Table II. Sustained virological response (SVR) at the end of follow-up

Variable	No. of patients achieving SVR (%)	
	peginterferon-α-2a + placebo	peginterferon-α-2a + ribavirin
Overall	23 (24.0)	57 (59.4)
Sex		
Male	18 (30.5)	39 (63.9)
Female	5 (13.5)	18 (51.4)
Age (y)		
≤29	5 (71.4)	2 (50.0)
30 to 39	5 (38.5)	7 (77.8)
40 to 49	4 (18.2)	16 (72.7)
50 to 59	4 (16.7)	20 (55.6)
60 to 69	5 (18.5)	12 (48.0)
≥70	0 (0.0)	
Weight (kg)		
<50	5 (41.7)	4 (66.7)
50 to <60	4 (12.5)	15 (45.5)
60 to <70	5 (17.2)	22 (66.7)
70 to <80	6 (33.3)	10 (62.5)
≥80	3 (60.0)	6 (75.0)
Serum HCV RNA (IU/mL)		
1 to <5 × 10^5	10 (38.5)	12 (57.1)
5 to <8.5 × 10^5	6 (22.2)	21 (65.6)
≥8.5 × 10^5	7 (16.3)	24 (55.8)
ALT activity (IU/L)		
<50	2 (10.5)	13 (76.5)
50 to <100	11 (23.4)	25 (62.5)
100 to <200	6 (27.3)	17 (51.5)
≥200	4 (50.0)	2 (33.3)
Fibrosis staging[a]		
F1	5 (21.7)	10 (55.6)
F2	13 (22.4)	38 (63.3)
F3	5 (33.3)	7 (43.8)
F4		1 (100.0)

a F1 = mild, F2 = moderate, F3 = severe, F4 =cirrhosis.
ALT = alanine aminotransferase; **HCV** = hepatitis C virus.

and the achievement of an SVR with combination therapy.

In contrast, in patients receiving monotherapy, lower baseline HCV RNA levels (OR 1.003; 95% CI 1.006, 1.001; p = 0.006), younger age (OR 1.081; 95% CI 1.125, 1.034; p = 0.0009) and a severe fibrosis stage (OR 6.194; 95% CI 1.037, 37.000; p = 0.0455) significantly increased the likelihood of achieving an SVR.

Effect of Medication Adherence on SVR

In the combination therapy group, patients maintaining a full dosage schedule of PEG IFN-α-2a and ribavirin and those requiring dose reductions of either study drug had similar SVR rates (figure 2). However, the SVR rate was reduced to 33.3% among patients who discontinued combination therapy. Only 3 out of the 31 patients who received the full dosage schedule were ≥60 years of age; the majority of elderly patients failed to complete the

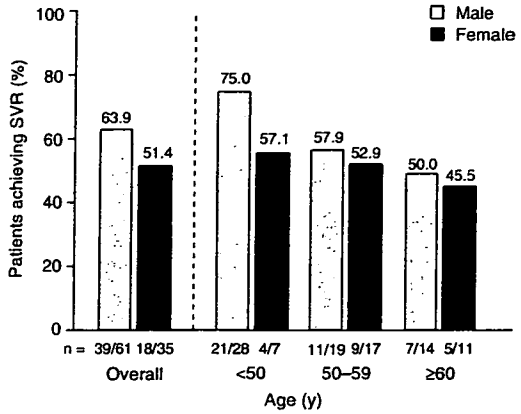

Fig. 1. Effect of patient age on sustained virological response (SVR).

full dosage schedule as a result of adverse events. Similarly, of the 15 patients who discontinued combination therapy, three were <50 years of age and six were ≥60 years old.

The SVR rate was reduced in patients receiving <60% of the cumulative PEG IFN-α-2a and ribavirin planned total doses (figure 3). Dose reductions negatively affected the SVR rate in elderly patients who had received <60% of the cumulative PEG IFN-α-2a and ribavirin doses, which was achieved by 0/10 (0%) and 3/13 (23%) patients who were ≥50 years of age, and by 0/6 (0%) and 2/7 (28.6%) patients who were ≥60 years of age, respectively.

Discussion

Combination therapy with PEG IFN-α-2a plus ribavirin was associated with significantly higher SVR rates compared with PEG IFN-α-2a monotherapy, in treatment-naive patients infected with HCV genotype 1b (61% vs 26%; $p < 0.001$).[3] This outcome is noteworthy, because individuals with HCV genotype 1 infections are considered to be relatively difficult to treat.[9]

Previously, there were no data on the association between sex or age and virological response following treatment with PEG IFN-α-2b plus ribavirin.[10] Our data indicate that the attainment of an SVR following combination therapy was not influenced by any of the pretreatment host-related factors (including age, sex, HCV RNA level, fibrosis stage and bodyweight) evaluated in this retrospective analysis, although younger males (<50 years) appeared to have a higher SVR rate compared with males aged ≥60 years (75% vs 50%). Younger age, lower baseline HCV RNA levels and a severe fibrosis stage significantly increased the likelihood of achieving an SVR with monotherapy. In contrast, a previous study[11] showed that a histological activity index score of >10 and a lack of cirrhosis or bridging fibrosis were independent factors associated with SVR attainment among patients treated with monotherapy, which suggests that severe fibrosis staging negatively impacts the SVR rate. In our study, a

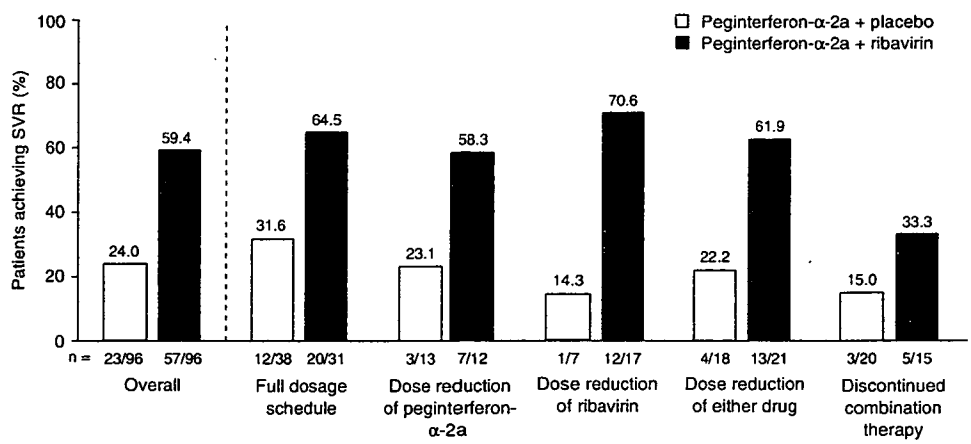

Fig. 2. Effects of dose reduction and discontinuation on sustained virological response (SVR).

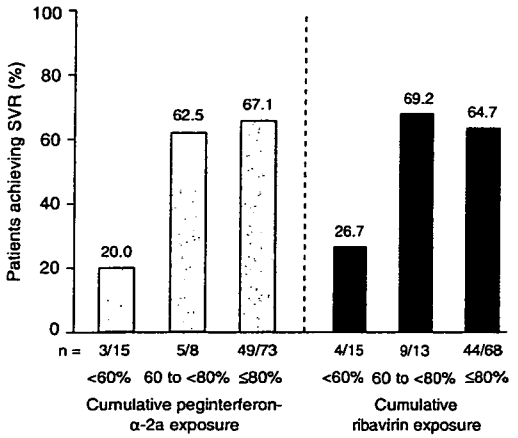

Fig. 3. Effects of peginterferon-α-2a and ribavirin exposure on sustained virological response (SVR). The cumulative exposure of patients to the study drug(s) was expressed as a percentage of the planned total dose.

severe fibrosis stage was reported in only 15.6% of patients. As a result, the small proportion of patients with severe fibrosis staging may have influenced the outcome of the current analysis.

Anaemia is a common adverse effect that can occur soon after the initiation of treatment with PEG IFN plus ribavirin for HCV infections. This complication can negatively impact patient quality of life, and is the most common reason for dose reductions and the temporary or permanent discontinuation of ribavirin. Such dose modifications have been shown to reduce the efficacy of treatment.[12] In general, females were predicted to have a higher likelihood of becoming anaemic than male patients.[13] In addition, the dose reduction rate of PEG IFN-α-2a and ribavirin is higher in elderly patients, which negatively impacts the achievement of an SVR.[5]

In a recent pooled analysis[14] of two phase III trials of 48 weeks of treatment with PEG IFN-α-2a plus ribavirin, the SVR rate was significantly reduced ($p = 0.0006$) in patients with a cumulative ribavirin dose of <60%. Prolonged periods of dose reduction, temporary interruptions or premature cessation of ribavirin were also associated with decreased SVR rates.

Previous studies have not assessed the impact of reducing the dose of PEG IFN independent of ribavirin, or differentiated between dose reduction, or interrupting or prematurely discontinuing treatment. An analysis of the HALT-C (Hepatitis C Antiviral Long-term Treatment against Cirrhosis) trial[15] investigated the impact of PEG IFN-α-2a and ribavirin dose reductions during the retreatment of patients infected with chronic HCV genotype 1 who did not respond to standard IFN with or without ribavirin treatment. A decrease in the cumulative dose of PEG IFN-α-2a received during the first 20 weeks of treatment (lead-in phase), from full dose (≥98%) to ≤60%, reduced the SVR rate from 17% to 5%. In contrast, reducing the dose of ribavirin from full dose to ≤60% did not affect the SVR rate as long as ribavirin administration was not interrupted for more than seven consecutive days. However, the premature discontinuation of ribavirin, even with full-dose PEG IFN-α-2a, reduced the SVR rate to 3%. This suggests that sufficient dosage during the early stages of therapy is required to achieve a high SVR rate with combination therapy. In our study, the SVR rate was also reduced in patients who received cumulative PEG IFN-α-2a and ribavirin doses of <60%, which was further decreased in patients who discontinued combination therapy. Therefore, it is important to alter the way adverse events of PEG IFN-α-2a and ribavirin therapy are managed to minimize the number of patients needing to reduce doses or discontinue therapy.

Conclusion

The attainment of an SVR following PEG IFN-α-2a plus ribavirin combination therapy was not influenced by any of the host-related factors evaluated in this analysis, although males aged ≥60 years tended to have a lower SVR rate. In contrast, younger age, male sex and lower baseline HCV RNA levels significantly increased the likelihood of achieving SVR with monotherapy. Dose reductions had a negative impact on SVR in elderly patients receiving combination therapy. Therefore, it is important to minimize PEG IFN-α-2a and ribavirin dose reductions by effectively managing treatment-related adverse events in elderly patients.

Acknowledgements

The authors are members of the Japanese PEG-IFN-α-2a plus ribavirin phase III study group. This study was funded by Chugai Pharmaceutical Co. Ltd, Japan, through the provision of drugs for the study. The retrospective analysis was supported by a grant from Chugai Pharmaceutical Co. Ltd. No other funding was received. The authors have no conflicts of interest that are directly relevant to the content of this study.

References

1. Tanaka Y, Hanada K, Mizokami M, et al. Inaugural article: a comparison of the molecular clock of hepatitis C virus in the United States and Japan predicts that hepatocellular carcinoma incidence in the United States will increase over the next two decades. Proc Natl Acad Sci USA 2002; 99: 15584-9
2. Hamada H, Yatsuhashi H, Yano M, et al. Impact of aging on the development of hepatocellular carcinoma in patients with post-transfusion chronic hepatitis C. Cancer 2002; 95: 331-9
3. Kuboki M, Iino S, Okuno T, et al. Peginterferon alpha-2a (40 KD) plus ribavirin for the treatment of chronic hepatitis C in Japanese patients. J Gastroenterol Hepatol 2007; 22: 645-52
4. Antonucci G, Longo MA, Angeletti C, et al. The effect of age on response to therapy with peginterferon alpha plus ribavirin in a cohort of patients with chronic HCV hepatitis including subjects older than 65 yr. Am J Gastroenterol 2007; 102: 1383-91
5. Iwasaki Y, Ikeda H, Araki Y, et al. Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. Hepatology 2006; 43: 54-63
6. Okamoto H, Sugiyama Y, Okada S, et al. Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. J Gen Virol 1992; 73: 673-9
7. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995; 22: 696-9
8. Dosmet VJ, Gerber M, Hoofnagle JH, et al. Classification of chronic hepatitis: diagnosis, grading, staging. Hepatology 1994; 19: 1515-20
9. Strader DB, Wright T, Thomas DL, et al. Diagnosis, management, and treatment of hepatitis C. Hepatology 2004; 39: 1147-71
10. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001; 358: 958-65
11. Lee SS, Heathcote EJ, Reddy KR, et al. Prognostic factors and early predictability of sustained viral response with peginterferon alfa-2a (40KD). J Hepatol 2002; 37: 500-6
12. McHutchison JG, Manns MP, Brown RS. Strategies for managing anemia in hepatitis C patients undergoing antiviral therapy. Am J Gastroenterol 2007; 102: 880-9
13. Snoeck E, Wade JR, Duff F, et al. Predicting sustained virological response and anaemia in chronic hepatitis C patients treated with peginterferon alfa-2a (40KD) plus ribavirin. Br J Clin Pharmacol 2006; 62: 699-709
14. Reddy KR, Shiffman ML, Morgan TR, et al. Impact of ribavirin dose reductions in hepatitis C virus genotype 1 patients completing peginterferon alfa-2a/ribavirin treatment. Clin Gastroenterol Hepatol 2007; 5: 124-9
15. Shiffman ML, Ghany MG, Morgan TR, et al. Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. Gastroenterology 2007; 132: 103-12

Correspondence: Dr *Gotaro Yamada*, Department of Internal Medicine, Kawasaki Medical School, Center for Liver Diseases, Kawasaki Hospital, Okayama City 2-1-80, Okayama, 700-0986, Japan.
E-mail: g.yamada@kawasaki-hp.jp

HEPATITIS

A large-scale, multicentre, double-blind trial of ursodeoxycholic acid in patients with chronic hepatitis C

Masao Omata, Haruhiko Yoshida, Joji Toyota, Eiichi Tomita, Shuhei Nishiguchi, Norio Hayashi, Shiro Iino, Isao Makino, Kiwamu Okita, Gotaro Toda, Kyuichi Tanikawa, Hiromitsu Kumada, for the Japanese C-Viral Hepatitis Network

Gut. doi: 10.1136/gut.2007.120956

See end of article for authors' affiliations

Correspondence to: Professor Masao Omata, Department of Gastroenterology, University of Tokyo Graduate School of Medicine, Hongo 7-3-1, Bunkyo, Tokyo 113-8655, Japan; omata-2im@h.u-tokyo.ac.jp

Revised 23 May 2007
Accepted 5 June 2007
Published Online First 20 June 2007

Background: Combined pegylated interferon and ribavirin has improved chronic hepatitis C (CH-C) therapy; however, sustained virological response is achieved in only about half of the patients with a 1b genotype infection. We assessed oral ursodeoxycholic acid (UDCA) on serum biomarkers as a possible treatment for interferon non-responders.

