Iwata K, Miyachi H, Tanaka H, Takeo M, et al. Value of the extracellular water ratio for assessment of cirrhotic patients with and without ascites. *Hepatol Res* 2009;**39**:1072–9.

47. Czaja A, Wolf A, Baggenstoss A. Clinical assessment of cirrhosis in severe chronic active liver disease: specificity and sensitivity of physical and laboratory findings. *Mayo Clin Proc* 1980;**55**:360–4.
48. Schrier R, Arroyo V, Bernardi M, Epstein M, Henriksen J, Rodés J. Peripheral arterial vasodilation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. *Hepatology* 1988;**8**:1151–7.
49. Parving H, Ranek L, Lassen N. Increased transcapillary escape rate of albumin in patients with cirrhosis of the liver. *Scand J Clin Lab Invest* 1977;**37**:643–8.
50. Henriksen J. Volume adaptation in chronic liver disease: on the static and dynamic location of water, salt, protein and red cells in cirrhosis. *Scand J Clin Lab Invest* 2004;**64**:523–33.
51. Banday A, Lokhandwala M. Oxidative stress-induced renal angiotensin AT1 receptor upregulation causes increased stimulation of sodium transporters and hypertension. *Am J Physiol Renal Physiol* 2008;**295**:F698–706.
52. Bar-Or D, Bar-Or R, Rael L, Gardner D, Slone D, Craun M. Heterogeneity and oxidation status of commercial human albumin preparations in clinical use. *Crit Care Med* 2005;**33**:1638–41.
53. Iwao Y, Anraku M, Yamasaki K, Kragh-Hansen U, Kawai K, Maruyama T, et al. Oxidation of Arg-410 promotes the elimination of human serum albumin. *Biochim Biophys Acta* 2006;**1764**:743–9.
54. Chalasani N, Gorski J, Horlander JS, Craven R, Hoen H, Maya J, et al. Effects of albumin/furosemide mixtures on responses to furosemide in hypoalbuminemic patients. *J Am Soc Nephrol* 2001;**12**:1010–6.
55. Inoue M, Okajima K, Itoh K, Ando Y, Watanabe N, Yasaka T, et al. Mechanism of furosemide resistance in analbuminemic rats and hypoalbuminemic patients. *Kidney Int* 1987;**32**:198–203.
56. Gentilini P, Casini-Raggi V, Di Fiore G, Romanelli R, Buzzelli G, Pinzani M, et al. Albumin improves the response to diuretics in patients with cirrhosis and ascites: results of a randomized, controlled trial. *J Hepatol* 1999;**30**:639–45.
57. Carballal S, Radi R, Kirk M, Barnes S, Freeman B, Alvarez B. Sulfenic acid formation in human serum albumin by hydrogen peroxide and peroxynitrite. *Biochemistry* 2003;**42**:9906–14.
58. Quinlan G, Margarson M, Mumby S, Evans T, Gutteridge J. Administration of albumin to patients with sepsis syndrome: a possible beneficial role in plasma thiol repletion. *Clin Sci (Lond)* 1998;**95**:459–65.
59. Quinlan G, Mumby S, Martin G, Bernard G, Gutteridge J, Evans T. Albumin influences total plasma antioxidant capacity favorably in patients with acute lung injury. *Crit Care Med* 2004;**32**:755–9.
60. Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gomez JM, et al. Bioelectrical impedance analysis—part I: review of principles and methods. *Clin Nutr* 2004;**23**:1226–43.

Redox state of albumin is not associated with colloid osmotic pressure

MASAHIRO SAKATA[1], TAKUMI KAWAGUCHI[1,2], EITARO TANIGUCHI[1], AKIRA NAKAYAMA[4], SONOKO ISHIZAKI[4], ICHIRO SONAKA[4], MIYAKO MAGANUMA[5], TORU NAKAMURA[1], MINORU ITOU[1], TETSUHARU ORIISHI[1], MITSUHIKO ABE[1], CHIKATOSHI YANAGIMOTO[1], HIRONORI KOGA[1], MASARU HARADA[6], TERUO SAKAMOTO[3], SHIGETO ODA[5] and MICHIO SATA[1,2]

