LIVER AND BILIARY DISEASE

Augmentation effect of postprandial hyperinsulinaemia on growth of human hepatocellular carcinoma

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Background: Cirrhotic patients with hepatocellular carcinoma (HCC) frequently have impaired glucose metabolism.

Aims: To investigate whether impaired glucose metabolism affects the growth rate of the tumour.

Patients and methods: Tumour doubling time (DT), assessed by ultrasound imaging analysis, was measured in 60 patients with single small HCC (diameter <30 mm). DT was compared with plasma insulin and glucose concentrations following the oral glucose tolerance test (OGTT). The effect of continuous infusion of octreotide (a somatostatin analogue 200 µg/day) for three months on DT in five cases was assessed.

Results: The 60 patients were divided into two groups because the median DT was 140 days: rapid growth group (DT ⩽140 days, n=30) and slow growth group (DT >140 days, n=30). Fasting plasma insulin concentration and area under the plasma insulin curve (AUC_{ins}) of the OGTT (10.4 (6.2) µU/ml and 262 (152) µU/ml/h, respectively; mean (SD)) in the rapid growth group were significantly higher than those in the slow growth group (7.6 (4.3) and 146 (140), respectively) (p=0.041 and p=0.0006, respectively). In contrast, fasting plasma glucose concentration and area under the plasma glucose curve (AUC_{gluc}) in the rapid growth group were significantly lower than those in the slow growth group (p=0.0003 and p=0.0012, respectively). Univariate and multivariate analyses of logistic regression models demonstrated that AUC_{ins} was a significant factor contributing to the growth rate of HCC (p=0.001 and p=0.016, respectively). AUC_{ins} significantly decreased after octreotide treatment (p<0.02) but AUC_{gluc} did not significantly change. DT after treatment increased in three of the five patients and could not be calculated in the remaining two patients because of no change in the diameter of the tumour.

Conclusions: These data suggest that postprandial hyperinsulinaemia is associated with accelerated HCC growth.

Hepatocellular carcinoma (HCC) is frequently accompanied by liver cirrhosis.[1] Patients with liver cirrhosis often have impaired glucose metabolism,[2] with 60–80% being glucose intolerant and 10–15% developing overt diabetes mellitus.[2-4] Hepatogenous diabetes differs from type 2 diabetes mellitus.[5] Its pathogenesis involves many factors, and hyperinsulinaemia and peripheral insulin resistance are thought to play major roles.[6-8]

The growth rate of a tumour is a critical factor for the prognosis of cancer patients. The growth of HCC can be measured by determining the doubling time (DT) with imaging analyses.[9 10] Several studies have surveyed clinical factors affecting DT in human HCC.[9-13] However, no significant factors affecting the growth rate of HCC have yet been reported.

Insulin is known to be an important hepatotrophic factor and also stimulates proliferation of hepatoma cells.[14 15] However, it is not known whether the growth rate of HCC is affected by hyperinsulinaemia based on underlying chronic liver disease.

The aim of the present study was to clarify whether hyperinsulinaemia in patients with HCC and cirrhosis affects the growth rate of tumours. We investigated the relationship of plasma insulin concentrations following the oral glucose tolerance test (OGTT) with DT assessment by ultrasound (US) analyses. The effect of reduction of hyperinsulinaemia by octreotide,[16 17] a somatostatin analogue, on the DT of the tumour was also tested.

MATERIALS AND METHODS

Study patients

From January 1995 to October 2000, 205 patients with chronic liver disease were diagnosed as having single small HCC (diameter <30 mm) at Osaka University Hospital and Yamagata University Hospital. These patients were found by periodic US imaging screening, and the diagnosis of HCC was performed by computed tomography or magnetic resonance imaging techniques.

Growth of HCC in 72 of the above 205 patients was followed by two US examinations at intervals of more than 60 days (the intervals depended on the patients' schedules) between the day of HCC diagnosis and the day before surgery and other anticancer treatment. Some of the 72 patients wanted to delay treatment against HCC because of their business schedules. The others declined any anticancer therapy but later consented to undergo surgery and other anticancer treatment. DT of HCC in 60 of the 72 patients was calculated because the tumour margins were clearly visible in the two US examinations and the tumours were spherical or nearly spherical in

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Abbreviations: AFP, α fetoprotein; AUC_{gluc}, area under the plasma glucose curve; AUC_{ins}, area under the plasma insulin curve; DT, doubling time; HCC, hepatocellular carcinoma; IGF, insulin-like growth factor; IGF-1R, insulin-like growth factor 1 receptor; IGFBP-1, insulin-like growth factor 1 binding protein; OGTT, oral glucose tolerance test; US, ultrasound.