Methods: CH-C patients with elevated alanine aminotransferase (ALT) were assigned randomly to 150 (n=199), 600 (n=200) or 900 mg/day (n=197) UDCA intake for 24 weeks. Changes in ALT, aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGT) were assessed. This study is registered at ClinicalTrial.gov, identifier NCT00200343.

Results: ALT, AST and GGT decreased at week 4 and then remained constant during drug administration. The median changes (150, 600 and 900 mg/day, respectively) were: ALT, −15.3, −29.2 and −36.2%; AST, −13.6, −25.0 and −29.8%; GGT, −22.4, −41.0 and −50.0%. These biomarkers decreased significantly less in the 150 mg/day than in the other two groups. Although changes in ALT and AST did not differ between the 600 and 900 mg/day groups, GGT was significantly lower in the 900 mg/day group. In subgroup analysis, ALT decreased significantly in the 900 mg/day group when the baseline GGT exceeded 80 IU/l. Serum HCV-RNA did not change in any group. Adverse effects were reported by 19.1% of the patients, with no differences between groups.

Conclusions: A 600 mg/day UDCA dose was optimal to decrease ALT and AST levels in CH-C patients. The 900 mg/day dose decreased GGT levels further, and may be preferable in patients with prevailing biliary injuries.

Chronic hepatitis C (CH-C) is a common liver disease worldwide. The prevalence of hepatitis C virus (HCV) infection increased recently in several countries[1] and has now resulted in a growing incidence of HCV-related hepatocellular carcinomas.[2 3] Following the discovery of HCV, interferon therapy was established as the only treatment to eliminate the viral infection. The introduction of combination therapy with pegylated interferon and ribavirin has substantially enhanced the efficacy of antiviral therapy.[4 5] However, the HCV genotype 1b, the major genotype in Japan, is refractory even to this combination therapy and only shows sustained virological response rates of about 50%. Moreover, interferon therapy is sometimes contraindicated or stopped early due to haematological, psychological and other complications.

Ursodeoxycholic acid (UDCA) is a hydrophilic stereoisomer of chenodeoxycholic acid which was used first to dissolve cholesterol gallstones and recently to treat primary biliary cirrhosis.[6 7] In 1985, Leuschner *et al* reported a decrease in serum aminotransferase levels in patients with HBV-negative chronic hepatitis who were given UDCA for concomitant gallstones.[8] Traditional Chinese medicine uses ursine bile for liver diseases; it contains plentiful UDCA and inspired the chemical name. Semi-synthetic UDCA became commercially available in Japan in 1957 and has been used since then for chronic liver disease. In 1994, Takano *et al* reported a randomised, controlled-dose study of UDCA for CH-C: 57 patients were assigned randomly to take 150, 600 or 900 mg/day of UDCA and compared with 17 control patients.[9] The authors showed that serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGT) decreased less with 150 mg/day, the dose recommended by the Japanese national health insurance policy at that time, than with 600 or 900 mg/day, while the results with the latter two doses were similar. Although the effects of UDCA on fibrosis progression rates have not been established, the strong association between serum ALT levels and fibrosis progression rates has been well documented,[10 11] and it can be speculated that a decreased ALT level is associated with delayed fibrosis progression. Thus, the present study was conducted primarily as a dose-finding trial, using the changes in ALT levels as the primary endpoint.

PATIENTS AND METHODS

Patients

Patients with CH-C who were 20 years of age or older and tested positive for HCV-RNA or HCV core proteins were recruited as candidates for this study. They were observed for 8 weeks prior to administration of the drug, and those who showed ALT of 61 IU/l or higher in week −4 were enrolled. Patients were excluded from the study if they had received antiviral treatment (interferon with or without ribavirin) within 20 weeks before the observation period or were treated with corticosteroids, immunosuppressive drugs, glycyrrhizic acid, cholestyramine or other drugs that may affect liver function or interfere with UDCA metabolism. Patients were also excluded if they: i) had decompensated cirrhosis, viral hepatitis

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CH-C, chronic hepatitis C; GGT, gamma-glutamyl transpeptidase; HCV, hepatitis C virus; UDCA, ursodeoxycholic acid

other than hepatitis C, autoimmune liver disease, alcoholic or drug-induced liver injury, malignant tumour, biliary disorder, fulminant hepatitis or peptic ulcer; ii) required hospitalisation for cardiac, renal or pancreatic disease; iii) were pregnant or lactating; iv) alcohol dependent or drinking more than approximately 22 g/day alcohol; v) were participants in another clinical study within 4 weeks before the observation period; or vi) were sensitive to UDCA or other bile acid preparations.

The protocol was approved by the ethics committee of each institution participating in the study. Patients were informed of the details of the clinical study and agreed to participate. We conducted this clinical study in accordance with the Declaration of Helsinki and good clinical practice.

Study design

After the 8-week observation period patients were treated with oral (prandial) UDCA (Urso, Mitsubishi Pharma, Osaka, Japan) for 24 weeks at 150, 600 or 900 mg/day, divided into three doses, under double-blind conditions. Double blinding used placebo, 50 and 100 mg tablets identical in appearance to the test drug. The UDCA doses were established from a previous clinical study of UDCA in patients with CH-C.[9] Concomitant use of drugs and therapies included in the exclusion criteria were prohibited throughout the observation and treatment periods.

Changes in serum ALT levels were previously reported to be −26% and −25.5% with 600 and 900 mg/day of UDCA, respectively, compared to untreated controls and no significant changes were observed with 150 mg/day.[9] Based on these data, we assumed a standard deviation of 30% for per cent changes in ALT, and the necessary sample size was calculated to be 200 in each group to detect any superiority of the 600 and 900 mg/day doses over 150 mg/day at a significance level of 0.05 and a power of 0.9.

We enrolled patients who met all criteria and gave written informed consent between July 2002 and May 2004 in 62 institutions with liver clinics throughout Japan. Each patient was assigned randomly to one of the three dose groups by using numbered containers provided based on a permuted block method (block size: 6).

When treatment or evaluation was discontinued because of patient request, aggravation of symptoms, adverse events or other reasons, prior data were included in the evaluation as final observation data.

To investigate the long-term effects of UDCA, the protocol included an option for additional UDCA administration for a minimum of 28 weeks and a maximum of 80 weeks (total 52–104 weeks including the initial 24 weeks) if the ALT level had decreased by at least 15% at week 20 compared to the baseline. In the additional period, the double-blind setting was discontinued and the dose of 600 mg/day was adopted, which could be increased to 900 mg/day by the decision of each patient and the physician responsible. Patients who entered the additional phase could discontinue UDCA administration anytime after week 52.

Laboratory tests

Blood was collected every 4 weeks from the start of the observation period to the end of drug administration. Serum ALT was measured as a primary endpoint of liver function, and AST and GGT as secondary endpoints, using conventional methods. Blood samples taken at the start of observation, at 0, 4 and 12 weeks of treatment, and at the final observation were analysed to determine leukocyte and erythrocyte counts, haemoglobin, haematocrit, thrombocyte count, and the levels of ALT, AST, GGT, alkaline phosphatase, lactate dehydrogenase, total protein, albumin, cholinesterase, total bilirubin, direct bilirubin, total cholesterol, urea nitrogen, creatinine, Na, K and Cl.

For bile acid composition analysis, blood was collected at the start of treatment and at the final observation in a fasted condition. Serum total bile acid was measured by the 3α-hydroxysteroid dehydrogenase method. Bile acid fractions were determined by a specific liquid chromatography-electrospray mass spectrometry, using an HPLC system (Agilent 1100 series, Agilent Technologies, CA, USA) equipped with a C18 cartridge (CAPCELL PAK C18 UG120A, Shiseido, Tokyo, Japan) and a mass spectrometer (Quattro Ultima, Micromass Technologies, Manchester, UK).

Serum HCV-RNA level was measured prior to treatment and at the final observation by a reverse transcriptional polymerase-chain-reaction method.

All analyses and measurements were performed in a single contract laboratory (SRL, Tokyo, Japan).

Statistical analysis

Patients' backgrounds were compared among the three dose groups by χ^2 test and ANOVA. Changes in serum ALT, AST and GGT levels due to UDCA administration were compared among the groups by repeated-measure ANOVA. Differences between groups were tested by using linear contrasts. Subgroup analyses of median changes in serum ALT at the final observation, relative to the pre-treatment levels, were performed according to gender, body weight and pre-treatment serum GGT level with Wilcoxon signed-ranks tests. Changes in bile acid and serum HCV-RNA levels were analysed by paired Student's t test. Fischer's exact probability test was applied to the incidences of adverse reactions. A p value <0.05 in a two-tailed test was considered significant. Analyses were done on the full analysis set. This study is registered at ClinicalTrial.gov, number NCT00200343, and is compliant with the published CONSORT guidelines for performance and publication of clinical trials.[12]

RESULTS

Patients

We enrolled 596 patients; 199 received UDCA at 150 mg/day, 200 at 600 mg/day, and 197 at 900 mg/day. Safety was evaluated in all patients as adverse events based on signs and symptoms and abnormal laboratory test results. Efficacy was evaluated in 586 patients (195, 150 mg/day; 198, 600 mg/day; and 193 at 900 mg/day), excluding 10 who lacked sufficient data. At the end of 24 weeks' administration, 392 patients were eligible for additional long-term administration. Of these patients, 280 chose to participate in the study and others refused mainly because of lack of time. Twenty three patients discontinued before week 52, one of them for biochemical relapse, and other 10 patients violated protocol. The effects of long-term administration were evaluated among the remaining 247 patients (fig 1).

Patients' backgrounds are summarised in table 1. Differences observed in gender, body weight and history of treatment with interferon between the three groups are indicated ($p<0.15$).