[1]Division of Gastroenterology, Department of Medicine, Departments of [2]Digestive Disease Information and Research, and [3]Emergency and Critical Care Medicine, Kurume University School of Medicine, Kurume, Fukuoka 830-0011; [4]Pharmaceutical Research Laboratories, Ajinomoto Co., Inc., Kanagawa 210-0801; [5]Department of Emergency and Critical Care Medicine, Chiba University Graduate School of Medicine, Chiba 260-8670; [6]Third Department of Internal Medicine, University of Occupational and Environmental Health, School of Medicine, Kitakyushu 807-8555, Japan

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Abstract. Serum albumin exists in oxidized and reduced forms. Although the oxidation of albumin affects some of its functions, the relationship between oxidized albumin and colloid osmotic pressure (COP) remains unclear. The aim of this study was to determine whether there is an association between oxidized albumin and COP. Blood samples from 20 healthy volunteers were divided into two aliquots in order to prepare reduced (n=20) and oxidized albumin samples (n=20). This was achieved by treatment with L-cysteine and a redox-stabilizing agent before and after incubation at 37˚C for 24 h. The percentage of oxidized albumin was determined by high-performance liquid chromatography. COP was measured using a colloid osmometer. Reduced and oxidized albumin samples showed 100% of reduced and 100% of oxidized albumin, respectively. There were no significant differences in albumin level and total protein level between the reduced and the oxidized albumin samples. No significant change was seen in COP between the reduced and the oxidized albumin samples (reduced albumin, 17.4±0.2 mmHg; oxidized albumin, 17.3±0.2 mmHg; P=0.465). Therefore, there is no significant difference in COP between reduced and oxidized albumin samples.

Correspondence to: Dr Masahiro Sakata, Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan
E-mail: sakata_masahiro@med.kurume-u.ac.jp

Abbreviations: COP, colloid osmotic pressure; HPLC, high-performance liquid chromatography; HSA, human serum albumin

Key words: oxidized albumin

Introduction

Human serum albumin (HSA) is synthesized by hepatocytes and is the most abundant protein in plasma. HSA acts not only as a transporter of various substances, but also as a component of colloid osmotic pressure (COP) (1,2). COP is an important factor that regulates the movement of fluids between intravascular and extravascular spaces (3). Since large plasma proteins cannot easily cross through the capillary walls, their effect on the osmotic pressure of the capillary interiors tends to pull fluid into the capillaries (4). HSA accounts for 80% of plasma COP, therefore it is believed that a decrease in serum albumin levels leads to low COP, which is associated with the development of fluid retention in the interstitial space or so-called edema (5). However, edema cannot always be explained by decreases in the serum albumin level (6).

HSA is divided into two forms according to the redox state of the Cys-34 locus of HSA: reduced albumin (human mercaptalbumin) and oxidized albumin (human non-mercaptalbumin). This has been demonstrated by high-performance liquid chromatographic (HPLC) analysis (7). In healthy adults, approximately 75% of the Cys-34 molecules in albumin contain a free sulfhydryl group (reduced albumin), while approximately 25% (oxidized albumin) form a disulfide with small sulfhydryl compounds, such as another cysteine, homocysteine or glutathione (8). Not only the quantity, but also the quality of albumin have been previously discussed (9,10). Oxidized albumin clearance in the body is more rapid compared to reduced albumin (11). In addition, ligand binding and antioxidant capacity are lower in oxidized than in reduced albumin (12). Thus, the oxidation of HSA is associated with structural and functional changes.

Recently, we reported that oxidized albumin is associated with edema in cirrhosis (13). Although one would think that the ability to synthesize albumin decreases with disease progression, leading to edema in cirrhotic patients, it remains unclear whether the oxidation of albumin directly affects

Table I. Reduced and oxidized albumin percentage of albumin samples treated with redox-stabilizing agent and L-cysteine.

	Reduced samples (n=20)	Oxidized samples (n=20)	P-value
Oxidized albumin (%)	0±0	100±0	<0.01
Reduced albumin (%)	100±0	0±0	<0.01

Table II. Comparison of laboratory data between reduced and oxidized albumin samples.