Table 1 Clinical and laboratory characteristics of the study subjects

Variable	Rapid growth DT ≤140 days (n=30)	Slow growth DT >140 days (n=30)	p Value
Sex			
Males	24	25	NS
Females	6	5	
Age (y)	64.1 (6.6)	64.3 (5.3)	NS
Hepatitis virus			
HBV ve+	2	6	NS
HCV ve+	28	24	
Haemoglobin (g/dl)	13.1 (1.4)	13.0 (1.0)	NS
Leucocytes (/µl)	5800 (2750)	5780 (2540)	NS
Platelet (10^3/µl)	105 (48)	103 (45)	NS
Albumin (g/dl)	3.36(0.54)	3.45(0.43)	NS
Total bilirubin (mg/dl)	1.36(0.77)	1.64(1.25)	NS
ALT (U/l)	57.3 (23.1)	75.8 (42.3)	NS
Creatinine (mg/dl)	0.74(0.21)	0.73(0.23)	NS
AFP (ng/ml)	188 (684)	237 (683)	NS
Fasting glucose (mg/dl)	100 (16.0)	120 (21.7)	0.0003
Fasting insulin (µU/ml)	10.4 (6.2)	7.6 (4.3)	0.041
AUC_{gluc} (mg/dl/h)	361 (102)	439 (119)	0.0012
AUC_{ins} (µU/ml/h)	263 (152)	146 (140)	0.0006
Tumour diameter (mm)	20.7 (4.5)	22.2 (5.2)	NS
Interval of US (days)	85.0 (25.2)	83.2 (27.9)	NS
(range)	(62–160)	(61–150)	
Underlying liver disease			
Chronic hepatitis	4	6	NS
Liver cirrhosis	26	24	
Histology			
Well	12	15	NS
Moderate/poor	18	15	

Values are mean (SD) or number.
ALT, alanine aminotransferase; AFP, α fetoprotein; AUC_{gluc}, area under the plasma glucose curve; AUC_{ins}, area under the plasma insulin curve; DT, doubling time; HBV, hepatitis B virus; HCV, hepatitis C virus; US, ultrasound.

shape, which is usually the case with small HCC. None of the patients had undergone any anticancer treatment or had received antidiabetic drugs or insulin before DT assessment.

Median age of the 60 patients (50 males and 10 females) with single small HCC, for whom DT was calculated, was 64 years (range 49–81). Hepatitis B surface antigen by radioimmunoassay (Abbott Laboratories, North Chicago, Illinois, USA) was detected in eight patients. Antibody to hepatitis C virus was detected in 52 patients by a second generation enzyme linked immunosorbent assay test (Abbott Laboratories). The diagnosis of HCC was confirmed histologically by examination of surgically resected tumour tissues from 35 patients or US guided needle biopsy specimens in 25. The histology of HCC was well differentiated in 27 patients and moderately/poorly differentiated in 33 patients. A diagnosis of chronic hepatitis or liver cirrhosis was carried out histologically.

Thirty five of the 60 patients underwent surgical treatment while 20 of the 60 patients were treated with percutaneous ethanol injection or microwave coagulation therapy with or without a combination of transcatheter arterial embolisation. The remaining five patients were treated with octreotide because of poor liver function reserve. DT during octreotide treatment was determined at intervals during treatment, and OGTT was also carried out at the end of treatment in five patients.

This study protocol was approved by Osaka University Medical School Ethical Committee and Yamagata University School of Medicine Ethical Committee.

Measurement of DT

For practical comparison of tumour sizes, the diameter (r) used was that for a circular tumour in the two dimensional image or an equivalent value ($(a×b)^{1/2}$), where a is the longest axis and b is the shortest axis perpendicular to it.[9][10] DT as a measure of growth was calculated in terms of volume from the radius or lengths in the largest section of the tumour. Thus the volume was either $4/3\pi r^3$ or $4/3\pi(a/2×b/2)^{2/3}$.

Two ultrasonographers (SI and HW) were involved in the study. The same ultrasonographer performed both volume measurements in each patient.

Oral glucose tolerance test

The 60 patients with single small HCC underwent an OGTT at admission for treatment of HCC. OGTT was performed after the second US examination for second tumour volume measurements. The test was performed at 8 am on day 2 following the recommendations of the American Diabetes Association.[18] A Teflon catheter was inserted into an antecubital vein for blood sampling, and samples were obtained for plasma glucose, free insulin, and C peptide concentrations in the basal period and after an oral glucose load (75 g) at 30 minute intervals for 180 minutes.