Changes in ALT, AST and GGT

Serum ALT, AST and GGT levels before and during treatment are shown in figs 2–4. The responses of ALT, AST and GGT over time were greater for 600 and 900 mg/day administration compared to 150 mg/day (ALT, $p<0.001$ and $p=0.021$; AST, $p<0.001$ and $p<0.001$; GGT, $p<0.001$ and $p<0.001$, respectively). No difference was observed between the 600 and 900 mg/day groups in ALT ($p=0.926$) or AST ($p=0.429$), but GGT differed significantly ($p<0.001$). Serum ALT, AST and GGT levels decreased by 4 weeks into treatment and remained

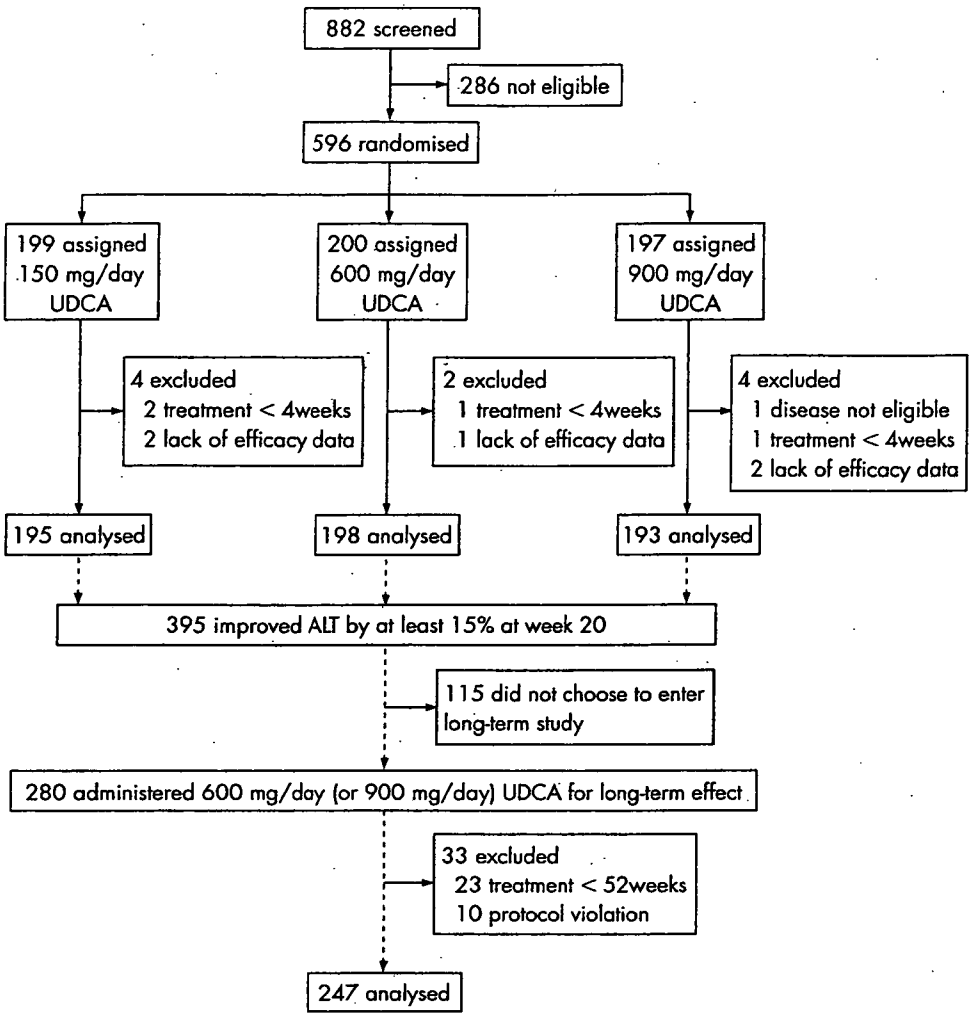

Figure 1 Trial profile.

constant. Serum ALT, AST and GGT levels at the final observation, together with median changes relative to 0 week (baseline), are shown in table 2. The mean decreases in serum ALT levels from the baseline value were 13.4, 30.6 and 29.3 IU/l in the 150, 600 and 900 mg/day groups, respectively. The median changes in ALT at the final observation were −15.3%, −29.2% and −36.2% in the corresponding groups (table 2).

Table 1 Characteristics of patients with chronic hepatitis C treated with UDCA (full analysis set)

	150 mg/day (n=195)	600 mg/day (n=198)	900 mg/day (n=193)	p Value
Gender				
Male	97 (49.7%)	117 (59.1%)	123 (63.7%)	0.018
Female	98 (50.3%)	81 (40.9%)	70 (36.3%)	
Age (years)	58.0±12.2	57.7±12.0	59.8±10.1	0.152
Height (cm)	160.1±9.5	161.9±9.2	160.8±8.7	0.163
Weight (kg)	58.8±11.4	61.8±11.2	61.6±11.9	0.017
ALT (IU/l)	109.2±49.7	106.3±59.4	110.6±57.3	0.745
AST (IU/l)	84.0±39.1	82.4±41.8	85.2±45.0	0.796
GGT (IU/l)	87.5±73.0	82.4±62.2	85.9±66.3	0.744
Interferon*				
Absent	119 (61.0%)	100 (50.5%)	96 (49.7%)	0.044
Present	76 (39.0%)	98 (49.5%)	97 (50.3%)	

Data represent the number of patients or mean±SD.
*Previous interferon treatment.

The mean decreases in serum AST levels from the baseline value were 8.5, 19.3 and 19.7 IU/l in the 150, 600 and 900 mg/day groups, respectively. The mean decreases in serum GGT levels from the baseline value were 17.1, 32.7 and 42.1 IU/l in the 150, 600 and 900 mg/day groups, respectively.

Long-term effects

The decreases in ALT, AST, GGT levels from the baseline value were maintained during long-term administration of UDCA, as shown in table 3.

Subgroup analyses

The decrease in serum ALT was significantly greater in the 600 and 900 mg/day groups than in the 150 mg/day group for most subgroups by gender, body weight or baseline serum GGT levels (table 4). Although the difference between the 600 and 900 mg/day groups as a whole was not significant, the subgroup of baseline GGT≥80 IU/l showed a significantly lower level of GGT with 900 mg/day administration ($p = 0.004$).

Bile acid in serum

Total bile acid concentration in serum increased in a dose-dependent manner from the start of drug administration to the final observation, as shown in table 5. The ratio of UDCA to total bile acid was increased significantly in all groups at the final observation compared to baseline. The ratio of UDCA at the final observation was similar in the 600 and 900 mg/day groups. The proportion of less hydrophilic bile acids was

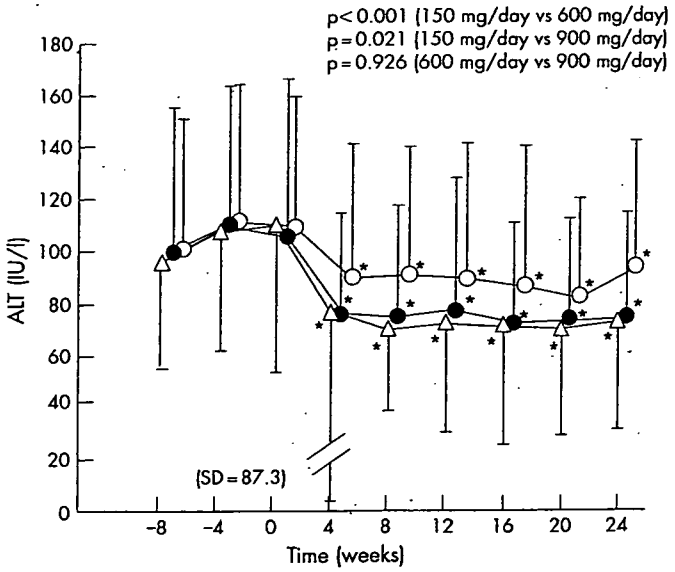

Figure 2 Changes in serum ALT levels in patients with chronic hepatitis C before and during the treatment period. Data are expressed as mean ± SD. Open circles, 150 mg/day; filled circles, 600 mg/day; open triangles, 900 mg/day; *p<0.01, paired t test (vs week 0). The p values refer to repeated measures ANOVA.

decreased accordingly. The proportion of chenodeoxycholic acid at the final observation was decreased significantly in all groups, and was similar in the 600 and 900 mg/day groups. The proportions of cholic acid and deoxycholic acid were also decreased significantly compared to baseline.

Virus load

HCV-RNA levels (mean ± SD) changed from the baseline of 1477 ± 1280 to 1366 ± 1224 kIU/ml in the 150 mg/day group, from 1463 ± 1299 to 1358 ± 1233 kIU/ml in the 600 mg/day group, and from 1553 ± 1318 to 1552 ± 1398 kIU/ml in the 900 mg/day group. None of these changes was significant.

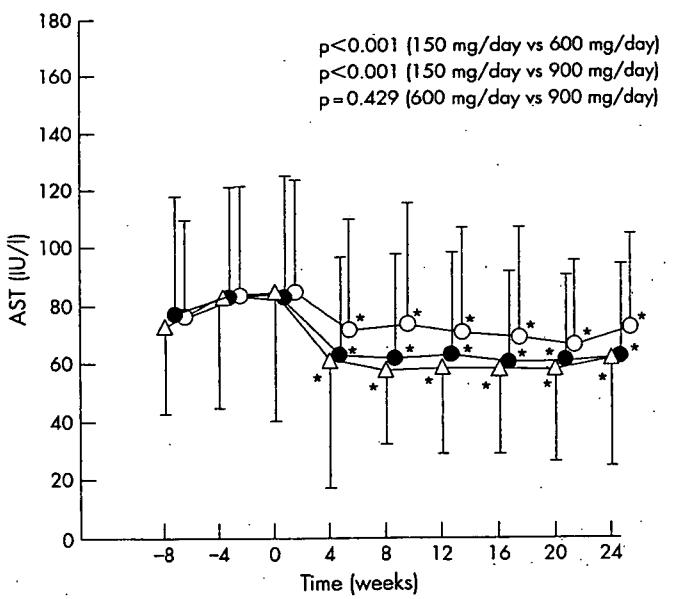

Figure 3 Changes in serum AST levels in patients with chronic hepatitis C before and during the treatment period. Data are expressed as mean ± SD. Open circles, 150 mg/day; filled circles, 600 mg/day; open triangles, 900 mg/day; *p<0.01, paired t test (vs week 0). The p values refer to repeated measures ANOVA.

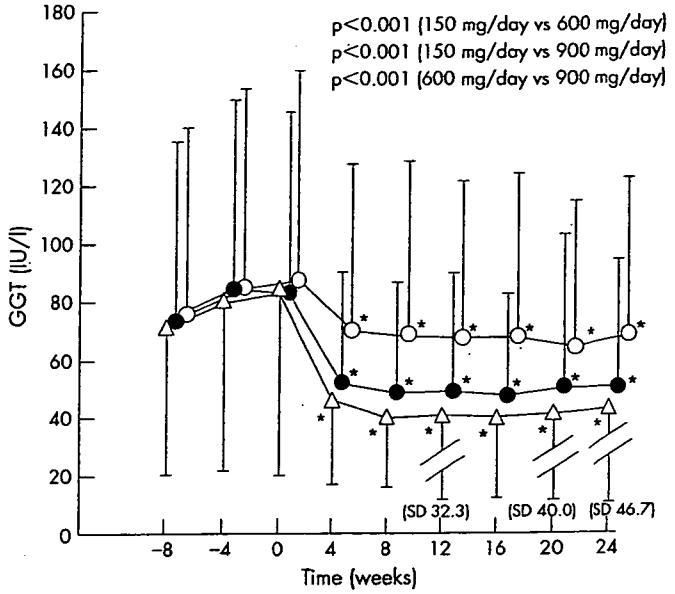

Figure 4 Changes in serum GGT levels in patients with chronic hepatitis C before and during the treatment period. Data are expressed as mean ± SD. Open circles, 150 mg/day; filled circles, 600 mg/day; open triangles, 900 mg/day; *p<0.01, paired t test (vs week 0). The p values refer to repeated measures ANOVA.

Safety

The observed adverse reactions possibly associated with UDCA administration are shown in table 6. The overall incidences of adverse reactions were 18.1%, 21.5% and 17.8% in the 150, 600 and 900 mg/day groups, respectively, with no significant difference between the groups. Diarrhoea was reported most often. No severe adverse reactions were seen.

DISCUSSION

UDCA is frequently used for cholestatic liver diseases, primary biliary cirrhosis in particular. UDCA improves biochemical indices such as serum GGT, ALT and bilirubin. Histopathological improvements have been shown[13] and prolonged survival reported.[14 15] Although its effect on survival remains controversial,[16 17] UDCA is the only approved medication for primary biliary cirrhosis. Suggested mechanisms for UDCA include reducing the cytotoxicity of hydrophobic bile acids, stimulating hepatobiliary secretion and anti-apoptosis.[18]

UDCA was used to decrease serum aminotransferase levels for so-called non-A non-B chronic hepatitis before the discovery of HCV.[8 19 20] Takano *et al* restricted their study to patients with CH-C and found the optimal dose of UDCA to be 600 mg/day.[9] There was a greater reduction in GGT (40.5%) than in ALT (26.0%), as also observed in the current study. The reported effect of UDCA was stronger among CH-C patients with morphological bile duct injury,[21] and UDCA administration was accompanied by histological improvement of biliary lesions but not of hepatitis.[22] These data suggest that UDCA may act on the biliary system in CH-C through enhanced bile formation and/or modification of bile acid composition. In fact, bile duct injury is characteristic of CH-C, although not specific.[23] In this study, the changes in bile acid composition were similar in the 600 and 900 mg/day groups but smaller in the 150 mg/day group, and this may have been associated with the changes in serum biomarkers.

Nakamura *et al* reported that UDCA had a greater effect in CH-C patients with autoimmune characteristics, that is high immunoglobulin G concentration or positive anti-nuclear or anti-smooth muscle antibodies,[24] which suggests involvement

Table 2 Serum ALT, AST and GGT levels in patients with chronic hepatitis C after treatment with UDCA

	Dose (mg/day)	Pre-treatment, mean±SD	Post-treatment, mean±SD	Change (%), median (range)
ALT (IU/l)	150	109.2±49.7	95.8±60.2	−15.3 (−80.7 to +375.9)
	600	106.3±59.4	75.7±41.9	**−29.2 (−88.3 to +95.2)**
	900	110.6±57.3	81.3±90.5	−36.2 (−81.4 to +1696.9)
AST (IU/l)	150	84.0±39.1	75.5±43.6	−13.6 (−74.2 to +347.2)
	600	82.4±41.8	63.1±32.9	−25.0 (−82.7 to +72.5)
	900	85.2±45.0	65.5±49.6	−29.8 (−79.0 to +1026.1)
GGT (IU/l)	150	87.5±73.0	70.4±58.3	−22.4 (−74.6 to +145.9)
	600	82.4±62.2	49.7±43.0	−41.0 (−81.1 to +153.1)
	900	85.9±66.3	43.8±44.8	−50.0 (−80.1 to +213.9)

Table 3 Serum ALT, AST and GGT levels in patients with chronic hepatitis C during long-term administration of UDCA

	Pre-treatment	Treatment period		
	Week 0	Week 24	Week 48	Week 104
Patients (n)	247	242*	243†	149‡
ALT (IU/l)	114.8±54.1	70.7±37.4	67.9±36.3	63.5±31.9
AST (IU/l)	86.6±41.7	59.0±31.5	56.6±27.4	54.1±23.7
GGT (IU/l)	87.3±67.6	49.5±42.6	47.3±40.5	41.8±30.1

Data are expressed as mean±SD.
*Corresponding data missing in five patients; †corresponding data missing in four patients; ‡administration between week 52 and week 104 was optional and 149 patients opted for the maximum term.

of immunomodulatory mechanisms. Indeed, studies in vitro have shown that UDCA suppresses NF-κB-dependent transcription by binding to the glucocorticoid receptor[25] and decreases proinflammatory cytokine-induced transcription of phospholipase A2.[26] These mechanisms may act cytoprotectively in vivo. The choleretic and cytoprotective mechanisms are not necessarily mutually exclusive.