	Reduced samples (n=20)	Oxidized samples (n=20)	P-value
Total protein (g/dl)	6.30±0.10	6.20±0.10	0.193
Serum albumin (g/dl)	4.06±0.06	4.05±0.08	0.882
Colloid osmotic pressure (mmHg)	17.4±0.20	17.3±0.20	0.465

COP. The aim of this study was to determine whether the redox state of albumin affects COP.

Materials and methods

Preparation of reduced and oxidized human serum albumin samples. Blood samples from 20 healthy adult volunteers (11 females and 9 males) were each divided into two aliquots in order to prepare reduced (n=20) and oxidized (n=20) albumin samples. Redox-stabilizing agent was prepared as previously described (14).

Reduced albumin samples. Samples were exposed to 1 mmol/ml L-cysteine (Sigma-Aldrich, St. Louis, MO, USA) and to redox-stabilizing agent simultaneously, and the mixture was incubated at 37˚C for 24 h. Thereafter, samples were stored at -20˚C until analysis.

Oxidized albumin samples. Samples were exposed to 1 mmol/ml L-cysteine and the mixture was incubated at 37˚C for 24 h. Thereafter, samples were mixed with the redox-stabilizing agent and stored at -20˚C until analysis.

Laboratory determinations. Blood samples were taken from the peripheral vein of the subjects while they were in the sitting position. After the oxidation or reduction treatment, plasma albumin levels were measured using nephelometry and plasma total protein levels were measured using Biuret methods (15,16). In untreated healthy human blood samples, the reference value of plasma albumin is 3,900-4,900 mg/dl; for the plasma total protein level, it is 6.7-8.3 g/dl.

Determination of oxidized and reduced albumin. HPLC was performed using 5 µl aliquots of each plasma sample and a Shodex Asahipak ES-502N column (Showa Denko, Tokyo, Japan; column temperature 35±0.5˚C). The HPLC system consisted of a Model SCL-10Avp system controler, a Model LC-10ATvp double-plunger pump with a Model FCV-10ALvp gradient and a Model SCL-10ADvp autosampler, a Model RF-10AXL fluorescence detector (excitation wavelength 280 nm; emission wavelength 340 nm). All instruments were purchased from Shimadzu Co. (Tokyo, Japan). Elution was carried out with a linear gradient of increasing ethanol concentration 0-5%, in 0.05 M sodium acetate-0.40 M sodium sulfate (pH 4.85) (acetate-sulfate buffer) at a flow rate of 1 ml/min.

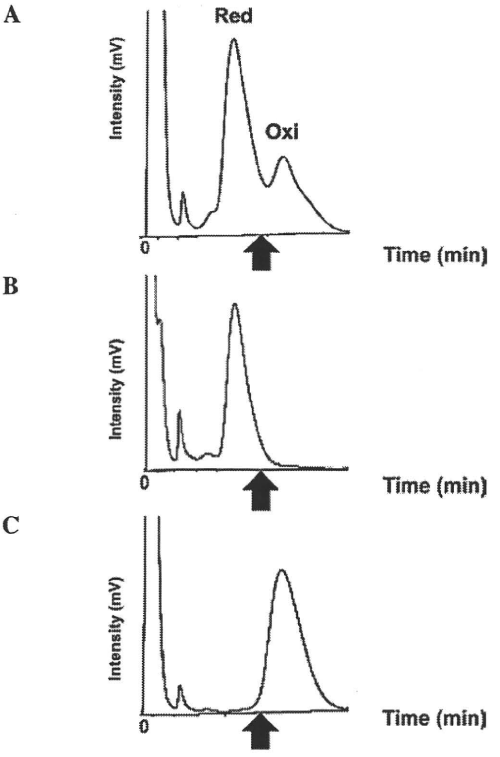

Figure 1. Representative chromatograms of L-cysteine-treated albumin samples. (A) Before treatment (n=4). (B) Reduced albumin sample (n=4). (C) Oxidized albumin sample (n=4). The arrow indicates 20 min past elution. Red, reduced albumin; Oxi, oxidized albumin.

Determination of colloid osmotic pressure. The colloid osmometer (Colloid 4420®; Wescor, UT, USA) was used to directly measure colloid osmotic pressure (17). To examine the reproducibility of measurements, COP was measured twice for each sample and the values were expressed as the means (Table II).

