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．

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Original Article

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

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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.

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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.

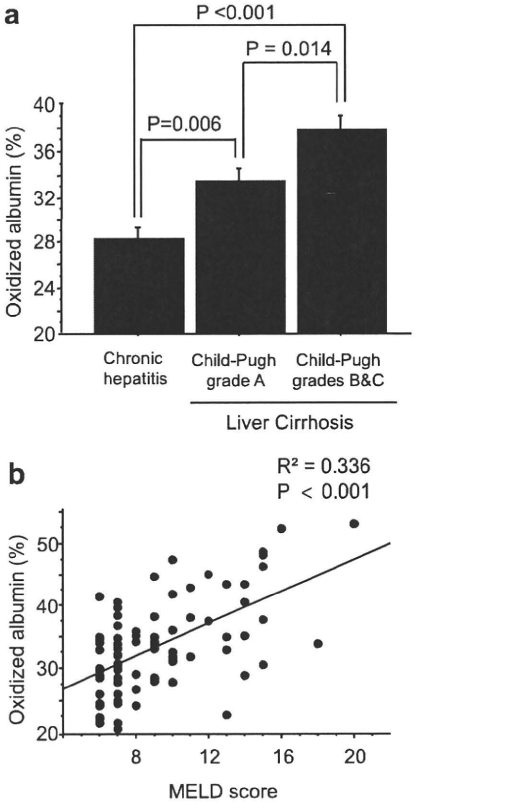

Fig. 1. Oxidized albumin percentage and disease progression in patients with chronic viral liver diseases. (a) Disease progression and oxidized albumin percentage. Values are expressed as mean ± SD. Comparisons among groups were made using analysis of variance followed by post hoc tests. (b) MELD score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

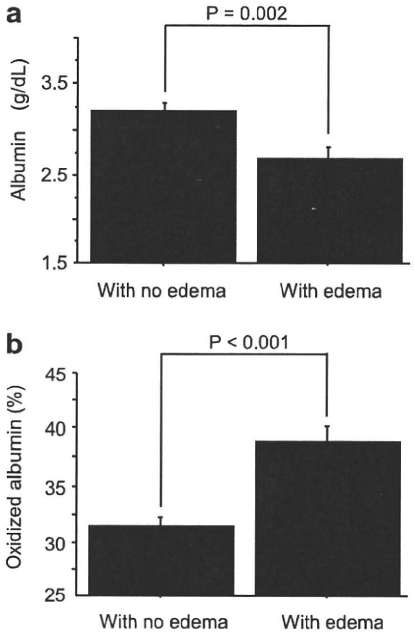

Fig. 2. Serum albumin levels and the oxidized albumin percentage in patients with edema or no edema. (a) Serum albumin levels and physical findings (with edema or no edema) in patients with chronic viral liver diseases. Values are expressed as mean ± SD. Comparisons between two groups were made using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

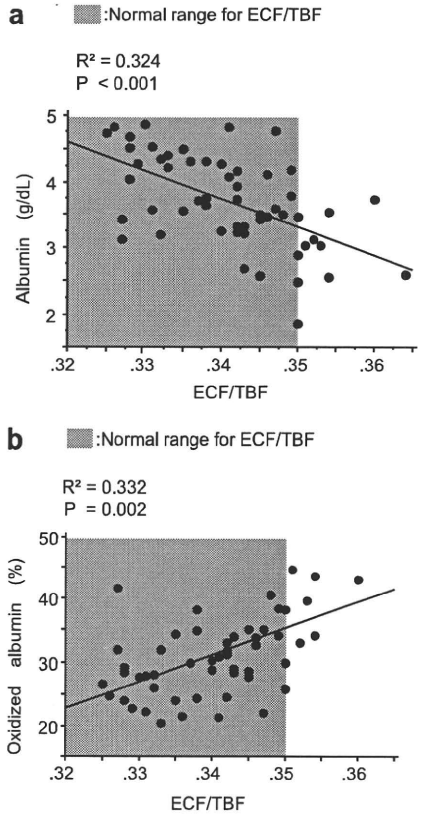

Fig. 3. Serum albumin levels and the oxidized albumin percentage in patients excluded due to edema or ascites on physical examination ($n = 55$). (a) The correlation between serum albumin levels and ECF/TBF. Values are expressed as mean ± SD. Correlations between two groups were made using Spearman rank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