We examined the effect of UDCA on CH-C in terms of serum biochemical markers in a large-scale, double-blind investigation. We confirmed that a dose of 600 mg/day, that is 10 mg/kg body weight on average, was more effective than 150 mg/day, while adverse effects remained similar and minimal. The doses of 600 and 900 mg/day induced similar decreases in serum ALT and AST. Consequently, it appears that 600 mg/day is the preferred dose of UDCA, assuming that serum transaminase levels reflect the degree of hepatocellular damage.

The decrease in serum GGT differed significantly between the 600 and 900 mg/day groups. In contrast to the decrease in ALT or AST, that of serum GGT may represent improved cholestasis from biliary injury in CH-C. Although the importance of biliary injury in CH-C is unclear, it is possible that a 900 mg/day dose has additional benefits compared to 600 mg/day, as the incidence of adverse effects did not differ between the two doses. It is of interest that the decrease in ALT was significantly different between the two doses in patients with high baseline GGT levels (table 4).

The long-term effects of UDCA therapy in CH-C patients are yet to be elucidated. Changes in liver histology following UDCA administration are not evident from short-term observation. However, it is possible that delayed progression of fibrosis by UDCA can be revealed only by much longer-term observation,

Table 4 Subgroup analyses of change in serum ALT in patients with chronic hepatitis C after treatment with UDCA

	Dose (mg/day)	No. of patients	Change (%), median (range)	p Value vs 150 mg	p Value vs 600 mg
Gender					
Male	150	97	−14.9 (−80.7 to +375.9)		
	600	117	−33.1 (−88.3 to +93.1)	<0.001	
	900	123	−36.4 (−79.1 to +1696.9)	<0.001	0.430
Female	150	98	−18.0 (−79.0 to +175)		
	600	81	−25.0 (−74.7 to +95.2)	0.058	
	900	70	−35.8 (−81.4 to +315.3)	0.002	0.076
Body weight (kg)					
<60	150	115	−14.9 (−80.7 to +375.9)		
	600	82	−28.6 (−74.7 to +95.2)	0.002	
	900	91	−35.2 (−81.4 to +315.3)	0.001	0.356
≥60	150	80	−16.7 (−73.4 to +166.1)		
	600	116	−30.3 (−88.3 to +93.1)	0.003	
	900	102	−36.6 (−77.1 to +1696.9)	<0.001	0.096
GGT (IU/l)					
≤39	150	45	−14.5 (−73.4 to +71.4)		
	600	39	−32.7 (−62.9 to +93.1)	0.049	
	900	45	−26.6 (−81.4 to +1696.9)	0.112	0.616
40–79	150	79	−15.2 (−69.1 to +175)		
	600	90	−30.3 (−74.7 to +95.2)	0.001	
	900	70	−36.3 (−77.7 to +200)	<0.001	0.633
≥80	150	71	−18.2 (−80.7 to +375.9)		
	600	69	−28.6 (−88.3 to +53.8)	0.057	
	900	78	−41.2 (−79.1 to +119.3)	<0.001	0.004

The p values refer to Wilcoxon signed-ranks tests.

Table 5 Composition of serum bile acid in patients with chronic hepatitis C treated with UDCA

	Dose (mg/day)	Before treatment	After treatment	p Value
Total bile acid concentration (μmol/l)	150	8.63±9.76	13.69±19.28	<0.001
	600	9.42±12.04	21.89±24.20	<0.001
	900	9.17±9.30	28.74±39.78	<0.001
Cholic acid (%)	150	17.69±10.33	11.35±7.08	<0.001
	600	17.75±10.35	5.93±4.53	<0.001
	900	18.15±9.54	5.14±4.19	<0.001
Deoxycholic acid (%)	150	21.62±16.24	13.84±11.39	<0.001
	600	19.86±16.84	6.50±7.06	<0.001
	900	18.74±15.29	5.68±6.58	<0.001
Chenodeoxycholic acid (%)	150	54.46±14.12	39.93±11.61	<0.001
	600	55.37±13.95	24.66±10.01	<0.001
	900	55.95±13.65	23.31±12.72	<0.001
Ursodeoxycholic acid (%)	150	5.93±8.72	34.25±13.75	<0.001
	600	6.70±9.72	62.26±13.69	<0.001
	900	6.83±10.6	65.12±16.84	<0.001
Lithocholic acid (%)	150	0.30±0.99	0.62±1.66	0.010
	600	0.33±1.23	0.66±1.35	0.010
	900	0.33±1.12	0.75±1.49	0.001

Data are expressed as mean±SD. The p values refer to paired t test (before vs after treatment).

because the natural progression of fibrosis in CH-C is usually slow, taking decades to establish cirrhosis.[27 28] The effect of UDCA lasted for at least 104 weeks without attenuation (table 3).

In the natural course of CH-C, those patients with normal serum aminotransferase levels show slow fibrosis progression[29] and a low incidence of hepatocellular carcinoma.[30 31] By multivariate analysis, the risk of hepatocellular carcinoma after interferon treatment without virological response was shown to be 0.26, 0.36 and 0.91 in patients whose ALT levels were normal, moderately elevated (less than twice the upper normal limit) and highly elevated, respectively, compared to untreated patients. It may be that when UDCA lowers serum ALT levels the risk of hepatocellular carcinoma is decreased. A retrospective study showed that hepatocellular carcinoma developed within 5 years from the onset of HCV-related early cirrhosis in 10 of 56 patients (18%) who took UDCA and 18 of 46 patients (39%) who did not.[32] Interestingly, ALT levels were similar in the two groups, possibly because UDCA was likely to be prescribed to those patients with high baseline ALT levels. Although these data were obtained from a non-randomised, retrospective study, they suggest that UDCA may provide cancer protective effects independent of decreasing ALT.

In summary, we confirmed, in a large-scale, double-blind study, that a UDCA dose of 600 mg/day was optimal to decrease serum ALT and AST levels in CH-C patients without serious adverse effects. A dose of 900 mg/day resulted in additional decreases in serum GGT levels, and may be preferred in patients with prevailing biliary injuries. The long-term effects of UDCA administration on prognosis, hepatocarcinogenesis in particular, remain to be investigated in future studies.

Table 6 Summary of adverse reactions

	150 mg/day	600 mg/day	900 mg/day
Overall incidence	18.1% (36/199)	21.5% (43/200)	17.8% (35/197)
Total adverse reactions, n	44	62	45
Common adverse reactions, n (%)*			
Abdominal distension	2 (1.0)	2 (1.0)	2 (1.0)
Upper abdominal pain	2 (1.0)	4 (2.0)	2 (1.0)
Constipation	3 (1.5)	4 (2.0)	2 (1.0)
Diarrhoea	7 (3.5)	8 (4.0)	8 (4.1)
Dyspepsia	3 (1.5)	2 (1.0)	2 (1.0)
Loose stool	1 (0.5)	6 (3.0)	5 (2.5)
Stomach discomfort	2 (1.0)	2 (1.0)	3 (1.5)
Pruritus	3 (1.5)	3 (1.5)	2 (1.0)

*The adverse reactions which were observed in 1% or more of the patients.

ACKNOWLEDGEMENTS

Investigators who participated in this study are as follows (listed in alphabetical order): Y Aizawa (Jikei University, Aoto Hospital), K Chayama (Hiroshima University), M Daikoku (National Hospital Organization Nagasaki Medical Center), K Dohmen (Okabe Hospital), K Egashira (Sakura Hospital), K Fujimura (Nara Social Insurance Hospital), K Fujise (Jikei University, Kashiwa Hospital), E Harada (National Hospital Organization Tokyo National Hospital), K Hayashi (University of Miyazaki), N Hayashi (Osaka University), K Hino (Delta Clinic), M Hirano (Tokyo Metropolitan Police Hospital), M Honda (Kanazawa University), N Horiike (Ehime University), H Ikematsu (Haradoi Hospital), Y Imai (Ikeda Municipal Hospital), F Imazeki (Chiba University), D Ito (Osaka Saiseikai Nakatsu Hospital), S Kakumu (Aichi Medical University), Y Katano (Nagoya University), M Kato (National Hospital Organization Osaka National Hospital), M Kawaguchi (Okayama Saiseikai General Hospital), T Kawanishi (Inazumi Park Hospital), S Kawata (Yamagata University), Y Kishimoto (San-in Rosai Hospital), M Kudo (Kinki University), H Kumada (Toranomon Hospital), T Kumada (Oogaki Municipal Hospital), M Matsumura (The Institute for Adult Diseases, Asahi Life Foundation), Y Matsuzaki (University of Tsukuba), H Moriwaki (Gifu University), Y Murawaki (Tottori University), I Nakamura (Jichi Medical University, Omiya Medical Center), K Nakamura (Asahikawa Medical College), R Nakata (Japanese Red Cross Medical Center), S Nishiguchi (Osaka City University), S Onishi (Kochi University), Y Osaki (Osaka Red Cross Hospital), H Saito (Keio University), I Sakaida (Yamaguchi University), S Sakisaka (Fukuoka University), Y Sasaki (Kumamoto University), M Sata (Kurume University), A Sato (St. Marianna University, Yokohama City-Seibu Hospital), M Suzuki (St. Marianna University), K Tachi (Kamiiida Hospital), K Tagawa (Mitsui Memorial Hospital), I Takagi (Jikei University, Third Hospital), A Takaki (Okayama University), Y Takei (Juntendo University), E Tanaka (Shinshu University), J Tazawa (Tsuchiura Kyodo General Hospital), K Togawa (Kawasaki Medical University), E Tomita (Gifu Municipal Hospital), J Toyota (Sapporo Kosei General Hospital), A Ueda (Miyazaki Prefectural Miyazaki Hospital), S Watanabe (Akita University), K Yasuda (Kiyokawa Hospital), T Yamanaka (Itabashi Central Hospital), J Yamao (Nara Medical University), H Yoshida (Yame General Hospital), K Yoshioka (Nagoya University), M Zeniya (Jikei University).

Competing interests: Declared (the declaration can be viewed on the *Gut* website at http://www.gutjnl.com/supplemental).

Authors' affiliations

Masao Omata, Haruhiko Yoshida, Department of Gastroenterology, University of Tokyo Graduate School of Medicine, Tokyo, Japan
Joji Toyota, Department of Gastroenterology, Sapporo Kosei General Hospital, Hokkaido, Japan
Eiichi Tomita, Department of Gastroenterology, Gifu Municipal Hospital, Gifu, Japan
Shuhei Nishiguchi, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan
Norio Hayashi, Department of Molecular Therapeutics, Osaka University Graduate School of Medicine, Osaka, Japan
Shiro Iino, Seizankai Kiyokawa Hospital, Tokyo, Japan
Isao Makino, Hokushinkai Megumino Hospitals, Hokkaido, Japan
Kiwamu Okita, Social Insurance Shimonoseki Kosei Hospital, Yamaguchi, Japan
Gotaro Toda, Sempo Tokyo Takanawa Hospital, Tokyo, Japan
Kyuichi Tanikawa, International Institute for Liver Research, Fukuoka, Japan
Hiromitsu Kumada, Department of Gastroenterology, Toranomon Hospital, Tokyo, Japan