Areas under the plasma glucose and insulin curves (AUC_{gluc} and AUC_{ins}) for the OGTT were calculated.

Treatment with octreotide

Octreotide was subcutaneously infused at a daily dose of 200 µg with an infusion pump for three months to continuously reduce plasma insulin concentration in five patients with HCC.

Statistical analysis

Fisher's exact probability test or the Mann-Whitney test was used to compare baseline characteristics in the rapid and slow growth groups. To estimate the significant factors contributing to tumour growth, univariate and multivariate analyses by the logistic regression model were used. The variables used were sex, age, hepatitis viral infection, fasting plasma glucose and insulin concentrations, AUC_{gluc} and AUC_{ins} of OGTT, liver function tests, renal function, haematological data, serum concentrations of α fetoprotein (AFP), underlying liver disease, and

Table 2 Factors contributing to doubling time of hepatocellular carcinoma using logistic regression model

	Univariate analysis			Multivariate analysis		
Variable	Odds ratio	95% CI	p Value	Odds ratio	95% CI	p Value
Sex						
Male	1			1		
Female	0.08	0.22–2.97	NS	0.650	0.10–4.28	NS
Age (y)						
≤65	1			1		
>65	2.10	0.71–6.22	NS	2.07	0.51–8.40	NS
Hepatitis virus						
HBV ve+	1			1		
HCV ve+	3.50	0.65–18.98	NS	2.84	0.24–32.81	NS
Haemoglobin (g/dl)						
≤13.0	1			1		
<13.0	1.25	0.38–3.75	NS	0.92	0.37–2.60	NS
Leucocyte (/µl)						
≤5500	1			1		
>5500	1.32	0.43–3.91	NS	1.57	0.48–4.62	NS
Platelet (10^3/µl)						
≤100	1			1		
>100	0.86	0.32–2.70	NS	1.15	0.37–3.63	NS
Albumin (g/dl)						
≤3.3	1			1		
>3.3	1.24	0.49–3.15	NS	1.35	0.47–3.95	NS
Total bilirubin (mg/dl)						
≤1.2	1			1		
>1.2	0.95	0.23–3.60	NS	0.73	0.23–2.39	NS
ALT (U/l)						
≤60	1			1		
>60	1.60	0.43–5.82	NS	1.31	0.44–4.03	NS
Creatinine (mg/dl)						
≤0.8	1			1		
>0.8	1.42	0.41–4.01	NS	0.82	0.31–2.20	NS
AFP (ng/ml)						
≤35	1			1		
>35	1.00	0.35–2.85	NS	0.61	0.16–2.37	NS
Fasting glucose (mg/dl)						
≤110	1			1		
>110	0.38	0.13–1.09	NS	1.25	0.21–7.53	NS
Fasting insulin (µU/ml)						
≤8	1			1		
>8	2.67	0.92–7.70	NS	0.30	0.04–2.16	NS
AUC_{glu} (mg/dl/h)						
≤400	1			1		
>400	0.38	0.12–19.76	NS	0.17	0.04–0.77	0.022
AUC_{ins} (µU/ml/h)						
≤205	1			1		
>205	6.42	2.08–19.76	0.001	22.23	1.79–276.7	0.016
Underlying liver disease						
Chronic hepatitis	1			1		
Liver cirrhosis	1.63	0.41–6.47	NS	0.96	0.12–7.76	NS
Histology						
Well	1			1		
Moderate/poor	1.50	0.54–4.17	NS	3.43	0.80–14.63	NS

ALT, alanine aminotransferase; AFP, α fetoprotein; AUC_{gluc}, area under the plasma glucose curve; AUC_{ins}, area under the plasma insulin curve; CI, confidence interval.

histology of HCC. The cutoff value was the median of each variable. Differences in AUC_{gluc} and AUC_{ins} before and after octreotide treatment were analysed by the Wilcoxon (matched pair) signed ranks test. A p value <0.05 was considered statistically significant.

RESULTS
Relation of DT to postprandial hyperinsulinaemia
Baseline characteristics of patients with single small HCC are presented in table 1. The 60 patients were divided into two groups (rapid growth group, ≤140 days, n=30 and slow growth group, >140 days, n=30) because the median DT of HCC was 140 days. Sex, age, hepatitis viral infection, serum AFP, and biochemical liver function tests, including serum alanine aminotransferase, bilirubin, and albumin concentrations, were not different between the rapid and slow growth groups. Also, no difference was noted in the ratio of liver cirrhosis and histological differentiation. Fasting plasma insulin concentration and AUC_{ins} were significantly higher (10.4 (6.2) µU/ml and 262 (152) µU/ml/h, respectively; mean (SD)) in the rapid growth group compared with those in the slow growth group (7.6 (4.3) and 146 (140), respectively) (p=0.041 and p=0.0006, respectively). In contrast, fasting plasma glucose concentration and AUC_{gluc} (100 (16.0) mg/dl and 361 (102) mg/dl/h, respectively) in the rapid growth group were significantly lower than those in the slow growth group (120 (21.7) and 439 (119), respectively) (p=0.0003 and p=0.0012, respectively).

To identify factors contributing to shortening of the DT in HCC patients, univariate and multivariate analyses of logistic regression models were performed (table 2). AUC_{ins} for the OGTT was a significant factor contributing to HCC growth in
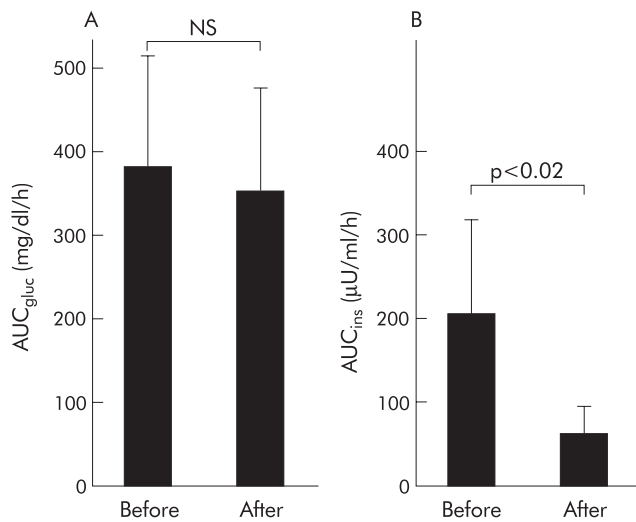
Figure 1 Effects of octreotide on plasma insulin and glucose concentrations during the oral glucose tolerance test (OGTT) in five patients with hepatocellular carcinoma (mean (SD)). (A) Comparison of the areas under the plasma glucose curve (AUC_{gluc}) for the OGTT before and after octreotide treatment (NS). (B) Comparison of the areas under the plasma insulin curve (AUC_{ins}) for the OGTT before and after octreotide treatment (p<0.02, Wilcoxon signed ranks test).
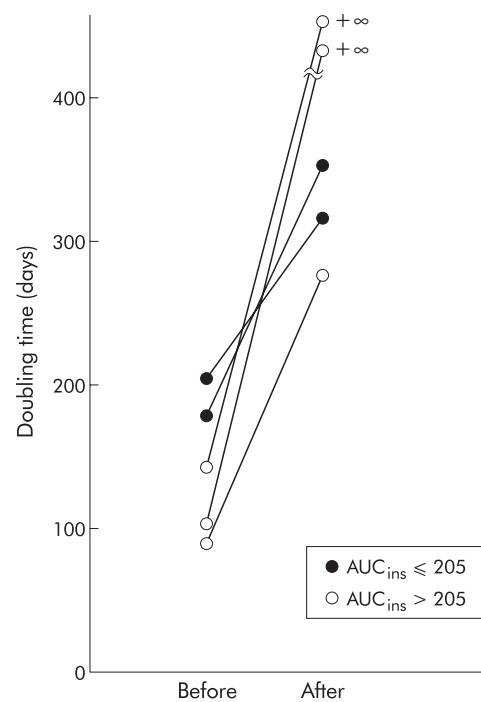

Figure 2 Changes in doubling time of hepatocellular carcinoma before and after octreotide treatment in five patients with an area under the plasma insulin curve (AUC_{ins}) of ≤205 µU/ml/h or AUC_{ins} >205 µU/ml/h before octreotide treatment.

both analyses (p=0.001 and p=0.016, respectively). These results imply that postprandial hyperinsulinaemia accelerates the growth rate of HCC. AUC_{gluc} was a negative significant factor for HCC growth in multivariate analysis (p=0.022).

Correlations of DT with fasting plasma glucose and insulin concentrations, AUC_{gluc}, and AUC_{ins} were assessed. DT was inversely correlated with AUC_{ins} ($r=-0.441$, p<0.01). DT was also significantly correlated with fasting plasma glucose concentration ($r=0.496$, p<0.01) but showed no correlation with AUC_{gluc} or fasting plasma insulin concentration ($r=0.319$ and $r=-0.268$, respectively).