Results

Oxidized albumin percentage of L-cysteine treated samples. After treatment with L-cysteine + redox-stabilizing agent, the proportion of samples that were reduced albumin became 100%. After treatment with L-cysteine without redox-stabilizing agent, the proportion of samples that were oxidized albumin became 100% (Table I and Fig. 1).

Albumin levels, total protein levels and colloid osmotic pressure in the reduced and oxidized samples. Data for the outcome variables are summarized in Table II. There were no significant differences in albumin levels and total protein levels between the reduced and oxidized albumin samples.

Similarly, there was no significant difference in COP between the two groups. Thus, none of the variables differed between reduced and oxidized albumin samples.

Discussion

In this study, we investigated the association between the oxidation of albumin and COP using 100% reduced and 100% oxidized albumin samples. There was no significant difference in COP between reduced and oxidized albumin samples.

The method for preparing 100% oxidized and 100% reduced albumin samples involved L-cysteine. Since cysteine has an SH residue, treatment with L-cysteine caused the albumin to shift to the reduced form, indicating that cysteine acted as a reductant. Incubation at 37˚C for 24 h caused albumin to became oxidized; since cystine (Cys-S-S-Cys) is produced under oxidizing conditions, the result is a shift of the albumin to the oxidized form. With these preparation methods, the only difference between the oxidized and reduced albumin samples was that, in the reduced samples, the cysteine formed disulfide bond with Cys34. Following treatment with L-cysteine, there were no significant differences in the albumin levels or total protein levels between the two groups. However, the concentrations of albumin and total protein decreased compared to the reference values of each parameter. One possible reason is that the samples were diluted by treatment with the redox-stabilizing agent.

We investigated a possible association between the oxidation of albumin and changes in COP. However, no significant difference in COP was detected between the reduced and oxidized albumin samples. COP is the equilibrium pressure exerted on a semi-permeable membrane separating two solutions of differing osmolality. Fluids pass across these membranes, while larger materials, such as proteins (also known as colloids), cannot (18). The molecular mass of human albumin is approximately 66,000, while the molecular mass of L-cysteine is 121.16. It is thought that there is little difference between the molecular mass of oxidized and reduced albumin. COP is dependent on the total concentration of molecules dissolved in a fluid (19). In this study, the concentrations of total protein and albumin did not differ between the reduced and oxidized samples. Therefore, the oxidation of albumin may not have influenced COP. We previously reported that oxidized albumin is associated with edema in cirrhosis (13). In line with this thinking, the indirect influence of albumin oxidation, such as drug binding properties and oxidative stress, may have been related to edema formation (11,20).

In conclusion, we investigated a possible association between the redox state of albumin and COP using 100% oxidized and 100% reduced albumin samples. However, we found that the oxidation of albumin is not associated with changes in COP.