REFERENCES

1 **Wasley A,** Alter MJ. Epidemiology of hepatitis C: geographic differences and temporal trends. *Semin Liver Dis* 2000;**20**:1–16.
2 **El-Serag HB,** Davila JA, Petersen NJ, *et al.* The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. *Ann Intern Med* 2003;**139**:817–23.
3 **Yoshizawa H.** Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future. *Oncology* 2002;**62**(Suppl 1):8–17.
4 **Manns MP,** McHutchison JG, Gordon SC, *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001;**358**:958–65.
5 **Fried MW,** Shiffman ML, Reddy KR, *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002;**347**:975–82.
6 **Poupon R,** Chretien Y, Poupon RE, *et al.* Is ursodeoxycholic acid an effective treatment for primary biliary cirrhosis? *Lancet* 1987;1:834–6.
7 **Leuschner U,** Fischer H, Kurtz W, *et al.* Ursodeoxycholic acid in primary biliary cirrhosis: results of a controlled double-blind trial. *Gastroenterology* 1989;**97**:1268–74.
8 **Leuschner U,** Leuschner M, Sieratzki J, *et al.* Gallstone dissolution with ursodeoxycholic acid in patients with chronic active hepatitis and two years follow-up. A pilot study. *Dig Dis Sci* 1985;**30**:642–9.
9 **Takano S,** Ito Y, Yokosuka O, *et al.* A multicenter randomized controlled dose study of ursodeoxycholic acid for chronic hepatitis C. *Hepatology* 1994;**20**:558–64.
10 **Marcellin P,** Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. *Hepatology* 2002;**36**:S47–S56.
11 **Ghany MG,** Kleiner DE, Alter H, *et al.* Progression of fibrosis in chronic hepatitis C. *Gastroenterology* 2003;**124**:97–104.
12 **Moher D,** Schulz KF, Altman DG, for the CONSORT Group. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. *Lancet* 2001;**357**:1191–4.
13 **Degott C,** Zafrani ES, Callard P, *et al.* Histopathological study of primary biliary cirrhosis and the effect of ursodeoxycholic acid treatment on histology progression. *Hepatology* 1999;**29**:1007–12.
14 **Poupon RE,** Poupon R, Balkau B, The UDCA-PBC Study Group. Ursodiol for the long-term treatment of primary biliary cirrhosis. *N Engl J Med* 1994;**330**:1342–7.
15 **Lindor KD,** Therneau TM, Jorgensen RA, *et al.* Effects of ursodeoxycholic acid on survival in patients with primary biliary cirrhosis. *Gastroenterology* 1996;**110**:1515–18.
16 **Poupon RE,** Lindor KD, Cauch-Dudek K, *et al.* Combined analysis of randomized controlled trials of ursodeoxycholic acid in primary biliary cirrhosis. *Gastroenterology* 1997;**113**:884–90.
17 **Goulis J,** Leandro G, Burroughs AK. Randomised controlled trials of ursodeoxycholic-acid therapy for primary biliary cirrhosis: a meta-analysis. *Lancet* 1999;**354**:1053–60.
18 **Paumgartner G,** Beuers U. Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. *Hepatology* 2002;**36**:525–31.
19 **Osuga T,** Tanaka N, Matsuzaki Y, *et al.* Effect of ursodeoxycholic acid in chronic hepatitis and primary biliary cirrhosis. *Dig Dis Sci* 1989;**34**(12 Suppl):49S–51S.
20 **Podda M,** Ghezzi C, Battezzati PM, *et al.* Effects of ursodeoxycholic acid and taurine on serum liver enzymes and bile acids in chronic hepatitis. *Gastroenterology* 1990;**98**:1044–50.
21 **Kiso S,** Kawata S, Imai Y, *et al.* Efficacy of ursodeoxycholic acid therapy in chronic viral hepatitis C with high serum gamma-glutamyltranspeptidase levels. *J Gastroenterol* 1996;**31**:75–80.
22 **Attili AF,** Rusticali A, Varriale M, *et al.* The effect of ursodeoxycholic acid on serum enzymes and liver histology in patients with chronic active hepatitis. A 12-month double-blind, placebo-controlled trial. *J Hepatol* 1994;**20**:315–20.
23 **Goodman ZD,** Ishak KG. Histopathology of hepatitis C virus infection. *Semin Liver Dis* 1995;**15**:70–81.
24 **Nakamura K,** Yoneda M, Takamoto S, *et al.* Effect of ursodeoxycholic acid on autoimmune-associated chronic hepatitis C. *J Gastroenterol Hepatol* 1999;**14**:413–18.
25 **Miura T,** Ouchida R, Yoshikawa N, *et al.* Functional modulation of the glucocorticoid receptor and suppression of NF-kappaB-dependent transcription by ursodeoxycholic acid. *J Biol Chem* 2001;**276**:47371–8.
26 **Ikegami T,** Matsuzaki Y, Fukushima S, *et al.* Suppressive effect of ursodeoxycholic acid on type IIA phospholipase A2 expression in HepG2 cells. *Hepatology* 2005;**41**:896–905.
27 **Poynard T,** Bedossa P, Opolon P, OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Natural history of liver fibrosis progression in patients with chronic hepatitis C. *Lancet* 1997;**349**:825–32.
28 **Shiratori Y,** Imazeki F, Moriyama M, *et al.* Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann Intern Med* 2000;**132**:517–24.
29 **Mathurin P,** Moussalli J, Cadranel JF, *et al.* Slow progression rate of fibrosis in hepatitis C virus patients with persistently normal alanine transaminase activity. *Hepatology* 1998;**27**:868–72.
30 **Yoshida H,** Shiratori Y, Moriyama M, *et al.* IHIT Study Group. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. Inhibition of hepatocarcinogenesis by interferon therapy. *Ann Intern Med* 1999;**131**:174–81.
31 **Tarao K,** Rino Y, Ohkawa S, *et al.* Sustained low alanine aminotransferase levels can predict the survival for 10 years without hepatocellular carcinoma development in patients with hepatitis C virus-associated liver cirrhosis of child stage A. *Intervirology* 2004;**47**:65–71.
32 **Tarao K,** Fujiyama S, Ohkawa S, *et al.* Ursodiol use is possibly associated with lower incidence of hepatocellular carcinoma in hepatitis C virus-associated liver cirrhosis. *Cancer Epidemiol Biomarkers Prev* 2005;**14**:164–9.

ORIGINAL ARTICLE

Ningen Dock 2007 ; **21** : 47－49

Carotid Ultrasonography in General Health Screening : Non-Invasive Assessment of Early Atherosclerosis

Nobukazu Ishizaka

As carotid ultrasonography is an easy, immediate, and non-invasive diagnosing modality that does not involve radiation exposure, it has gained considerable recognition for use in the screening of carotid atherosclerosis even in asymptomatic subjects. Carotid plaque may be defined when the maximum of the IMT measured at the several arterial segments surpasses a certain cut-off value, and, although not always, has a focal protrusion. The size, number, surface morphology, and echogenicity of such carotid plaque may provide useful information for estimating the likelihood of future ischemic cerebrovascular events. Although significant luminal narrowing (≥70% stenosis) of carotid arteries that may increase the incidence of stroke will rarely be encountered in the setting of Ningen Dock, the presence of carotid atherosclerosis may also indicate an increased likelihood of the presence of coronary artery stenosis or future cardiac events. In addition, visualization of the presence of plaque may also increase the subjects' motivation for making lifestyle modifications, such as smoking cessation. Due to its non-invasive nature, carotid ultrasonography has become reasonable and feasible modality for the diagnosis of early atherosclerosis in the setting of general health screening or Ningen Dock. (*Ningen Dock* 2007 ; **21** : 47－49)

Key Words : ultrasonography, intima-media thickness (IMT), screening, ningen dock

Duplex carotid ultrasonography, which enables the observation of both B mode ultrasonography and Doppler mode ultrasonography, has gained considerable recognition for use in the screening of carotid atherosclerosis. Because of its non-invasive nature, this method is appropriate for use on asymptomatic subjects, such as those who undergo general health screening or Ningen Dock. Although the exact screening region of the carotid artery that is screened may differ according to the institute, it usually includes the common carotid artery and bilateral internal carotid artery. We can also get images of vertebral arteries by ultrasonography, although some of these arteries may be obscured by vertebral bones. Ultrasonography can observe three layer structure, and the first high echoic layer and the second low echoic layer are collectively considered to represent the intima-media complex (**Fig. 1**). Carotid IMT increases with age and is larger in men than in women[1].

Diagnosis of Carotid Atherosclerosis

Carotid atherosclerosis may be diagnosed when there is a plaque or a thickening of the intima-media complex within the internal carotid arteries, bifurcations, and common carotid arteries[2]. When the thickness of the intima-media complex, commonly referred to as the IMT, is greater than a certain cut-off point, the subjects is said to have intima-media thickening. The cut-off value for diagnosing intima-media thickening may vary slightly across different studies.

Plaque may be defined when the maximum of the IMT measured at the several arterial segments surpasses a certain cut-off value, and, although not always, has a focal protrusion. Here again, cut-off values may differ slightly according to the study[3-9]. In Japan, guidelines for diagnosing carotid plaque have been advocated by several committees and study groups, including the Joint Committee on Guidelines for Management Stroke, the Japan Academy of Neurosonology (http : //wwwsoc.nii.ac.jp/jan/index.html), and the Society for the Study of Early Atherosclerosis (http : //www.imt-ca.com/).

In addition to the plaque size, the number of plaques, surface morphology, and echogenicity may provide useful information for estimating the likelihood of future ischemic cerebrovascular events[10]. Surface morphology may be classified as smooth, irregular or ulcerated. Heterogeneous plaque is considered to be at higher risk of rupture than homogenous plaque. Plaque may be categorized according to its density (hypodense, isodense, hyperdense, or calcified)[5,11]. Calcification within the plaque will make an acoustic shadow.

Screening for High-Grade Carotid Stenosis

Although significant luminal narrowing (≥70% stenosis) of carotid arteries increases the incidence of stroke, such high-grade carotid stenosis, which may require surgical or catheter intervention, will rarely be encountered in the setting of general health screening. Even when moderate-grade carotid stenosis is found in

From the Departments of Cardiovascular Medicine and Gastroenterology, University of Tokyo Graduate School of Medicine.
Address for Reprints : Nobukazu Ishizaka, Department of Cardiovascular Medicine, University of Tokyo Graduate School of Medicine, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Tel. +81-3-3815-5411 (ext. 37156) ; Fax. +81-3-5842-5586 ; E-mail. nobuishizka-tky@umin.ac.jp
Received December 16, 2006 ; Accepted February 2, 2007

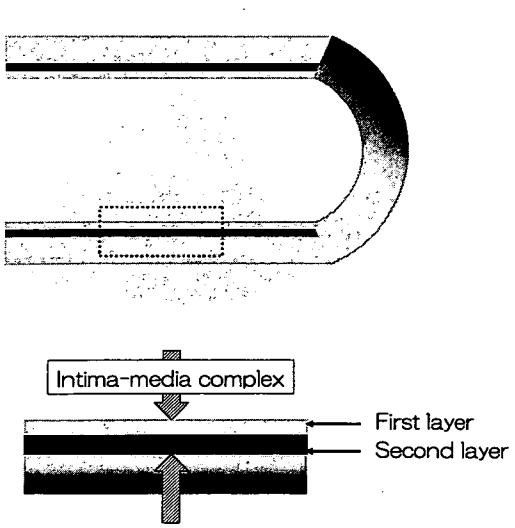

Fig. 1. *Schematic of the intima-media complex*

asymptomatic subjects, the indication for carotid artery endarterectomy should be carefully assessed because peri-operative mortality and/or morbidity following this procedure may not be negligible. Some investigators argue that carotid ultrasonography may not be cost-effective as a method of screening for significant carotid stenosis in the *asymptomatic* population[12], whereas other investigators have proposed that ultrasonographic screening for carotid artery screening may be more cost effective especially in the senior population, if a new and more rapid protocol of carotid artery screening protocol is adopted[13].

Although we may need to be aware of the cost-effectiveness of the ultrasonographic screening of extracranial carotid artery in *asymptomatic* individuals, cost-effectiveness may not be the chief concern of those undergoing general health screening in our country. As the presence of carotid plaque is a risk factor for ischemic stroke and coronary heart disease[7,14,24], anti-platelet and/or vasodilating agents might be recommended for a subset of subjects with such low-grade carotid stenosis. We may be able to identify the subjects at higher risk for ischemic stroke and/or coronary artery disease by plaque morphology combined with other diagnostic tests, such as a treadmill exercise test. At present, however, there is no standard therapeutic protocol is present for the treatment of subjects with such low-grade carotid stenosis.

Risk Factor Properties of Carotid Atherosclerosis

Atherosclerosis can develop simultaneously in different vascular beds. In this sense, it is reasonable to assume that the presence of carotid atherosclerosis may indicate an increased likelihood of the presence of coronary artery stenosis or future cardiac events. Indeed, much evidence shows that the presence of carotid intima-media thickening[15–17] and carotid plaque[18,19] are predictors for future cardio- and cerebrovascular events, and thus represent a subclinical atherosclerosis. A recent study showed that in elderly community-dwelling Japanese people, a 0.3 mm increase in the left and the right, respectively, carotid IMT was associated with a relative risk of 1.7 and 3.3 for all cause mortality, and that of 2.4 and 2.9 for cardiovascular mortality[20].

Craven *et al*. have reported that in individuals older than 50 years old, the B-mode score was associated with coronary stenosis, which was independent of other traditional coronary risk factors, and that considering the results of the B mode score, in addition to conventional risk factors, may increase the sensitivity and specificity for determining coronary artery disease status in such a population[21]. Simon *et al*. reported that the presence of carotid atherosclerosis in *asymptomatic* subjects was associated with a coronary heart disease with an incidence of 1.2% to 3.3% per year, which was, surprisingly, greater than the incidence associated with major risk factors, such as hypertension, diabetes, and smoking[22]. Importantly, they found that an absence of intima-media thickening was associated with a yearly incidence of coronary heart disease of 0.1% to 0.8%. Collectively, these studies indicate that the findings of carotid ultrasonography can provide useful information as an aid to the diagnosis of coronary heart disease.