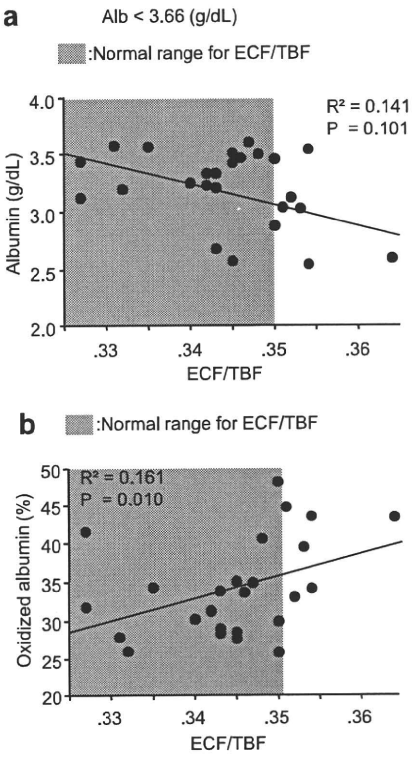

Fig. 4. Stratification of subjects according to median serum albumin levels (3.66 g/dL) in patients excluded due to edema or ascites on physical examination ($n = 55$). (a) The correlation between serum albumin levels and ECF/TBF. Values are expressed as mean ± SD. Correlations between two groups were 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.

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Redox state of albumin is not associated with colloid osmotic pressure

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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.

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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.

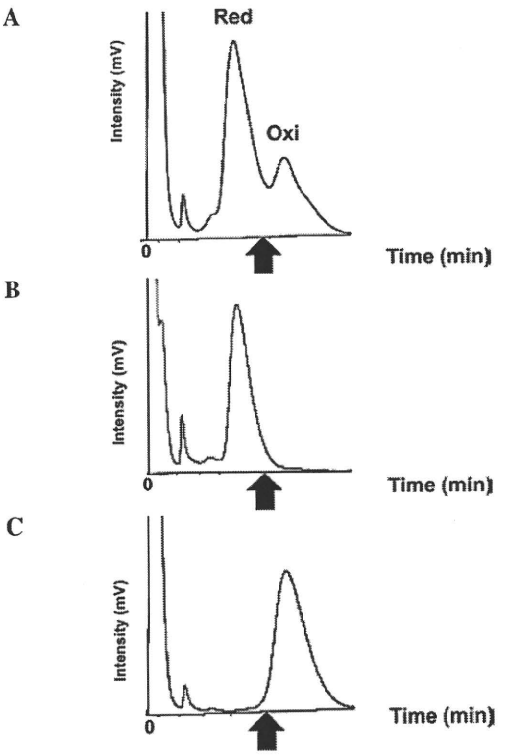

Figure 1. Representative chromatograms of L-cysteine-treated albumin samples. (A) Before treatment (n=4). (B) Reduced albumin sample (n=4). (C) Oxidized albumin sample (n=4). The arrow indicates 20 min past elution. Red, reduced albumin; Oxi, oxidized albumin.

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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EDITORIAL

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

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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

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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

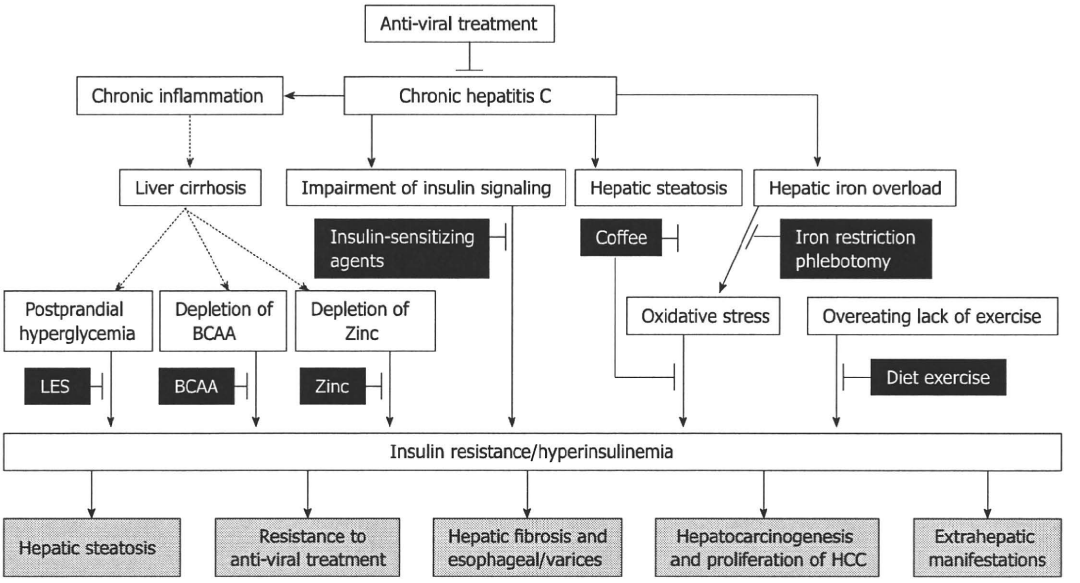

Figure 1 Pathogenic mechanisms and therapeutic strategies for hepatitis C virus (HCV)-associated insulin resistance. Black squares indicate therapeutic strategies for HCV-associated insulin resistance. Proper diet, exercise, iron restriction, phlebotomy, and coffee intake are recommended to any stage 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

insulin signaling, phosphatase and tensin homologue[69], and SH2 domain-containing inositol phosphatase- 2[20] occur in HCC. Thus, HCC may be sensitive to insulin stimulation.

Extrahepatic manifestations

HCV causes extrahepatic manifestations including mixed cryoglobulinemia, Sjögren's syndrome, and non-Hodgkin lymphoma, oral lichen planus, oral squamous cell carcinoma, and malignancies other than HCC[22-24,70-73]. In patients with extrahepatic manifestations of HCV, fasting insulin levels and homeostasis model assessment for insulin resistance are significantly higher than for patients without extrahepatic manifestations[22]. Among various extrahepatic manifestations, insulin resistance is associated with oral lichen planus[23], oral squamous cell carcinoma[24], and multiple primary cancers including gastric cancer[24]. Although reasons for this association remain unclear, a high prevalence of precancerous lesions and cancers are seen in patients with type 2 diabetes mellitus[74,75], suggesting that insulin resistance or hyperinsulinemia may enhance carcinogenic activities.

DISTINCTIVE THERAPEUTIC STRATEGY FOR HCV-ASSOCIATED INSULIN RESISTANCE

Despite awareness of the increased risk of insulin resistance, therapeutic guidelines to inhibit distinctive complications of HCV-associated insulin resistance have not yet been established. HCV itself has a significant impact on the development of insulin resistance, and eradication of HCV improves insulin resistance[44,46,76]. Thus, anti-viral therapy is a fundamental therapeutic strategy for patients with HCV infection. In addition, amelioration of insulin resistance is considered to inhibit complications and improve prognosis. Here, we summarize treatments which could improve HCV-associated insulin resistance as therapeutic strategies (Figure 1).

Late evening snack

Proper diet and exercise are fundamental for patients with lifestyle-associated insulin resistance as well as patients with HCV-associated insulin resistance[77-80]. As a nutritional treatment for liver cirrhosis, divided energy intake (4 to 6 meals/d) has been recommended[77,79]. As postprandial hyperglycemia is characteristic of HCV-associated insulin resistance[77-80], a decrease in energy intake per meal reduces postprandial hyperglycemia and hyperinsulinemia. In particular, a late evening snack is reported not only to improve glucose intolerance[81-84], but also to suppress hepatocarcinogenesis in cirrhotic patients[85].

Coffee consumption

Coffee consumption reduces the risk of elevated serum alanine aminotransferase activity[86], hepatic fibrosis[87], and disease progression in chronic hepatitis C[88]. Coffee consumption also reduces the risk of HCC independent of HCC etiology[89]. Caffeine is metabolized by hepatic cytochrome P450 1A2 into 3 metabolites, the dimethylxanthines paraxanthine, theobromine, and theophylline. Of these metabolites, theophylline inhibits transforming growth factor-β-stimulated CTGF expression through PPARγ and Smad 2/3-dependent pathways. Since CTGF and transforming growth factor-β are important factors associated with progression of hepatic fibrosis and hepatocarcinogenesis, a metabolite of caffeine, theophylline, may have an inhibitory effect on the development of complications associated with HCV infection. In addition, coffee has significant effects on glucose metabolism[90]. In an animal experiment, the insulin-sensitizing effects of coffee have been demonstrated[91]. Similarly, in a human study, coffee consumption reduced fasting glucose and insulin levels[90,92]. Although the mechanisms for the coffee-induced insulin-sensitizing effect remain unclear, some possibilities exist. Chlorogenic acids, a constituent of coffee, inhibits hepatic glucose-6-phosphate translocation[90,93], limits glucose absorption from the gut by inhibiting Na^+-dependent transport[94], and increases the secretion of glucose regulating hormone, glucagon-like peptide (GLP)-1, from the gut[90,95,96]. These findings suggest that a constituent of coffee, chlorogenic acid, directly ameliorates HCV-associated insulin resistance. Furthermore, coffee modulates lipid metabolism[97,98] and lowers body weight[90], indicating that coffee may suppress the lipid-induced increase in oxidative stress and ameliorates HCV-associated insulin resistance.

Phlebotomy

Hepatic iron overload produces oxidative stress and is a factor responsible for the development of HCV-associated insulin resistance[4,99-101]. Although the pathogenesis of hepatic iron overload remains unclear, recent studies showed that iron-regulating molecules are modulated by HCV infection. Hepcidin is a negative regulator of duodenal iron absorption and macrophage iron release[100] and decreased hepatic expression of hepcidin is seen in both HCV polyprotein transgenic mice[102] and patients with HCV infection[103-105]. In addition, upregulation of hepatic expression of transferrin receptor 2, a mediator of iron uptake, is responsible for hepatic iron overload[106].

In order to reduce hepatic iron deposition, dietary iron restriction and phlebotomy are effective. Dietary iron restriction (less than 7 mg/d) decreases serum alanine aminotransferase levels in patients with HCV infection[107]. Phlebotomy reduces oxidative stress as well as insulin resistance in patients with HCV infection[101,108,109]. A long-term combination treatment with phlebotomy and dietary iron restriction reduces the risk of development of HCC in patients with HCV infection[110].

Supplementation of zinc

Zinc plays a crucial role in the metabolism of protein, carbohydrate, lipid, nucleic acid, and ammonia[111-113]. In fact, zinc supplementation improves glucose disposal

in patients with cirrhosis[114]. Zinc also inhibits hepatic inflammation[115] and hepatic fibrosis[116]. More recently, zinc supplementation was shown to lower the cumulative incidence of HCC in patients with HCV infection[117]. It is unclear whether these inhibitory effects of zinc on progression of liver disease are mediated by amelioration of insulin resistance. However, zinc participates in the synthesis, storage and secretion of insulin[118] and regulates the binding ability of insulin to bind to its receptor[113]. As the serum zinc level is decreased in patients with HCV infection[115,117], supplementation of zinc could be a therapeutic option.

Anti-diabetic agents

Exogenous insulin and sulfonylurea agents: Anti-diabetic agents are effective for decreasing plasma glucose and HbA1c levels, leading to prevention of diabetes mellitus-associated complications including cardiovascular diseases[119,120]. However, it has never been determined whether anti-diabetic agents prevent complications or improve prognosis in patients with HCV infection. Use of exogenous insulin or sulfonylurea agents may worsen hyperinsulinemia. In fact, we, along with others, recently reported an association between exogenous insulin or sulphonylurea treatment and the development of HCC in patients with HCV infection[29,30,121]. Use of exogenous insulin is also reported to be associated with the development of colon cancer[122] and other malignancies[123]. Although a causal relationship between exogenous insulin and the development of HCC remains controversial[124], the reduction of serum insulin levels is a first line therapeutic strategy for insulin resistance[125-128].

Insulin-sensitizing agents: Insulin resistance is associated with a poor response to anti-viral treatment in patients with HCV infection[10,43-46]. Amelioration of insulin resistance may improve the response to anti-viral treatment. However, the impact of insulin-sensitizing agents, biguanides and thiazolidinediones, on sustained virologic response (SVR) rates has not yet been established. Recently, metformin, a biguanide agent, has been reported to ameliorate HCV-associated insulin resistance, and increase the SVR rate in HCV genotype 1 infected patients with normalization of homeostasis model assessment for insulin resistance at week 24 of therapy[129]. Pioglitazone, a thiazolidinedione agent, has also been reported to ameliorate insulin resistance and increase SVR rates in patients with HCV genotype 4 infection[130]. Although the insulin-sensitizing mechanisms of metformin and of pioglitazone are different, both agents are known to up-regulate IRS[131,132], which is the molecule responsible for HCV-associated insulin resistance[3,6,50], and to improve HCV-associated insulin resistance. Because both agents have severe adverse effects, neither is recommended for patients with liver cirrhosis. Biguanides predispose cirrhotic patients to lactic acidosis[133]. Thiazolidinediones cause overproduction of hydrogen peroxide leading to severe hepatotoxicity[134]. Thus, further validation for safety is required.

Dipeptidyl peptidase IV (DPPIV) inhibitor is a new therapeutic agent[135] and its clinical efficacy in type 2 diabetes has been shown[136]. Although no study has examined the effect of DPPIV inhibitor on HCV-associated insulin resistance, we found that activation of DPPIV is a factor responsible for HCV-associated insulin resistance[27]. Thus, a DPPIV inhibitor may be suited for ameliorating HCV-associated insulin resistance.

BCAA supplementation, a possible insulin-sensitizing agent

BCAA are constituents of proteins and are required for protein synthesis[19,78,137,138]. In addition, BCAA are reported to modulate glucose metabolism. Leucine and isoleucine induce glucose transporter 1 and 4 translocation to the plasma membrane of muscle cells and improve glucose metabolism in a carbon tetrachloride-induced cirrhotic rat model[139]. In addition, leucine enhances the insulin-induced activation of the Akt/mammalian target of the rapamycin pathway in adipocytes of db/db mice[140]. Moreover, isoleucine increases hepatic phosphatidylinositol 3-kinase activity and improves insulin resistance in Zucker fa/fa rats, a model of severe insulin resistance[141]. Recently, knockout of the mitochondrial BCAA aminotransferase gene in mice, in which results in elevated plasma BCAA levels, was found to ameliorate insulin resistance[142]. Thus, BCAA improve insulin signaling in various animal models *via* several pathways. In good agreement with these results in animals, in human studies, we have recently shown that BCAA-enriched supplementation reduces insulin resistance in patients with HCV infection[143,144]. In a multicenter, randomized, controlled trial, BCAA supplementation led to a reduction in the risk of HCC in cirrhotic patients[145]. This suppressive effect on hepatocarcinogenesis was more evident in obese patients with HCV infection[145]. Both obesity and HCV induce the development of insulin resistance. Thus, BCAA may improve insulin resistance and subsequently inhibit insulin resistance-induced hepatocarcinogenesis[19,145].

CONCLUSION

In this review, we summarize the distinctive complications of, and therapeutic strategies for, HCV-associated insulin resistance. Although cardiovascular diseases, renal failure, and infections are well-known complications of lifestyle-associated insulin resistance, these complications are not major causes of death in cirrhotic patients with insulin resistance. HCV-associated insulin resistance rather causes (1) hepatic steatosis, (2) resistance to anti-viral treatment, (3) hepatic fibrosis and esophageal varices, (4) hepatocarcinogenesis and proliferation of HCC, and (5) extrahepatic manifestations. These complications are life-threatening, and therapeutic strategies for HCV-associated insulin resistance have to be considered on the basis of its pathogenic mechanisms.

Pathogenic mechanisms for HCV-associated insulin resistance differ from those for lifestyle-associated insulin resistance. Postprandial hyperglycemia, lipid-induced oxi-

dative stress, hepatic iron overload, and depletion of zinc are responsible for the development of HCV-associated insulin resistance. Therefore, a late evening snack, coffee consumption, dietary iron restriction, phlebotomy, and supplementation of zinc are recommended therapeutic strategies. No clinical guidelines for the use of anti-diabetic agents are available for patients with HCV-associated insulin resistance. However, use of exogenous insulin or sulphonylurea may increase the risk for HCC. On the other hand, insulin-sensitizing agents may improve the SVR rate of anti-viral treatment. In addition, BCAA supplementation has an insulin-sensitizing effect as well as a suppressive effect on hepatocarcinogenesis. Thus, in order to ameliorate HCV-associated insulin resistance, various therapeutic approaches are required.

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