Effect of reduction of hyperinsulinaemia on DT

Five of the 60 patients were treated with octreotide for three months with no significant adverse effects. AUC_{gluc} values in the five patients were not different before and after treatment (380 (133) mg/dl/h v 350 (126)) (fig 1). AUC_{ins} after treatment significantly decreased in comparison with before treatment (65 (34) µU/ml/h v 205 (112); p<0.02, Wilcoxon signed ranks test). DT after octreotide treatment increased in three of the five patients (from about 1.5-fold to threefold) and could not be calculated because of no change in the diameters of the tumour for three months during octreotide treatment in the remaining two patients (fig 2). DT in the three patients with an AUC_{ins} >205 µU/ml/h became longer than that in the two patients with an AUC_{ins} <205 µU/ml/h.

DISCUSSION

The growth rate of a tumour is a critical factor for the prognosis of cancer patients. The DT of human HCC has been measured in an attempt to clarify the clinical factors affecting tumour growth rate.[9][12][13] However, no clinical factor, including age, sex, liver function, and hepatitis viral infection, was found to correlate with the growth rate of HCC. DT was also independent of the stage of liver cirrhosis, histological type, and grade of malignancy. Only vascularity showed a correlation with growth rate in well differentiated HCC.[13] The present study showed that hyperinsulinaemia after oral glucose intake was a significant factor contributing to the growth rate of HCC.

Glucose intolerance, overt diabetes mellitus, and insulin resistance are characteristic features of patients with cirrhosis.[2–8][19] A recent study suggested that liver cirrhosis is a condition characterised by marked peripheral insulin resistance, and diabetes develops only in patients in which β cells cannot compensate with increased secretory performance.[20] In fact, insulin resistance in cirrhosis is normalised after liver transplantation.[20][21] Several lines of evidence suggest that hyperinsulinaemia in cirrhotic patients results from three abnormalities: impaired hepatic insulin clearance, portosystemic/intrahepatic shunting, and enhanced insulin secretion related to insulin resistance.[19][22]

Insulin functions as a potent growth factor via phosphorylation of insulin receptor substrate 1 and activation of mitogen activated protein kinases.[23][24] Human and rat hepatoma derived cell lines have insulin receptors and their proliferation is dose dependently stimulated by addition of physiological concentrations of insulin into the medium.[24][25] Thus high concentrations of insulin after a meal in cirrhotic patients may stimulate growth of HCC. In this study, peripheral insulin was measured in contrast with portal insulin which is more likely to exert any growth stimulating effects on liver tumours. Further study is needed to clarify whether insulin can sufficiently act to stimulate growth of hepatoma cells in vivo.

AUC_{gluc} was a negative factor for the growth of HCC in multivariate analysis. Fasting plasma glucose also correlated with DT. However, this result is difficult to explain. Cirrhotic patients with elevated fasting plasma glucose concentrations and higher AUC_{gluc} values appear to have lower AUC_{ins} values due to development of overt diabetes and reduced insulin secretion. It is possible that the relatively slow growth of HCC results from lower AUC_{ins} values.

Recently, Kouroumalis and colleagues[16] reported that octreotide administration significantly improved quality of life and survival in patients with inoperable HCC in a randomised controlled trial. Continuous infusion of octreotide at a daily dose of 200 µg reduces hyperinsulinaemia and restores plasma insulin concentrations to the normal range during OGTT without changes in plasma glucose concentration in patients with cirrhosis.[17] In this study, we observed a reduction in hyperinsulinaemia and a decrease in HCC growth rate in five

patients during octreotide treatment. Furthermore, the reduction in growth rate was more marked in the three patients with hyperinsulinaemia with an AUC for OGTT of >205 μU/ml/h (fig 2). These data support the fact that postprandial hyperinsulinaemia may participate in HCC growth although there is no direct evidence that the reduction in hyperinsulinaemia during octreotide treatment caused the decreased HCC growth rate in these patients. Insulin-like growth factors 1 (IGF-1) and 2 (IGF-2) and their receptor (IGF-1R) are expressed by human hepatoma cells and have been implicated in the pathogenesis of liver cancer.[26–29] Octreotide is also reported to inhibit growth of liver tumours via suppression of circulating IGF-1 levels and induction of insulin-like growth factor 1 binding protein (IGFBP-1).[30] In this study, IGF-1 and IGFBP-1 levels were not measured, even in the five patients who received octreotide. Examination of IGF-1 and IGFBP-1 levels should be done in further studies.

The present study demonstrated that postprandial hyperinsulinaemia was associated with accelerated HCC growth. Treatment of postprandial hyperinsulinaemia may be beneficial for therapeutic management of cirrhotic patients with HCC. However, this study could not clarify whether the presence of postprandial hyperinsulinaemia predicts the prognosis of patients with HCC. Further study is needed to examine whether hyperinsulinaemia affects the prognosis of patients with HCC.

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