Comparison with Other Non-Invasive Diagnostic Modalities

In addition to carotid ultrasonography, several non-invasive or least-invasive diagnostic modalities for atherosclerotic diseases have become available with recent advances in technology, including computed tomography (CT) and magnetic resonance (MR) angiography. In a recent meta-analysis, Wardlaw *et al.* compared the power and accuracy of several diagnostic tools for the diagnosis of carotid stenosis. They found that both the sensitivity and specificity of carotid ultrasonography are comparable to those of MR and CT angiography when 70–99% stenosis is present. On the other hand, the sensitivity of carotid ultrasonography and MR angiography for carotid stenosis may not be satisfactory for detecting carotid stenosis when there is an angiographically-proven level of 50–69% stenosis[23].

Carotid Ultrasonography in General Health Screening

In the setting of general health screening or Ningen Dock, many other hemodynamic and metabolic data can be obtained from the health screening participants at the time of carotid artery screening ; therefore, such data may enable us to analyze the possible association between early atherosclerosis and various non-traditional putative risk factors such as metabolic syndrome, microalbuminuria, CRP, and circulating WBC count (**Fig. 2**).

Carotid ultrasonography is an easy, immediate, and non-invasive diagnosing modality that does not involve radiation exposure. Visualization of the presence of plaque may help to assess the extent of atherosclerosis in the subjects, and may also increase the subjects' motivation for making lifestyle modifications, such as smoking cessation[25]. Considering that the presence of carotid plaque and intima-media thickening not only increases the risk of stroke, but also increases the like-

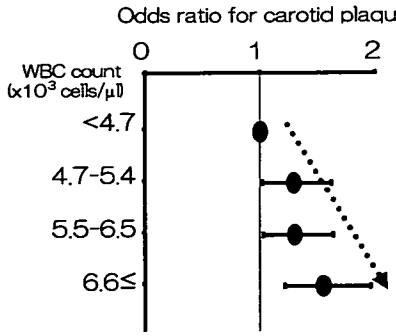

Fig. 2. *Increased WBC count is a risk factor for carotid plaque in men. Odds ratios were calculated after adjusting for age, BMI, systolic BP, total and HDL cholesterol, TG, and fasting glucose.*

lihood of the coronary artery disease, carotid ultrasonography is a feasible tool for detecting early atherosclerosis in the carotid arterial wall in the setting of general health screening or Ningen Dock.

Acknowledgement

The work was supported in part by a grant from the Smoking Research Foundation.

References

1. Ishizaka N, Ishizaka Y, Takahashi E, *et al.* : The Gender Difference in the Prevalence of Carotid Atherosclerosis-Results from General Health Screening. *Ningen Dock* 2005 ; 19 : 25–28.
2. Salonen R, Seppanen K, Rauramaa R, *et al.* : Prevalence of carotid atherosclerosis and serum cholesterol levels in eastern Finland. *Arteriosclerosis* 1988 ; 8 : 788–792.
3. Zureik M, Touboul PJ, Bonithon-Kopp C, *et al.* : Differential association of common carotid intima-media thickness and carotid atherosclerotic plaques with parental history of premature death from coronary heart disease : the EVA study. *Arterioscler Thromb Vasc Biol* 1999 ; 19 : 366–371.
4. Handa N, Matsumoto M, Maeda H, *et al.* : Ultrasonic evaluation of early carotid atherosclerosis. *Stroke* 1990 ; 21 : 1567–1572.
5. Honda O, Sugiyama S, Kugiyama K, *et al.* : Echolucent carotid plaques predict future coronary events in patients with coronary artery disease. *J Am Coll Cardiol* 2004 ; 43 : 1177–1184.
6. Wyman RA, Fraizer MC, Keevil JG, *et al.* : Ultrasound-detected carotid plaque as a screening tool for advanced subclinical atherosclerosis. *Am Heart J* 2005 ; 150 : 1081–1085.
7. Kitamura A, Iso H, Imano H, *et al.* : Carotid intima-media thickness and plaque characteristics as a risk factor for stroke in Japanese elderly men. *Stroke* 2004 ; 35 : 2788–2794.
8. Loimaala A, Rontu R, Vuori I, *et al.* : Blood leukocyte count is a risk factor for intima-media thickening and subclinical carotid atherosclerosis in middle-aged men. *Atherosclerosis* 2006 ; 188 : 363–369.
9. Iwamoto T, Fukuda S, Shimizu S, *et al.* : Long-term effects of lipoprotein(a) on carotid atherosclerosis in elderly Japanese. *J Gerontol A Biol Sci Med Sci* 2004 ; 59 : 62–67.
10. Prabhakaran S, Rundek T, Ramas R, *et al.* : Carotid plaque surface irregularity predicts ischemic stroke : the northern Manhattan study. *Stroke* 2006 ; 37 : 2696–2701.
11. Yamagami H, Kitagawa K, Nagai Y, *et al.* : Higher levels of interleukin-6 are associated with lower echogenicity of carotid artery plaques. *Stroke* 2004 ; 35 : 677–681.
12. Lee TT, Solomon NA, Heidenreich PA, *et al.* : Cost-effectiveness of screening for carotid stenosis in asymptomatic persons. *Ann Intern Med* 1997 ; 126 : 337–346.
13. Lavenson GS Jr., Pantera RL, Garza RM, *et al.* : Development and implementation of a rapid, accurate, and cost-effective protocol for national stroke prevention screening. *Am J Surg* 2004 ; 188 : 638–643.
14. Iwamoto T, Jingzi J, Sugiyama T, *et al.* : Prognosis of the elderly with asymptomatic atherosclerotic plaques of the carotid arteries. *Intern Med* 2002 ; 41 : 526–531.
15. Bots ML, Hoes AW, Koudstaal PJ, *et al.* : Common carotid intima-media thickness and risk of stroke and myocardial infarction : the Rotterdam Study. *Circulation* 1997 ; 96 : 1432–1437.
16. Chambless LE, Heiss G, Folsom AR, *et al.* : Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors : the Atherosclerosis Risk in Communities (ARIC) Study, 1987–1993. *Am J Epidemiol* 1997 ; 146 : 483–494.
17. O'Leary DH, Polak JF, Kronmal RA, *et al.* : Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med* 1999 ; 340 : 14–22.
18. Salonen JT, Salonen R : Ultrasonographically assessed carotid morphology and the risk of coronary heart disease. *Arterioscler Thromb* 1991 ; 11 : 1245–1249.
19. Stork S, van den Beld AW, von Schacky C, *et al.* : Carotid artery plaque burden, stiffness, and mortality risk in elderly men : a prospective, population-based cohort study. *Circulation* 2004 ; 110 : 344–348.
20. Murakami S, Otsuka K, Hotta N, *et al.* : Common carotid intima-media thickness is predictive of all-cause and cardiovascular mortality in elderly community-dwelling people : Longitudinal Investigation for the Longevity and Aging in Hokkaido County (LILAC) study. *Biomed Pharmacother* 2005 ; 59 Suppl 1 : S49–53.
21. Craven TE, Ryu JE, Espeland MA, *et al.* : Evaluation of the associations between carotid artery atherosclerosis and coronary artery stenosis. A case-control study. *Circulation* 1990 ; 82 : 1230–1242.
22. Simon A, Chironi G, Levenson J : Performance of subclinical arterial disease detection as a screening test for coronary heart disease. *Hypertension* 2006 ; 48 : 392–396.
23. Wardlaw JM, Chappell FM, Best JJ, *et al.* : Non-invasive imaging compared with intra-arterial angiography in the diagnosis of symptomatic carotid stenosis : a meta-analysis. *Lancet* 2006 ; 367 : 1503–1512.
24. O'Holleran LW, Kennelly MM, McClurken M, *et al.* : Natural history of asymptomatic carotid plaque. Five year follow-up study. *Am J Surg* 1987 ; 154 : 659–662.
25. Bovet P, Perret F, Cornuz J, *et al.* : Improved smoking cessation in smokers given ultrasound photographs of their own atherosclerotic plaques. *Prev Med* 2002 ; 34 : 215–220.

ELSEVIER

Atherosclerosis 192 (2007) 131–137

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

Higher serum uric acid is associated with increased arterial stiffness in Japanese individuals

Nobukazu Ishizaka*, Yuko Ishizaka, Ei-Ichi Toda, Hideki Hashimoto, Ryozo Nagai, Minoru Yamakado

Departments of Cardiovascular Medicine (N.I., R.N.) and Health Management and Policy (H.H), University of Tokyo Graduate School of Medicine, and Center for Multiphasic Health Testing and Services, Mitsui Memorial Hospital (Y.I., E-I.T., M.Y.), Tokyo, Japan

Received 1 August 2005; received in revised form 23 January 2006; accepted 11 April 2006
Available online 23 May 2006

Abstract

Hyperuricemia is postulated to be a risk factor for atherosclerotic diseases, although whether it is independent of classical atherogenic risk factors is controversial. The automatic computer-assisted measurement of brachial-ankle pulse wave velocity (baPWV) is a valid and reproducible method by which to assess arterial stiffness, a potential surrogate marker of early atherosclerosis. By analyzing cross-sectional data from 982 individuals who underwent health screening, we have investigated whether serum uric acid is associated with high baPWV, which was determined as the highest quartile of baPWV values, in a sex-specific manner. Multivariate analysis showed that the odds ratios (95% CI) of the highest baPWV quartile across the sex-specific quartiles of serum uric acid were 1.0, 2.80 (0.93–8.40), 2.13 (0.74–6.19), and 2.76 (1.01–7.55) in women, and 1.0, 1.10 (0.55–2.20), 1.97 (1.04–3.75), and 2.24 (1.10–4.56) in men after adjusting for age, total and HDL-cholesterol, BMI, systolic blood pressure, triglycerides, fasting glucose and smoking status. The association between uric acid and high baPWV was observed in both subjects with metabolic syndrome and those without. These data suggest that in both genders, serum uric acid level is associated with increased baPWV, a marker of arterial stiffness, and is in part independent of other conventional risk factors for atherosclerosis and metabolic syndrome.

Keywords: Aortic stiffness; Pulse wave velocity; Metabolic syndrome; Multivariate analysis

1. Introduction

Aortic pulse wave velocity (PWV) and augmentation index are indices of arterial stiffness, and are a predictor for cardiovascular diseases, stroke, and cognitive impairment [1–3]. Recent studies have shown that brachial-ankle PWV (baPWV), which can be measured fairly reproducibly by an automated device [4], well correlates well with aortic stiffness determined by an invasive method [5]. Because of their non-invasive nature, both baPWV measurements and carotid artery ultrasonography are used to assess arteriosclerosis during health screening. Several risk factors for increased PWV have been reported, including raised C-reactive protein (CRP) levels [6], menopause [7], and increased vascular calcification observed in patients with end-stage renal disease (ESRD) [8].

Several previous studies have suggested that hyperuricemia is a risk factor for cardiovascular diseases [9,10]. Hyperuricemia increases the risk for metabolic syndrome, which has reported to be a risk factor for cardiovascular disease [11,12]. Therefore, it is possible that observed association between uric acid (UA) and atherosclerotic disease may be in part attributed to the link between hyperuricemia and metabolic syndrome. On the other hand, however, recent stud-

* Corresponding author at: Department of Cardiovascular Medicine, University of Tokyo Graduate School of Medicine, Hongo 7-3-1 Bunkyo-ku, Tokyo 113-8655, Japan. Tel.: +81 3 3815 5411x37156; fax: +81 3 5842 5586.
E-mail address: nobuishizka-tky@umin.ac.jp (N. Ishizaka).

0021-9150/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2006.04.016

ies also suggested that association between serum UA and death from cardiovascular disease and that between serum UA and carotid plaque may be independent of variables related to metabolic syndrome [13,14].

It has recently been reported that metabolic syndrome is a strong risk factor for increased baPWV [15]. Thus, in the present study, we have investigated whether serum UA is associated with increased baPWV, and the independency of this association from metabolic syndrome as well as classical risk factors.

2. Methods

2.1. Study subjects

The study was approved by The Ethical Committee of Mitsui Memorial Hospital. A total of 982 (women, 297, and men, 695) subjects aged between 23 and 87 (mean 59.2) years, who underwent health screening targeted towards atherosclerosis between April 2003 and March 2005 at our institute participated in the study. In Japan, regular health check-ups for employees are legally mandated, and all or most of the costs of the screening are usually paid by the company to which they belong or by each subject. At our institute, several types of health screening program are available, and the current one is termed atherosclerosis-screening course. The choice of which course to be chosen is dependent on the decision of individuals and/or the companies to which they belong. Among 982 enrolled subjects (mean age, 59 yr), 89 (9.0%) were taking anti-hyperlipidemic, 36 (3.7%) were taking anti-diabetic, and 166 (16.9%) were taking antihypertensive drugs. These percentages were significantly greater than those in the subjects who underwent 'ordinary' heath screening course during this study period (mean age, 52 yr, anti-hyperlipidemic drugs, 4.1%; anti-diabetic drugs, 2.2%; anti-hypertensive drugs, 10.0%). Therefore, it could be said that there might have been some selection bias for participants planning atherosclerosis-screening course. However, this was never either the decision or the recommendation of any attending physician.

2.2. Laboratory data

Serum levels of TC, HDL-C, and TG were determined enzymatically. Serum UA was measured by the uricase-peroxidase method, haemoglobin A_{1C} was determined by the latex agglutination immunoassay, and serum total bilirubin was measured by the bilirubin oxidase method.