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References

1. Rothschild M, Oratz M and Schreiber S: Serum albumin. Hepatology 8: 385-401, 1988.
2. Kragh-Hansen U: Structure and ligand binding properties of human serum albumin. Dan Med Bull 37: 57-84, 1990.
3. Krogh A, Landis EM and Turner AH: The movement of fluid through the human capillary wall in relation to venous pressure and to the colloid osmotic pressure of the blood. J Clin Invest 11: 63-95, 1932.
4. Vercueil A, Grocott MP and Mythen MG: Physiology, pharmacology, and rationale for colloid administration for the maintenance of effective hemodynamic stability in critically ill patients. Transfus Med Rev 19: 93-109, 2005.
5. Coward W: Serum colloidal osmotic pressure in the development of kwashiorkor and in recovery: its relationship to albumin and globulin concentrations and oedema. Br J Nutr 34: 459-467, 1975.
6. Noguera Vinas EC, Hames W, Mothe G and Barrionuevo MP: Extracellular fluid, plasma and interstitial volume in cirrhotic patients without clinical edema or ascites. Medicina 49: 320-324, 1989.
7. Sogami M, Era S, Nagaoka S, *et al*: Hplc-studies on nonmercaptmercapt conversion of human serum albumin. Int J Pept Protein Res 25: 398-402, 1985.
8. Beck J, Ambahera S, Yong S, Sheil M, de Jersey J and Ralph S: Direct observation of covalent adducts with cys34 of human serum albumin using mass spectrometry. Anal Biochem 325: 326-336, 2004.
9. Shaklai N, Garlick R and Bunn H: Nonenzymatic glycosylation of human serum albumin alters its conformation and function. J Biol Chem 259: 3812-3817, 1984.
10. Yamada N, Nakayama A, Kubota K, Kawakami A and Suzuki E: Structure and function changes of oxidized human serum albumin: physiological significance of the biomarker and importance of sampling conditions for accurate measurement. Rinsho Byori 56: 409-415, 2008.
11. Iwao Y, Anraku M, Hiraike M, *et al*: The structural and pharmacokinetic properties of oxidized human serum albumin, advanced oxidation protein products (aopp). Drug Metab Pharmacokinet 21: 140-146, 2006.
12. Kawakami A, Kubota K, Yamada N, *et al*: Identification and characterization of oxidized human serum albumin. A slight structural change impairs its ligand-binding and antioxidant functions. FEBS J 273: 3346-3357, 2006.
13. Sakata M, Kawaguchi T, Taniguchi E, *et al*: Oxidized albumin is associated with water retention and severity of disease in patients with liver cirrhosis. Gastroenterology 134: A793-A793, 2008.
14. Kubota K: A simple stabilization method of reduced albumin in blood and plasma for the reduced/oxidized albumin ratio measurement. Int J Biomed Sci 5: 293-301, 2009.
15. Okutucu B, Dincer A, Habib O and Zihnioglu F: Comparison of five methods for determination of total plasma protein concentration. J Biochem Biophys Methods 70: 709-711, 2007.
16. Deaton CD, Maxwell KW, Smith RS and Creveling RL: Use of laser nephelometry in the measurement of serum proteins. Clin Chem 22: 1465-1471, 1976.
17. Blackwell MM, Riley J, McCall M, Ecklund J and Southworth R: An evaluation of three methods for determining colloid osmotic pressure. J Extra Corpor Technol 26: 18-22, 1994.
18. Krediet RT, Ho-dac-Pannekeet MM, Imholz AL and Struijk DG: Icodextrin's effects on peritoneal transport. Perit Dial Int 17: 35-41, 1997.
19. Sinclair S and Webb AR: Colloid osmotic pressure measurement in critically ill patients. Intensive Care World 8: 120-122, 1991.
20. Banday A and Lokhandwala M: Oxidative stress-induced renal angiotensin at1 receptor upregulation causes increased stimulation of sodium transporters and hypertension. Am J Physiol Renal Physiol 295: F698-F706, 2008.

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EDITORIAL

Importance of hepatitis C virus-associated insulin resistance-therapeutic strategies for insulin sensitization

Takumi Kawaguchi, Michio Sata

Takumi Kawaguchi, Michio Sata, Department of Digestive Disease Information & Research and Department of Medicine, Kurume University School of Medicine, Kurume 830-0011, Japan

Author contributions: Kawaguchi T and Sata M contributed equally to this paper.

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Correspondence to: Takumi Kawaguchi, MD, PhD, Assistant Professor, Department of Digestive Disease Information & Research and Department of Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan. takumi@med.kurume-u.ac.jp
Telephone: +81-942-317902 Fax: +81-942-317820

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Abstract

Insulin resistance is one of the pathological features in patients with hepatitis C virus (HCV) infection. Generally, persistence of insulin resistance leads to an increase in the risk of life-threatening complications such as cardiovascular diseases. However, these complications are not major causes of death in patients with HCV-associated insulin resistance. Indeed, insulin resistance plays a crucial role in the development of various complications and events associated with HCV infection. Mounting evidence indicates that HCV-associated insulin resistance may cause (1) hepatic steatosis, (2) resistance to anti-viral treatment, (3) hepatic fibrosis and esophageal varices, (4) hepatocarcinogenesis and proliferation of hepatocellular carcinoma, and (5) extrahepatic manifestations. Thus, HCV-associated insulin resistance is a therapeutic target at any stage of HCV infection. Although the risk of insulin resistance in HCV-infected patients has been documented, therapeutic guidelines for preventing the distinctive complications of HCV-associated insulin resistance have not yet been established. In addition, mechanisms for the development of HCV-associated insulin resistance differ from lifestyle-associated insulin resistance. In order to ameliorate HCV-associated insulin resistance and its complications, the efficacy of the following interventions is discussed: a late evening snack, coffee consumption, dietary iron restriction, phlebotomy, and zinc supplements. Little is known regarding the effect of anti-diabetic agents on HCV infection, however a possible association between use of exogenous insulin or a sulfonylurea agent and the development of HCC has recently been reported. On the other hand, insulin-sensitizing agents are reported to improve sustained virologic response rates. In this review, we summarize distinctive complications of, and therapeutic strategies for, HCV-associated insulin resistance. Furthermore, we discuss supplementation with branched-chain amino acids (BCAA) as a unique insulin-sensitizing strategy for patients with HCV-associated insulin resistance.

Key words: Hepatitis C virus; Diabetes mellitus; Insulin resistance; Complications; Treatments; Branched-chain amino acid

Peer reviewer: Atsushi Tanaka, MD, PhD, Associate Professor, Department of Medicine, Teikyo University School of Medicine, 2-11-1, Kaga, Itabashi-ku, Tokyo 173-8605, Japan

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INTRODUCTION

Insulin resistance is frequently seen in patients with

hepatitis C virus (HCV) infection[1,2]. Although in the general population, lack of exercise and overeating are major causes of insulin resistance, in patients with HCV infection, hepatic inflammation, activated inflammatory cytokines, and HCV-induced impairments of insulin and lipid signaling molecules are also important factors for the development of insulin resistance[3-14]. Therefore, the prevalence of insulin resistance is higher in patients with HCV infection compared to that in the general population and patients with other hepatobiliary disorders[6,15].

Generally, insulin resistance results in the development of type 2 diabetes mellitus and increases the risk of life-threatening complications such as cardiovascular diseases, renal failure, and infections. However, these complications are not major causes of death in cirrhotic patients with insulin resistance[16]. On the other hand, the development of intrahepatic complications, including hepatocellular carcinoma (HCC), is known to be associated with insulin resistance[17-21]. Insulin resistance is also reported to be involved in the development of extrahepatic manifestations of HCV infection including gastric cancer[22-24].

Reduction of fasting blood glucose and hemoglobin A1c (HbA1c) is a well-established therapeutic strategy for prevention of complications in diabetic patients[25,26]. However, in patients with chronic liver diseases, fasting blood glucose and HbA1c are not always available for evaluation of glucose metabolism because of decreased hepatic glycogen content[27] and increased turnover of hemoglobin[28]. Furthermore, an association between the use of exogenous insulin or sulfonylurea agents and the development of HCC has recently been reported[29,30]. Although therapeutic guidelines for inhibiting the distinctive complications of HCV-associated insulin resistance are not yet available, amelioration of insulin resistance is considered to inhibit complications and improve prognosis. Here, we summarize treatments that could reduce HCV-associated insulin resistance.

In this review, we summarize distinctive complications of, and therapeutic strategies for, HCV-associated insulin resistance. In addition, we discuss the merits of branched-chain amino acid (BCAA) supplementation as a unique insulin-sensitizing strategy for patients with HCV-associated insulin resistance.

DISTINCTIVE COMPLICATIONS OF HCV-ASSOCIATED INSULIN RESISTANCE

Complications of HCV-associated insulin resistance are different from those of lifestyle-associated insulin resistance[16]. Cardiovascular diseases are major causes of death in patients with lifestyle-associated insulin resistance[31]. However, these complications are not major causes of death in patients with HCV-associated insulin resistance[16]. In contrast, HCV-associated insulin resistance is involved in the development of various complications associated with HCV infection. Here, we summarize events associated with insulin resistance that are distinctive complications of HCV-associated insulin resistance (Figure 1).

Hepatic steatosis

Hepatic steatosis is commonly observed[32,33] and is an independent risk factor for disease progression in patients with HCV infection[34]. Various mechanisms are operative in the development of hepatic steatosis. HCV core protein induces production of reactive oxygen species and lipid peroxidation[35]. HCV core protein also regulates secretion of very low-density lipoprotein, triglycerides, and apoliprotein B through regulation of fatty acid synthase, microsomal triglyceride transport protein, peroxisome proliferator-activated receptor gamma (PPARγ), and sterol regulatory element binding protein-1c[9,36-38]. Thus, HCV itself is directly involved in the development of hepatic steatosis. In addition, insulin is an anabolic hormone and promotes hepatic lipogenesis through activation of hydroxymethylglutaryl-CoA reductase and acetyl-CoA carboxylase[39]. In addition, insulin inhibits lipolysis through regulation of phosphodiesterase type 3B[19]. In HCV core gene transgenic mice, the development of insulin resistance precedes the development of hepatic steatosis, suggesting that insulin resistance may induce hepatic steatosis[8,40]. However, hepatic steatosis could also cause insulin resistance[41,42], and therefore, the initial step in HCV-related metabolic disorders remains unclear in patients with HCV infection.

Resistance to anti-viral treatment

Insulin resistance is associated with a poor response to anti-viral treatment in patients with HCV genotype 1, 2, and 3 infections[10,43-46]. Although the reason for an association between insulin resistance and resistance to anti-viral treatment is largely unknown, the following are possibilities. Insulin resistance is known to increase hepatic lipid synthesis[47]. Since the lipid droplet is an important organelle for hepatitis C virus replication[48], accumulation of hepatic lipid droplets may increase HCV replication and result in poor responses to anti-viral treatment, even in patients with HCV genotype 2 and 3[45].

Alternatively, HCV core protein is reported to upregulate suppressor of cytokine signaling (SOCS) 3[6,49-52], which acts as an adaptor to facilitate the ubiquitination of signaling proteins, leading to subsequent proteasomal degradation of SOCS3[19]. HCV core protein-induced SOCS3 upregulation promotes proteasomal degradation of insulin receptor substrate (IRS) 1 and IRS2, resulting in the development of insulin resistance in patients with HCV infection[6,19,44]. Simultaneously, SOCS3 is also known to inhibit interferon-alpha-induced expression of the anti-viral proteins 2',5'-oligoadenylate synthetase and myxovirus resistance A through inactivation of Janus kinase, a signal transducer and activator of the transcription pathway[49]. Thus, SOCS3 seems to be a key molecule for a cross-talk between insulin resistance and resistance in patients with HCV infection. In fact, hepatic expression of SOCS3 has predictive value for the outcome of anti-viral therapy in patients with HCV infection[53,54].

Hepatic fibrosis and esophageal varices

Insulin resistance is closely associated with progression

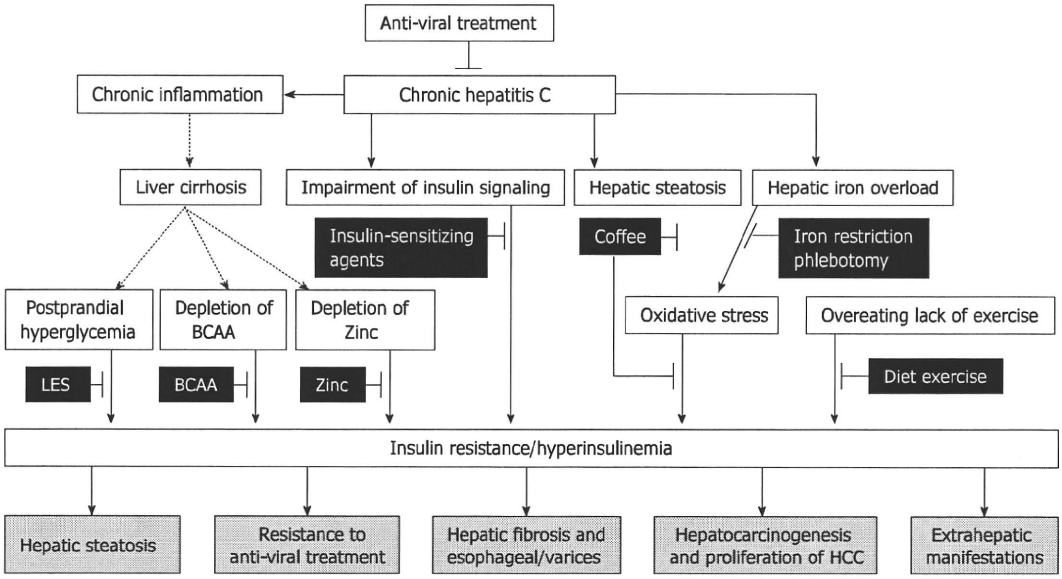

Figure 1 Pathogenic mechanisms and therapeutic strategies for hepatitis C virus (HCV)-associated insulin resistance. Black squares indicate therapeutic strategies for HCV-associated insulin resistance. Proper diet, exercise, iron restriction, phlebotomy, and coffee intake are recommended to any stage of liver disease. In cirrhotic patients, a late evening snack (LES), branched-chain amino acid (BCAA) supplementation, and a zinc supplement are also recommended. Insulin-sensitizing agents can be used in patients with chronic hepatitis C, however, the agents are not always recommended for patients with liver cirrhosis because of severe adverse effects.

of hepatic fibrosis in patients with HCV infection[6,11,55]. The hepatocyte is known to degrade circulating insulin, and, therefore, hepatic fibrosis may reduce insulin clearance, resulting in increased serum insulin levels regardless of the presence of insulin resistance[56]. However, insulin resistance is seen in early stages of chronic hepatitis C[6]. Furthermore, even in patients that have received a liver transplantation for HCV-related liver cirrhosis, insulin resistance is a risk factor for rapid progression of hepatic fibrosis[57]. These findings suggest that insulin resistance promotes hepatic fibrosis. Insulin resistance may directly affect hepatic stellate cells and increase connective tissue growth factor (CTGF), which causes production of extracellular matrix[58]. Alternatively, insulin resistance-induced hepatic lipid accumulation may increase oxidative stress, resulting in progression of hepatic fibrosis[32].

Insulin resistance is also a risk factor for esophageal varices in cirrhotic patients with HCV infection[59]. As the hepatic fibrosis is correlated with the development of esophageal varices, insulin resistance may be associated with the development of esophageal varices through progression of hepatic fibrosis[60]. In addition, insulin modulates the endothelial synthesis of nitric oxide and endothelin[61], regulators of sinusoidal blood flow[62]. Thus, insulin-induced hepatic fibrosis and vasoconstriction may be possible mechanisms for the development of esophageal varices.

Hepatocarcinogenesis and proliferation of HCC

Liver cirrhosis, aging, and being a male are well-known risk factors for the development of HCC in patients with HCV infection[18,63]. In addition, insulin resistance is now recognized as an independent risk factor for the development of HCC worldwide[18,63]. Diabetes is reported as the only independent risk factor for HCC in patients with chronic hepatitis C[21]. Moreover, development of diabetes-related HCC is reported to be independent of viral hepatitis and alcoholism[64]. These findings suggest that insulin resistance has direct effects on hepatocarcinogenesis. Although precise mechanisms for this effect remain unclear, the following explanations may be put forward. Insulin resistance causes lipid accumulation[19]. Visceral adiposity results in changes in serum adipocytokine levels, including reduction of adiponectin, which suppresses effects for hepatocarcinogenesis[65]. Hepatic lipid accumulation also increases oxidative stress, which may be responsible for the development of HCC[18,63]. Besides these possibilities, insulin has a mitogenic effect[19,30], suggesting that insulin may be directly linked to hepatocarcinogenesis[19].

Insulin resistance may be associated not only with hepatocarcinogenesis, but also with proliferation of HCC. We have examined the significance of insulin resistance on the prognosis in patients with HCV-associated HCC and found that insulin resistance is an independent risk factor for poor prognosis[20]. As no significant difference was seen in disease-free survival between patients with and without insulin resistance, these findings indicate that insulin resistance accelerates the proliferation of HCC[20]. In good accordance with our results, Saito *et al*[66] reported that reduction of serum insulin levels by continuous infusion of octreotide significantly suppressed proliferation of HCC. Although the mechanisms for insulin-induced proliferation of HCC remain obscure, insulin exerts growth-promoting activity through activation of a mitogen-activated protein kinase pathway[19]. In addition, overexpression of transducing molecules for insulin signaling, IRS1[67] and IRS2[68], and downregulation of suppressing molecules for