The mean UA level was found to be significantly lower in women (4.7 ± 0.9 mg/dL) than in men (6.1 ± 1.2 mg/dL, $P < 0.0001$). Therefore, sex-specific quartiles of serum UA were used. The median (range) UA values of 3.6 (2.1–4.0), 4.3 (4.1–4.6), 5.0 (4.7–5.3) and 5.9 (5.4–8.0) mg/L were used for women, and 4.6 (2.9–5.2), 5.7 (5.3–6.0), 6.5 (6.1–6.9) and 7.8 (7.0–11.0) mg/L were used for men (Table 1).

2.3. Criteria for metabolic syndrome

Diagnosis of metabolic syndrome was made according to the criteria of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP-III), with body mass index (BMI) used as a surrogate for waist circumference [16]. Metabolic syndrome was diagnosed when three or more of following components were present. (1) triglyceride levels ≥ 150 mg/dL, (2) HDL-C levels < 40 mg/dL in men or < 50 mg/dL in women, (3) fasting plasma glucose levels ≥ 110 mg/dL, or taking an antidiabetic medication, (4) systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mm Hg or taking an antihypertensive medication, or (5) BMI > 25 kg/m^2.

2.4. Measurement of baPWV and ankle-brachial pressure index (ABI)

baPWV was assessed by using a fully automatic device (form® PWV/ABI, model BP-203RPE II, COLIN Medical Technology Co. Ltd., Komaki, Japan) as described elsewhere [5]. Due to its automated nature, the applied this method has been shown to possess high inter- and intra-observer reproducibility [5,17,18]. High baPWV was defined as the highest quartile of the values among the study subjects, which was equal to or more than 1594 cm/s in women and 1721 cm/s in men. Ankle-brachial pressure index (ABI), a simple marker of peripheral arterial stenosis was measured simultaneously with baPWV measurement. To test inter- and intra-observer reproducibility, three observers measured baPWV of three subjects at the four different time points. Pearson's correlation coefficients of intraobserver and intraobserver reproducibility were $r = 0.984$ and $r = 0.970$, respectively, indicating that baPWV measurement using an automatic device was fairly reproducible as has been reported elsewhere [5].

2.5. Carotid ultrasonography

Carotid artery status was studied and analyzed as described previously [19]. In brief, it was examined by high resolution B-mode ultrasonography, using an instrument (Sonolayer SSA270A, Toshiba, Japan) equipped with a 7.5 MHz transducer (PLF-703ST, Toshiba). The carotid arteries were examined bilaterally at the levels of the common carotid, the bifurcation, and the internal carotid arteries from transverse and longitudinal orientations by trained sonographers. The intima-media thickness was measured using a computer-assisted method by experienced sonographers who were unaware of the subjects' clinical and laboratory findings. Plaque was defined as a clearly isolated focal thickening of the intima-media layer with thickness of ≥ 1.3 mm at the common or internal carotid artery or the carotid bulb.

In the current study population, carotid plaque was positive in 39% (116/297) of women and 52% (338/655) of men, values that were significantly greater than that we have reported before (16% (440/2671) of women, and 29%

Table 1
Baseline characteristics

Variables	Quartiles of serum uric acid				*P*-value
	1	2	3	4	
Women					
Uric acid range (median), mg/dL	2.1–4.0 (3.7)	4.1–4.6 (4.3)	4.7–5.3 (5.0)	5.4–8.0 (5.7)	
Number of subjects	76	70	78	73	
Age, years	60 ± 11	60 ± 10	58 ± 12	63 ± 10	0.062
Body mass index, kg/m^2	21.4 ± 2.6	21.5 ± 3.0	21.7 ± 2.6	23.3 ± 3.7	0.0001
Systolic blood pressure, mmHg	118 ± 22	119 ± 22	122 ± 18	137 ± 23	<0.0001
Diastolic blood pressure, mmHg	71 ± 12	73 ± 13	74 ± 111	83 ± 12	<0.0001
Heart rate, bpm	65 ± 8	64 ± 8	66 ± 11	67 ± 9	0.33
Laboratory data					
Uric acid, mg/dL	3.6 ± 0.5	4.3 ± 0.2	5.0 ± 0.2	5.9 ± 0.6	<0.0001
Total bilirubin, mg/dL	0.76 ± 0.27	0.76 ± 0.22	0.76 ± 0.26	0.80 ± 0.25	0.63
Creatinine, mg/dL	0.62 ± 0.10	0.61 ± 0.09	0.64 ± 0.10	0.69 ± 0.10	<0.0001
gamma-GTP, IU/L	23.3 ± 14.5	26.8 ± 17.4	28.1 ± 19.1	30.0 ± 31.4	0.27
C-reactive protein, mg/dL	0.08 ± 0.17	0.21 ± 0.96	0.10 ± 0.16	0.17 ± 0.25	0.38
Serum lipid data					
Total cholesterol, mg/dL	222 ± 31	216 ± 33	220 ± 38	235 ± 29	0.0062
HDL-cholesterol, mg/dL	72 ± 14	70 ± 21	69 ± 15	68 ± 17	0.46
Triglycerides, mg/dL	82 ± 34	81 ± 33	90 ± 51	107 ± 49	0.0009
Glucose metabolisms					
Fasting glucose, mg/dL	91 ± 18	91 ± 10	95 ± 21	94 ± 10	0.22
Haemoglobin A1C, %	5.10 ± 0.66	5.15 ± 0.38	5.19 ± 0.60	5.23 ± 0.46	0.52
Smoking status					
Non-smokers (%)	66 (87)	63 (90)	66 (85)	64 (88)	0.87
Former smokers (%)	4(5)	4(6)	7(9)	6(8)	
Current smokers (%)	6(8)	3(4)	5(6)	3(4)	
Men					
Uric acid range (median), mg/dL	2.9–5.2 (4.7)	5.3–6.0 (5.7)	6.1–6.9 (6.5)	7.0–11.0 (7.7)	
Number of subjects	159	162	177	157	
Age, years	60 ± 10	58 ± 11	60 ± 10	56 ± 11	0.0002
Body mass index, kg/m^2	23.2 ± 2.8	24.2 ± 2.9	24.2 ± 2.8	25.1 ± 2.7	<0.0001
Systolic blood pressure, mmHg	127 ± 21	131 ± 21	131 ± 18	133 ± 20	0.07
Diastolic blood pressure, mmHg	78 ± 12	82 ± 13	82 ± 11	83 ± 11	0.0021
Heart rate, bpm	64 ± 11	64 ± 9	65 ± 9	64 ± 8	0.76
Laboratory data					
Uric acid, mg/dL	4.6 ± 0.6	5.7 ± 0.2	6.5 ± 0.3	7.8 ± 0.7	<0.0001
Total bilirubin, mg/dL	0.89 ± 0.29	0.92 ± 0.37	0.90 ± 0.30	0.87 ± 0.32	0.59
Creatinine, mg/dL	0.83 ± 0.12	0.88 ± 0.13	0.88 ± 0.12	0.94 ± 0.18	<0.0001
gamma-GTP, IU/L	46.0 ± 36.4	49.8 ± 38.5	64.1 ± 74.8	79.0 ± 86.0	<0.0001
C-reactive protein, mg/dL	0.24 ± 1.39	0.18 ± 0.20	0.19 ± 0.44	0.19 ± 0.40	0.6
Serum lipid data					
Total cholesterol, mg/dL	205 ± 30	208 ± 35	208 ± 32	212 ± 30	0.34
HDL-cholesterol, mg/dL	57 ± 13	55 ± 12	54 ± 13	53 ± 14	0.015
Triglycerides, mg/dL	124 ± 82	129 ± 64	154 ± 234	165 ± 93	0.022
Glucose metabolisms					
Fasting glucose, mg/dL	105 ± 23	107 ± 31	104 ± 18	103 ± 20	0.53
Haemoglobin A1C, %	5.56 ± 0.84	5.46 ± 0.92	5.41 ± 0.64	5.33 ± 0.60	0.058
Smoking status					
Non-smokers (%)	43 (27)	47 (29)	48 (27)	46 (29)	0.57
Former smokers (%)	72 (45)	59 (36)	82 (46)	64 (41)	
Current smokers (%)	44 (28)	56 (35)	47 (27)	48 (30)	

(1607/5473) of men) [14]. This difference was partly because the mean age was greater than in our previous study (57 ± 11 yr in women, and 57 ± 11 yr in men in the previous study; and 60 ± 11 yr in women and 59 ± 11 yr in men in the current study).

2.6. Statistical analysis

The data in this study were analyzed by the χ^2-test, ANOVA with a Bonferroni post hoc test, and multivariate logistic regression analysis using computer software,

StatView ver. 5.0 (SAS Institute, NC, USA). A value of $p < 0.05$ was taken to be statistically significant. Results are expressed as the mean ± S.D. unless stated otherwise.

3. Results

3.1. Baseline characteristics

The age of the enrolled subjects ranged from 23 to 87 yr (women, 25–85 yr; men, 23–87 yr) with a mean age of 59.2 ± 10.8 yr (women, 60.2 ± 10.8 yr; men, 58.8 ± 10.8 yr). The mean age did not significantly differ among each UA quartile group in women. In men, by contrast, the mean age in the fourth quartile was less than that in the first quartile ($P = 0.0001$) (Table 1). Of the 982 subjects, an ABI value of < 0.95 was obtained in only 13 subjects (1.3%).

3.2. Relationship between UA and PWV

Pearson's correlation coefficients for the relationship between UA and baPWV were 0.19 ($P = 0.0033$) in women and 0.033 (P = NS) in men. The mean baPWV value was 1549 ± 342 in men, which was significantly higher than in women (1447 ± 315, $P < 0.0001$). The sex-specific interquartile cut off points for baPWV were 1207, 1370, and 1594 cm/s in women, and those of 1311, 1493, 1721 cm/s in men. Thus, the high baPWV was defined as equal to or more than 1594 cm/s in women and 1721 cm/s in men.

The prevalence of the high baPWV in the first, second, third, and fourth UA quartiles in each gender is shown in Fig. 1. When multivariate analysis was performed after adjusting for gender, age, TC, BMI, systolic blood pressure, HDL-C, triglycerides, fasting glucose and smoking status, the association between UA and high baPWV was statistically significant, and statistical significance was maintained after subdividing subjects according to their gender (Table 2).

In this model, the odds ratios (95% CI) of the other covariates were as follows. In women, age (per 1 yr), 1.13 (1.08–1.19), $P < 0.0001$, TC (per 1 mg/dL), 1.01 (1.00–1.02), $P = 0.075$; former smoking, 1.07 (0.24–4.82), $P = 0.93$; current smoking, 5.67 (0.79–40.41), $P = 0.084$; BMI (per 1 kg/m^2), 0.95 (0.84–1.08), $P = 0.43$; systolic blood pressure (per 1 mmHg), 1.05 (1.03–1.08), $P < 0.0001$; HDL-C

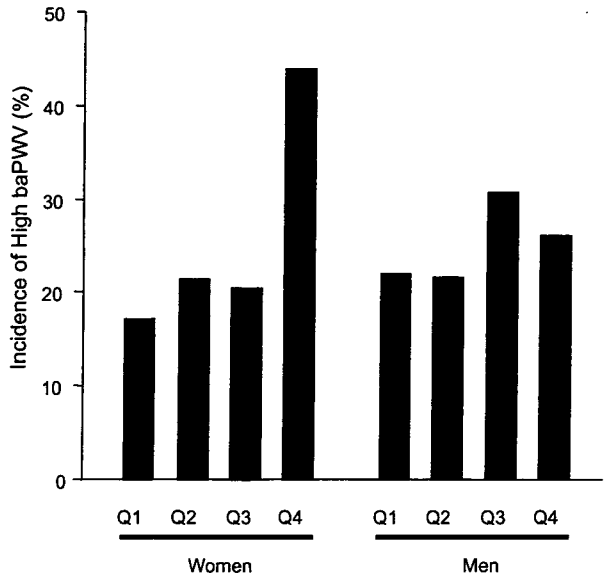

Fig. 1. Incidence of high baPWV according to the UA quartile and gender.

(per 1 mg/dL), 1.00 (0.97–1.01), $P = 0.79$; triglycerides (per 1 mg/dL), 1.00, (CI 1.00–1.01), $P = 0.30$; fasting glucose (per 1 mg/dL), 1.01 (0.99–1.02), $P = 0.57$. In men, age (per 1 yr), 1.18 (1.14–1.22), $P < 0.0001$, TC (per 1 mg/dL), 1.00 (0.99–1.01), $P = 0.82$; former smoking, 1.77 (0.97–3.22), $P = 0.61$; current smoking, 1.74 (0.88–3.46), $P = 0.11$; BMI (per 1 kg/m^2), 0.98 (0.89–1.08), $P = 0.68$; systolic blood pressure (per 1 mmHg), 1.05 (1.04–1.06), $P < 0.001$; HDL-C (per 1 mg/dL), 1.01 (0.99–1.03), $P = 0.18$; triglycerides (per 1 mg/dL), 1.00 (1.00–1.00), $P = 0.27$; fasting glucose (per 1 mg/dL), 1.01 (0.99–1.02), $P = 0.37$.

Serum creatinine showed graded increase according to the UA levels in both genders (Table 1). After further adjustment for serum creatinine level, mode of association between serum UA quartile and high baPWV did not greatly changed; odds ratios (95% CI) for the first, second, third, and fourth UA quartiles were 1.0 (reference), 2.81 (0.93–8.46, $P = 0.066$), 2.22 (0.76–6.49, $P = 0.15$), and 2.97 (1.05–8.39, $P = 0.040$), respectively, in women, and 1.0 (reference), 1.19 (0.58–2.42, $P = 0.64$), 2.13 (1.11–4.09, $P = 0.024$), and 2.63 (1.26–5.50, $P = 0.010$), respectively, in men.

We also assessed whether increased CRP levels, white blood cell count, and serum bilirubin levels were associated with increased baPWV using the above-described

Table 2
Multivariate analysis assessing the association between UA and high baPWV

	Quartiles of serum uric acid			
	Q1	Q2	Q3	Q4
Whole	1 (reference)	1.41 (0.78–2.51)	2.00 (1.17–3.43)*	2.40 (1.36–4.26)**
Women	1 (reference)	2.80 (0.93–8.40)	2.13 (0.74–6.19)	2.76 (1.01–7.55)*
Men	1 (reference)	1.10 (0.55–2.20)	1.97 (1.04–3.75)*	2.24 (1.10–4.56)*

Age, sex (for whole), BMI, systolic blood pressure, TC, HDL-C, triglycerides, fasting glucose, and smoking status were used as covariates.
* $P < 0.05$.
** $P < 0.01$ versus the lowest quartile (reference).

factors as covariates. For the high baPWV, the odds ratio of increased CRP levels (per 1 mg/dL) was 1.13 (95% CI 1.08–1.19, $P<0.0001$) in women and 1.79 (95% CI 0.99–3.24, $P=0.056$) in men; that of the white blood cell count (per $1 \times 10^3/\mu L$) was 0.98 (95%CI 0.74–1.29, $P=0.86$) in women and 1.08 (95% CI 0.90–1.29, $P=0.42$) in men; and those of serum bilirubin levels (per 1 mg/dL) were 1.98 (95%CI 0.46–8.60, $P=0.36$) in women and 0.61 (95%CI 0.29–1.29, $P=0.20$) in men.

3.3. Effect of metabolic syndrome on the association between UA and baPWV

Out of 952 subjects, 159 (17%) were found to have metabolic syndrome (16/297 (5.4%) in women, 143/655 (21.8%) in men). After adjusting for gender, age, TC, and smoking status, serum UA level was found to be associated with metabolic syndrome with an odds ratio of 1.32 (95% CI 1.14–1.53, $P=0.0003$, per 1 mg/dL increase) which was in agreement with previous findings [20]. In addition, after adjusting for gender, age, TC, and smoking status, metabolic syndrome was associated with the high baPWV with an odds ratio of 2.43 (95% CI 1.55–3.83, $P=0.0001$).

Thus we next investigated the association between UA and baPWV according to the status of metabolic syndrome. After adjusting for gender, age, TC, BMI, systolic blood pressure, HDL-C, triglycerides, fasting glucose and smoking status, the odds ratio (95% CI) of the first, second, third, and fourth UA quartiles for the high baPWV was 1.0 (reference), 1.19 (0.62–2.27, $P=0.60$), 1.86 (1.01–1.32, $P=0.047$), and 2.02 (1.04–3.90, $P=0.037$), respectively, in subjects without metabolic syndrome, and 1.0 (reference), 3.34 (0.74–14.9, $P=0.11$), 3.36 (0.83–13.61, $P=0.089$), and 4.52 (1.09–18.72, $P=0.038$), respectively, in the subjects with metabolic syndrome.

3.4. Relationship between carotid plaque, carotid intima-media thickening, and baPWV

The incidence of carotid plaque in subjects with and without high baPWV were 49/76 (64%) and 67/221 (30%), respectively, in women ($P<0.0001$), and 117/165 (71%) and 221/490 (45%), respectively, in men ($P<0.0001$). After adjusting for age, BMI, systolic blood pressure, TC, HDL-C, triglycerides, fasting glucose and smoking status, the odds ratio (95% CI) of the first, second, third, and fourth baPWV quartiles for the carotid plaque was 1.0 (reference), 1.69 (0.72–3.95, $P=0.23$), 1.23 (0.49–3.06, $P=0.66$), and 3.00 (1.07–8.39, $P=0.037$), respectively, in women, and 1.0 (reference), 1.42 (0.87–2.31, $P=0.16$), 1.70 (1.00–2.90, $P=0.052$), and 1.96 (1.03–3.75, $P=0.041$), respectively, in men.

The incidence of carotid intima-media thickening in subjects with and without high baPWV were 28/76 (37%) and 18/221 (8%), respectively, in women ($P<0.0001$), and 47/165 (28%) and 60/490 (12%), respectively, in men ($P<0.0001$). After adjusting for age, BMI, systolic blood pressure, TC, HDL-C, triglycerides, fasting glucose and smoking status, the odds ratio (95% CI) of the first, second, third, and fourth baPWV quartiles for the carotid intima-media thickening was 1.0 (reference), 4.49 (0.51–39.45, $P=0.17$), 4.35 (0.50–38.14, $P=0.18$), and 10.76 (1.20–96.59, $P=0.034$), respectively, in women, and 1.0 (reference), 1.37 (0.52–3.66, $P=0.52$), 2.93 (1.17–7.32, $P=0.022$), and 1.97 (0.71–5.47 −3.75, $P=0.19$), respectively, in men.

When compared to the lower three baPWV quartiles, high baPWV was associated with carotid plaque with odds ratio (95% CI) of 2.24 (1.10–4.48, $P=0.025$) in women, and 1.26 (0.79–2.20, $P=0.92$) in men, and associated with intima-media thickening with odds ratio (95% CI) of 2.67 (1.17–6.07, $P=0.019$) in women, and 0.83 (0.47–1.46, $P=0.51$) in men, after adjusting for above-mentioned variables.

4. Discussion

In the present study, by analyzing the subjects undergoing health screening test, we found that serum UA levels showed a graded association with the incidence of high baPWV, which was determined as ≥1594 cm/s in women and ≥1721 cm/s in men, and that this association was independent of other confounding atherogenic risk factors. A high baPWV value, as determined by these cut-off points may have clinical significance, because baPWV over 1400 cm/s and that over 1600 cm/s have been shown to be useful to predictors for cardiovascular diseases as assessed by the Framingham risk score [21] and for mild cardiac diastolic dysfunction [22], respectively, in the general population. It has been reported that both increased baPWV and hyperuricemia are associated with an increased incidence of some pathophysiological conditions, such as ischemic heart disease [9,23], poor prognosis in patients with coronary artery disease [10,24], and heart failure [22,25].

Non-invasive aortic stiffness measurement is postulated to be a surrogate marker of early atherosclerosis. An automated computer-assisted baPWV measurement facilitates a valid and reproducible assessment of arterial stiffness that is closely associated with invasive measurements of aortic stiffness [5]. Not surprisingly, carotid atherosclerosis and PWV have been shown to be related to each other in several previous studies [22,26,27], as well as in the current one. It might be considered that screening of both baPWV may not provide any information additional to carotid ultrasonographic evaluation. However, PWV may not be significantly associated with carotid atherosclerosis in certain cases [28,29], and a combination of carotid atherosclerosis and PWV may have a more power for detecting atherosclerotic diseases than PWV alone [27]. In addition, besides being an indicator of atherosclerosis, arterial stiffness itself may be involved in the process of atherosclerosis [30]. These data support the utility

of baPWV measurements in the setting of a general health screening test.

Although baPWV is easily performed, caution should be taken because the accuracy of the baPWV value obtained is diminished when the ABI is less than 0.95 due to the peripheral artery stenosis [31]. In the current study, however, only 1.3% subjects were found to have an ABI of less than 0.95, which supports the validity of using the baPWV measurement in our study. This finding also supports the notion that the baPWV measurement is more useful for participants undergoing general health screening than for those who are known to have severe atherosclerotic diseases.

In the current study, we have shown that serum UA levels is associated with metabolic syndrome, in agreement with our previous findings [14]. Together with a report from another group [32], our current study shows that metabolic syndrome is a risk factor for increased baPWV. Thus, the link between serum UA levels and increased PWV might be mediated by the metabolic syndrome. We found here, however, that an association between serum UA levels and baPWV could be observed in both subjects with metabolic syndrome and in those without, suggesting that this association is in part independent of metabolic syndrome. We have previously shown that serum UA is associated with carotid plaque that is independent of other confounding atherogenic risk factors [14]. In that study, we found that metabolic syndrome was found to be a risk factor for carotid plaque and that UA was associated with carotid plaque in a metabolic syndrome-independent manner. Thus, the overall relationship between serum UA and high baPWV is similar to that between serum UA and carotid plaque.

In the current study, we showed that association between serum UA and high baPWV was at least in part independent of conventional risk factors for atherosclerosis. What is the possible underlying mechanism that links between hyperuricemia and arterial stiffening? It has been reported that UA promotes vascular smooth muscle proliferation and upregulates the expression of platelet-derived growth factor [33] and monocyte chemoattractant protein-1 [34]. Hypoxanthine is converted to uric acid via xanthine. This reaction can be catalyzed by xanthine hydrogenase and xanthine oxidase, the latter of which produce uric acid and superoxide. Thus, it is possible that, in certain diseased conditions, hyperuricemia is accompanied by the increased production of reactive oxygen species [35], which may result the modulation of vascular contractility [36]. Consistent with this notion that allopurinol, a xanthine oxidase inhibitor, not only reduced the serum UA levels but also improved vascular endothelial function in patients with chronic heart failure [37]. Another possible explanation is that hyperuricemia may induce endothelial dysfunction by decreasing the production of nitric oxide in the vascular endothelial cells [38]. Although these mechanisms may underlie the hyperuricemia-associated arterial stiffening, it should be kept in mind that whether the relationship between serum UA and increased arterial stiffness is circumstantial or causal cannot be determined by this cross-sectional study.

In conclusion, by analyzing the data of individuals who had undergone general health screening that was targeted towards screening for atherosclerosis, we have found that serum UA is associated with baPWV, and thus increased arterial stiffness. Although serum UA is associated with an incidence of metabolic syndrome, association between serum UA and baPWV is, in part, independent of metabolic syndrome.

References

[1] Hirai T, Sasayama S, Kawasaki T, Yagi S. Stiffness of systemic arteries in patients with myocardial infarction. A noninvasive method to predict severity of coronary atherosclerosis. Circulation 1989;80:78–86.
[2] Laurent S, Boutouyrie P, Asmar R, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension 2001;37:1236–41.
[3] Scuteri A, Brancati AM, Gianni W, Assisi A, Volpe M. Arterial stiffness is an independent risk factor for cognitive impairment in the elderly: a pilot study. J Hypertens 2005;23:1211–6.
[4] Matsui Y, Kario K, Ishikawa J, Eguchi K, Hoshide S, Shimada K. Reproducibility of arterial stiffness indices (pulse wave velocity and augmentation index) simultaneously assessed by automated pulse wave analysis and their associated risk factors in essential hypertensive patients. Hypertens Res 2004;27:851–7.
[5] Yamashina A, Tomiyama H, Takeda K, et al. Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement. Hypertens Res 2002;25:359–64.
[6] Nagano M, Nakamura M, Sato K, Tanaka F, Segawa T, Hiramori K. Association between serum C-reactive protein levels and pulse wave velocity: a population-based cross-sectional study in a general population. Atherosclerosis 2005;180:189–95.
[7] Zaydun G, Tomiyama H, Hashimoto H, et al. Menopause is an independent factor augmenting the age-related increase in arterial stiffness in the early postmenopausal phase. Atherosclerosis 2005.
[8] Guerin AP, London GM, Marchais SJ, Metivier F. Arterial stiffening and vascular calcifications in end-stage renal disease. Nephrol Dial Transplant 2000;15:1014–21.
[9] Fessel WJ. High uric acid as an indicator of cardiovascular disease. Independence from obesity. Am J Med 1980;68:401–4.
[10] Freedman DS, Williamson DF, Gunter EW, Byers T. Relation of serum uric acid to mortality and ischemic heart disease. The NHANES I Epidemiologic Follow-up Study. Am J Epidemiol 1995;141:637–44.
[11] Nagahama K, Iseki K, Inoue T, Touma T, Ikemiya Y, Takishita S. Hyperuricemia and cardiovascular risk factor clustering in a screened cohort in Okinawa. Japan Hypertens Res 2004;27:227–33.
[12] Bonora E, Kiechl S, Willeit J, et al. Carotid atherosclerosis and coronary heart disease in the metabolic syndrome: prospective data from the Bruneck study. Diabetes Care 2003;26:1251–7.
[13] Niskanen LK, Laaksonen DE, Nyyssonen K, et al. Uric acid level as a risk factor for cardiovascular and all-cause mortality in middle-aged men: a prospective cohort study. Arch Intern Med 2004;164:1546–51.
[14] Ishizaka N, Ishizaka Y, Toda E, Nagai R, Yamakado M. Association between serum uric acid, metabolic syndrome, and carotid atherosclerosis in Japanese individuals. Arterioscler Thromb Vasc Biol 2005;25:1038–44.
[15] Nakanishi N, Shiraishi T, Wada M. Brachial-ankle pulse wave velocity and metabolic syndrome in a Japanese population: the Minoh study. Hypertens Res 2005;28:125–31.
[16] Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation,