Table 5 Comparison of changes in clinical parameters of HIV/HBV-coinfected patients with or without antiretroviral therapy (ART)

a. Baseline statistical data

	Natural course[a] (without ART)	With ART	p value (with vs. without ART)
Number (male:female)	84:6	159:3	0.105[†]
Age (year)	37.0 ± 10.3	39.0 ± 9.1	0.362
Observation period (month)	34.5 ± 55.5	50.9 ± 43.9	0.022*
Presumed transmission route	Blood products:homosexual contact:heterosexual contact:injection drug use:other		
	5:60:12:2:3	9:126:12:0:1	0.052[†]
Recognized acute hepatitis	10	11	0.243[†]
HBeAg (positive:negative)	42:18	100:40	0.394[†]
HBV DNA (high:low)	29:18	83:37	0.356[†]
HBV genotype	A:B:C:D:F:G:H		
	17:0:1:1:1:0:1	31:3:6:0:1:1:2	0.372[†]
Ascites	1/56	2/144	1.000[†]
Hepatocellular carcinoma	0/62	1/159	1.000[†]
Acquired immunodeficiency syndrome (AIDS)	10/64	52/162	0.012*[,†]

b. Comparison of clinical parameters between pre- and post-ART among patients with and without ART

	Natural course (without ART)	With ART	p value (with vs. without ART)
CD4 count (per μl)			
Start[b]	402.9 ± 180.1	242.5 ± 187.6	0.000*
End[c]	406.4 ± 212.4	398.1 ± 195.9	0.883
p value (start vs. end)	0.893	0.000*	
Albumin (g/dl)			
Start	4.1 ± 0.4	3.8 ± 0.8	0.292
End	3.9 ± 0.8	4.2 ± 0.4	0.025*
p value	0.473	0.001*	
Bilirubin[d] (mg/dl)			
Start	0.7 (0.30, 4.26)	0.5 (0.30, 2.62)	0.138
End	0.5 (0.25, 1.30)	0.9 (0.36, 4.32)	0.000*
p value	0.046*	0.000*	
ALT[d] (IU/l)			
Start	46.0 (15.0, 1418.2)	34.0 (12.8, 1,068.8)	0.120
End	27.0 (9.9, 229.9)	31.5 (12.73, 89.3)	0.713
p value	0.003*	0.000*	
Prothrombin time index (%)			
Start	89.4 ± 13.1	78.8 ± 23.0	0.650
End	78.8 ± 27.3	84.2 ± 16.3	0.531
p value	0.377	0.218	
WBC ($\times 10^3$/μl)			
Start	6.1 ± 2.4	4.8 ± 2.1	0.000*
End	5.4 ± 1.4	5.1 ± 1.6	0.404
p value	0.044*	0.247	
Platelet ($\times 10^4$ /μl)			
Start	22.2 ± 6.5	19.3 ± 6.3	0.010*
End	21.2 ± 6.5	20.8 ± 6.1	0.649
p value	0.204	0.001*	

* $p < 0.05$

† Chi-square test was performed

a Two patients with habitual alcohol intake were included in this group

b Start of observation period

c End of observation period

d Means were compared by log transformation because of the nonnormality of the distribution; median and percentiles (5th percentile, 95th percentile) are provided

difference in serum total bilirubin level between the beginning and the end of the observation period [Dbilirubin level = (bilirubin level at the end) − (bilirubin level at the beginning)] in individual patients and compared it between the PI group and the non-PI group. The mean Dbilirubin level in the PI group was 0.5 ± 3.4 mg/dl and that in the non-PI group was −0.2 ± 1.6 mg/dl ($p = 0.250$). The Dbilirubin level in a patient in the PI group who was coinfected with HCV besides HIV/HBV as well was −27.4 mg/dl. Excluding this single outlier, the mean Dbilirubin level was significantly different between the PI and non-PI groups (mean Dbilirubin level 0.8 vs. −0.2; $p = 0.01$).

Discussion

We have summarized here the data from our comprehensive survey of HIV/HBV coinfection in Japan, focusing particularly on the clinical features of the patients and the effect of ART on liver function. As we reported earlier, HIV/HBV coinfection was observed in 6.3 % of Japanese HIV-positive patients [7]. Certain considerations for HBV coinfection are important in HIV patient care.

The major transmission route of HIV was male homosexual contact, which accounted for the infection in about 80 % of the patients; thus, male patients were the majority in the present cohort. The most frequently found genotype of HBV was genotype A, which is infrequent in HIV-negative patients in Japan. Genotype A is often found in the United States, Europe, India, and the west coast of Sub-Saharan Africa [25]. Although the data on HBV subgenotypes were not available in our study, some reports showed that most genotype A strains detected in HIV/HBV-coinfected individuals are of genotype Ae [26]. These findings suggest that HBV infection among Japanese HIV carriers is not caused by the spread of indigenous HBV, such as transmission in the perinatal period, but rather specific strains are circulating among the homosexual population in Japan. Genotypes B and C accounted for more than 96 % of the entire Japanese chronic HBV infection [27, 28]. These findings are compatible with the report that the presumed transmission route of HBV in HIV/HBV-coinfected patients is not from Japanese female partners but from male partners, as shown by Koibuchi et al. [29].

Seventy-five percent of HIV/HBV-coinfected patients received ART with two agents against HBV, and its efficacy against HBV as well as HIV is considered to be high. As recommended by the United States Department of Health and Human Services (DHHS) and the Japanese guidelines on HIV treatment, the initiation of ART with NRTIs with anti-HBV activity as the backbone is indicated for HIV/HBV-coinfected patients regardless of HIV viral load or CD4+ T lymphocyte count [30]. Nucleoside analogues can improve liver function in HBV-monoinfected patients [31]. Our study shows that ART decreased the levels of ALT and albumin in HIV/HBV-coinfected patients. It is noteworthy that the regimen used in ART includes multiple drugs with anti-HBV activity such as lamivudine plus abacavir, which is unusual for HBV-monoinfected patients.

When we compared the characteristics of patients on ART with those not on ART, there were some notable differences in their immune status and liver function. At the beginning of the observation period, patients on ART showed a lower CD4+ T-cell count and poorer liver function. Our study is a retrospective observation, and patients were not grouped randomly. These observations are rather understandable because those who had a low CD4+ T cell count were more likely candidates for ART. Additionally, patients on ART had a longer observation period and were more likely to develop AIDS. These findings are also understandable because the longer the duration of HIV infection, the more likely is the immune system of the patient to deteriorate. Moreover, once ART is started, patients need to visit clinics or hospitals regularly for a long period; in reality, for the rest of their life. Following current recommendations for the initiation of ART for HIV infection, patients with worse immune status are more likely to receive the treatment. These findings can explain our observation.

Our data show that the serum albumin level and platelet count improved in the patients who were on ART. As the regimen of ART usually contains two drugs against HBV, ART suppresses HBV replication, which may lead to an improved liver function, as observed in HBV-monoinfected patients treated with nucleoside analogues [31]. Long-term treatment with lamivudine was shown to regress the fibrosis of the liver [32, 33] and decrease the proportion of patients with hepatocellular carcinoma complication [34]. In view of these findings, ART for HIV/HBV-coinfected patients may markedly improve the prognosis of patients. In our study, only a small number of patients with advanced liver diseases associated with HBV infection such as cirrhosis or hepatocellular carcinoma were observed, which could be attributable in part to the short observation period and the short duration of HBV infection. If we had a longer observational period, we would be able to clarify the difference in clinical course between the ART and non-ART groups, and the actual significance of ART for HIV/HBV-coinfected patients should become clearer.

We found that some parameters related to liver function changed paradoxically, particularly in the ART group. Although the mean serum albumin level, ALT level, and platelet count improved, the mean serum bilirubin level worsened, from 0.5 to 0.9 mg/dl. On the other hand, the serum bilirubin level in the non-ART group decreased. Both changes are statistically significant, which suggests

that the observed hyperbilirubinemia was not associated with HBV activity. The increase in serum bilirubin level is presumably caused by PIs. Hyperbilirubinemia following PI administration was previously reported [35]. Although it is unclear whether hyperbilirubinemia itself may lead to liver injury, PIs should be used carefully particularly for patients with advanced liver diseases.

Our present study has one major limitation; that is, the effect of alcohol on liver function was not analyzed because the history of alcohol consumption could not be obtained in the majority of the studied patients. Excessive alcohol consumption has been found to be an important risk factor for the development of severe hepatic injury in HIV-infected patients with [3] or without HCV coinfection [5]. Our present study showed that among the 26 patients whose history of alcohol consumption was available, only 2 patients were habitual drinkers. The results suggested that the effect of alcohol on liver function is small in HIV/HBV-coinfected patients in Japan.

In conclusion, ART with anti-HBV drugs may retard the progression of liver diseases and prevent liver-related death in HIV/HBV-coinfected patients. Multiple agents with anti-HBV activity seem essential for the efficacy. PIs should be carefully used particularly for patients with advanced liver diseases.

Acknowledgments We thank Ms. Ogawa for assistance in the survey. This work was supported in part by Health Sciences Research Grants from the Ministry of Health, Labor, and Welfare of Japan (Research on HIV/AIDS). We thank the hospitals in the HIV/AIDS Network of Japan for cooperation in this survey.

References

1. Ministry of Health, Labour and Welfare of Japan (ed). Annual Health, Labour and Welfare Report 2010–2011, Tokyo, Japan, 2011
2. Weber R, Sabin CA, Friis-Moller N, Reiss P, El-Sadr WM, Kirk O, et al. Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. Arch Intern Med. 2006;166:1632–41.
3. Sulkowski MS. Drug-induced liver injury associated with antiretroviral therapy that includes HIV-1 protease inhibitors. Clin Infect Dis. 2004;38(Suppl 2):S90–7.
4. Lemoine M, Serfaty L, Capeau J. From nonalcoholic fatty liver to nonalcoholic steatohepatitis and cirrhosis in HIV-infected patients: diagnosis and management. Curr Opin Infect Dis. 2012;25:10–6.
5. Chaudhry AA, Sulkowski MS, Chander G, Moore RD. Hazardous drinking is associated with an elevated aspartate aminotransferase to platelet ratio index in an urban HIV-infected clinical cohort. HIV Med. 2009;10:133–42.
6. Tanaka J, Koyama T, Mizui M, Uchida S, Katayama K, Matsuo J, et al. Total numbers of undiagnosed carriers of hepatitis C and B viruses in Japan estimated by age- and area-specific prevalence on the national scale. Intervirology. 2011;54:185–95.
7. Koike K, Kikuchi Y, Kato M, Takamatsu J, Shintani Y, Tsutsumi T, et al. Prevalence of hepatitis B virus infection in Japanese patients with HIV. Hepatol Res. 2008;38:310–4.
8. Spradling PR, Richardson JT, Buchacz K, Moorman AC, Brooks JT. Prevalence of chronic hepatitis B virus infection among patients in the HIV Outpatient Study, 1996–2007. J Viral Hepat. 2010;17:879–86.
9. Alter MJ. Epidemiology of viral hepatitis and HIV co-infection. J Hepatol. 2006;44:S6–9.
10. Sherman KE, Peters M, Koziel MJ. HIV and liver disease forum: conference proceedings. Hepatology. 2007;45:1566–77.
11. Weinbaum CM, Sabin KM, Santibanez SS. Hepatitis B, hepatitis C, and HIV in correctional populations: a review of epidemiology and prevention. AIDS 2005;19(suppl 3):S41–S416.
12. Salmon-Ceron D, Lewden C, Morlat P, Bevilacqua S, Jougla E, Bonnet F, et al. Liver disease as a major cause of death among HIV infected patients: role of hepatitis C and B viruses and alcohol. J Hepatol. 2005;42:799–805.
13. Thio CL, Seaberg EC, Skolasky R Jr, Phair J, Visscher B, Munoz A, et al. HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort Study (MACS). Lancet. 2002;360:1921–6.
14. Mendes-Correa M, Nunez M. Management of HIV and hepatitis virus coinfection. Expert Opin Pharmacother. 2010;11:2497–516.
15. Bessesen M, Ives D, Condreay L, Lawrence S, Sherman KE. Chronic active hepatitis B exacerbations in human immunodeficiency virus-infected patients following development of resistance to or withdrawal of lamivudine. Clin Infect Dis. 1999;28:1032–5.
16. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology. 2009;50:661–2.
17. Jain MK, Comanor L, White C, Kipnis P, Elkin C, Leung K, et al. Treatment of hepatitis B with lamivudine and tenofovir in HIV/HBV-coinfected patients: factors associated with response. J Viral Hepat. 2007;14:176–82.
18. Quarleri J, Moretti F, Bouzas MB, Laufer N, Carrillo MG, Giuliano SF, et al. Hepatitis B virus genotype distribution and its lamivudine-resistant mutants in HIV-coinfected patients with chronic and occult hepatitis B. AIDS Res Hum Retroviruses. 2007;23:525–31.
19. Sulkowski MS, Thomas DL, Chaisson RE, Moore RD. Hepatotoxicity associated with antiretroviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection. JAMA. 2000;283:74–80.
20. den Brinker M, Wit FW, Wertheim-van Dillen PM, Jurriaans S, Weel J, van Leeuwen R, et al. Hepatitis B and C virus co-infection and the risk for hepatotoxicity of highly active antiretroviral therapy in HIV-1 infection. AIDS 2000;14:2895–2902.
21. Alter MJ, Hadler SC, Margolis HS, Alexander WJ, Hu PY, Judson FN, et al. The changing epidemiology of hepatitis B in the United States. Need for alternative vaccination strategies. JAMA. 1990;263:1218–22.
22. Usuda S, Okamoto H, Iwanari H, Baba K, Tsuda F, Miyakawa Y, et al. Serological detection of hepatitis B virus genotypes by ELISA with monoclonal antibodies to type-specific epitopes in the preS2-region product. J Virol Methods. 1999;80:97–112.
23. Usuda S, Okamoto H, Tanaka T, Kidd-Ljunggren K, Holland PV, Miyakawa Y, et al. Differentiation of hepatitis B virus genotypes D and E by ELISA using monoclonal antibodies to epitopes on the preS2-region product. J Virol Methods. 2000;87:81–9.
24. Soriano V, Vispo E, Barreiro P. New 2011 updated DHHS antiretroviral treatment guidelines and chronic hepatitis B. AIDS. 2011;25:1013–4.
25. Miyakawa Y, Mizokami M. Classifying hepatitis B virus genotypes. Intervirology. 2003;46:329–38.
26. Shibayama T, Masuda G, Ajisawa A, Hiruma K, Tsuda F, Nishizawa T, et al. Characterization of seven genotypes (A to E, G and H) of hepatitis B virus recovered from Japanese patients infected with human immunodeficiency virus type 1. J Med Virol. 2005;76:24–32.

27. Orito E, Ichida T, Sakugawa H, Sata M, Horiike N, Hino K, et al. Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. Hepatology. 2001;34:590–4.
28. Matsuura K, Tanaka Y, Hige S, Yamada G, Murawaki Y, Komatsu M, et al. Distribution of hepatitis B virus genotypes among patients with chronic infection in Japan shifting toward an increase of genotype A. J Clin Microbiol. 2009;47:1476–83.
29. Koibuchi T, Hitani A, Nakamura T, Nojiri N, Nakajima K, Jyuji T, et al. Predominance of genotype A HBV in an HBV-HIV-1 dually positive population compared with an HIV-1-negative counterpart in Japan. J Med Virol. 2001;64:435–40.
30. Ratcliffe L, Beadsworth MB, Pennell A, Phillips M, Vilar FJ. Managing hepatitis B/HIV co-infected: adding entecavir to truvada (tenofovir disoproxil/emtricitabine) experienced patients. AIDS. 2011;25:1051–6.
31. Hyun JJ, Seo YS, Yoon E, Kim TH, Kim DJ, Kang HS, et al. Comparison of the efficacies of lamivudine versus entecavir in patients with hepatitis B virus-related decompensated cirrhosis. Liver Int. 2012;32:656–64.
32. Dienstag JL, Goldin RD, Heathcote EJ, Hann HW, Woessner M, Stephenson SL, et al. Histological outcome during long-term lamivudine therapy. Gastroenterology. 2003;124:105–17.
33. Suzuki Y, Arase Y, Ikeda K, Saitoh S, Tsubota A, Suzuki F, et al. Histological improvements after a three-year lamivudine therapy in patients with chronic hepatitis B in whom YMDD mutants did not or did develop. Intervirology. 2003;46:164–70.
34. Hiraoka A, Michitaka K, Kumagi T, Kurose K, Uehara T, Hirooka M, et al. Efficacy of lamivudine therapy for decompensated liver cirrhosis due to hepatitis B virus with or without hepatocellular carcinoma. Oncol Rep. 2005;13:1159–63.
35. Zucker SD, Qin X, Rouster SD, Yu F, Green RM, Keshavan P, et al. Mechanism of indinavir-induced hyperbilirubinemia. Proc Natl Acad Sci USA. 2001;98:12671–6.

Jpn. J. Infect. Dis., 65, 84-87, 2012

Short Communication

In Vitro Combination Effects of Aztreonam and Aminoglycoside against Multidrug-Resistant *Pseudomonas aeruginosa* in Japan

Hideki Araoka[1]*, Masaru Baba[1], Kazuhiro Tateda[2], Yoshikazu Ishii[2], Toyoko Oguri[3], Katsuko Okuzumi[4], Tsuyoshi Oishi[5], Shinichiro Mori[6], Toshihiro Mitsuda[7], Kyoji Moriya[8], Yoshitaka Nakamori[9], Norio Ohmagari[10], Keizo Yamaguchi[2], Akiko Yoneyama[1], and ABX Combination Therapy Study Group

[1]*Department of Infectious Diseases, Toranomon Hospital, Tokyo 105-8470;*
[2]*Department of Microbiology and Infectious Diseases, Toho University School of Medicine, Tokyo 143-8540;*
[3]*Laboratory Medicine, Kameda Medical Center, Chiba 296-8602;*
[4]*Department of Medical Safety Administration Division of Infection Control Dokkyo Medical University Hospital, Tochigi 321-0293;*
[5]*Department of Infectious Diseases, Tokyo Medical University Ibaraki Medical Center, Ibaraki 300-0395;*
[6]*Hematopoietic Stem Cell Transplantation Unit, National Cancer Center Hospital, Tokyo 104-0045;*
[7]*Department of Infection Prevention and Control, Yokohama City University Hospital, Kanagawa 236-0004;*
[8]*Department of Infection Control and Prevention, Graduate School of Medicine Faculty of Medicine, The University of Tokyo, Tokyo 113-0033;*
[9]*Department of Respiratory Diseases, Misyuku Hospital, Tokyo 153-0051; and*
[10]*Division of Infectious Diseases, Shizuoka Cancer Center, Shizuoka 411-8777, Japan*

(Received August 8, 2011. Accepted November 8, 2011)

SUMMARY: The aim of this study was to evaluate the in vitro combination effects of aztreonam (AZT) and aminoglycosides against multidrug-resistant (MDR) *Pseudomonas aeruginosa* strains in Japan. We investigated 47 MDR *P. aeruginosa* strains collected from 8 facilities. We selected the aminoglycosides amikacin (AMK), gentamicin (GM), and arbekacin (ABK) to examine their effects when combined with AZT using the checkerboard method. Of the 47 MDR *P. aeruginosa* strains, 41 tested positive for metallo-β-lactamase (MBL). In all combinations, aminoglycosides decreased the minimum inhibitory concentrations of AZT in a dose-dependent manner, and there was no apparent antagonism. The combination effects were scored on a scale of 0 to 4, and statistical analysis was performed using the Wilcoxon signed-rank test. In all 47 strains, AZT + ABK (mean score, 2.02) had the highest score, followed by AZT + AMK (1.68) and AZT + GM (1.38) (ABK versus GM, $P < 0.0001$). In 41 MBL-positive strains, AZT + ABK (mean score, 2.05) had the highest score, followed by AZT + AMK (1.56) and AZT + GM (1.37) (ABK versus AMK, $P = 0.02$, and ABK versus GM, $P < 0.0001$). AZT + ABK was the most promising combination regimen against MDR *P. aeruginosa* strains; the other promising combinations were AZT + AMK and AZT + GM.

Pseudomonas aeruginosa is a clinically significant Gram-negative rod and an important cause of hospital-acquired infection, particularly in immunocompromised patients. Multidrug-resistant (MDR) *P. aeruginosa* is emerging as a serious problem in clinical settings worldwide. Since intravenous colistin is not available in Japan, combination therapy is required. Tateda et al. have suggested the usefulness of a "Break-point Checkerboard Plate" to screen appropriate antibiotic combinations against MDR *P. aeruginosa* (1). A "Break-point Checkerboard Plate" is used to evaluate the effect of combination therapy with reference to the breakpoint concentration, which correlates with clinical efficacy. It allows for simultaneous evaluation of the effect of combination antimicrobial therapy using 8 clinically important agents (ceftazidime, piperacillin, imipenem [IMP], aztreonam [AZT], gentamicin [GM], ciprofloxacin, polymyxin B, and rifampicin) on a single plate. In Japan, a commercially available "Break-point Checkerboard Plate" is the BC plate EIKEN (Eiken Chemical, Tokyo, Japan), which includes amikacin (AMK), meropenem, and colistin instead of GM, IMP, and polymyxin B. IMP-type metallo-β-lactamase (MBL) production is frequently observed in highly resistant *P. aeruginosa* strains isolated in Japan (2). MBL-producing *P. aeruginosa* strains often remain susceptible to monobactams (3,4). Our previous study also demonstrated that the combination of AZT and aminoglycoside was often effective against *P. aeruginosa*, both in vitro and in vivo (5). In this study, we investigated the in vitro effects of a combination of AZT and aminoglyco-

*Corresponding author: Mailing address: Department of Infectious Diseases, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan. Tel: +81-3-3588-1111, Fax: +81-3-3582-7068, E-mail: h-araoka@toranomon.gr.jp

side against MDR *P. aeruginosa* strains collected from multiple centers.

MDR *P. aeruginosa* was defined as *P. aeruginosa* resistant to aminoglycosides (minimum inhibitory concentration [MIC] of AMK $\geq 32\,\mu g/mL$), carbapenems (MIC of IMP $\geq 16\,\mu g/mL$), and fluoroquinolones (MIC of ciprofloxacin $\geq 4\,\mu g/mL$).

Between 2003 and 2006, 47 MDR *P. aeruginosa* strains were collected by the ABX Combination Therapy Study Group (ACTs) from unrelated patients under treatment at 8 facilities throughout Japan. The MDR *P. aeruginosa* strains were obtained from the urinary tract in 24 patients, blood in 6, respiratory tract in 6, and other sites in 4. The survey did not indicate the source of the remaining 7 strains. AMK, GM, and arbekacin (ABK) were selected as aminoglycosides to be combined with AZT using the checkerboard method. The Clinical and Laboratory Standards Institute recommended standards of antibiotic susceptibility testing were applied in this study (6,7). MBL was detected using the sodium mercaptoacetate (SMA) disc method, employing SMA, ceftazidime, and IMP disks.

The clinically therapeutic MIC for AZT was 16 $\mu g/mL$ (intermediate). The combination of AZT and each aminoglycoside was defined as effective at the following concentrations: $16\,\mu g/mL$ AZT (intermediate) + $32\,\mu g/mL$ AMK (intermediate), $16\,\mu g/mL$ AZT (intermediate) + $8\,\mu g/mL$ GM (intermediate), and 16 $\mu g/mL$ AZT (intermediate) + $8\,\mu g/mL$ ABK (defined as intermediate). Since the breakpoint of ABK against *P. aeruginosa* was not defined, the GM criterion was used as an alternative (7). Combination effects were scored and evaluated. In this study, scoring was performed using a 0–4 scale to evaluate the combination effects with reference to the breakpoint concentration established by the "Break-point Checkerboard Plate" correlated with clinical efficacy. A score of 4 (inhibited bacterial growth in the following combination: AZT [susceptible] + aminoglycoside [susceptible]) indicated the most promising combined effect, while a score of 0 (bacterial growth not inhibited even by the following combination: AZT [intermediate] + aminoglycoside [intermediate]) indicated that there was no combination effect (Fig. 1). Statistical analysis was performed using the Wilcoxon signed-rank test. A P value <0.05 was considered significant. All analyses were conducted using SPSS (version 11.0 for Windows; SPSS Inc., Chicago, Ill., USA).

Of the 47 MDR *P. aeruginosa* strains, 41 were MBL positive and 6 were negative. The MICs of AZT as a single agent were as follows: $8\,\mu g/mL$ (2 strains), 32 $\mu g/mL$ (18 strains), $64\,\mu g/mL$ (18 strains), and >128 $\mu g/mL$ (9 strains). The clinically therapeutic MIC of AZT was $16\,\mu g/mL$ (intermediate). AZT as a single agent achieved an effect that is likely to be clinically significant in only 4% of strains (2 of 47 strains). In all combinations, aminoglycoside decreased the MICs of AZT in a dose-dependent manner, and there was no apparent antagonism. The combinations of AZT and aminoglycosides required to achieve these effects were as follows: $16\,\mu g/mL$ AZT + $32\,\mu g/mL$ AMK (77%, 36 of 47 strains), $16\,\mu g/mL$ AZT + $8\,\mu g/mL$ GM (43%, 20 of 47 strains), and $16\,\mu g/mL$ AZT + $8\,\mu g/mL$ ABK (79%, 37 of 47 strains).

In addition, the combination effects were scored and evaluated as described above. For the combination effect score, AZT + ABK (mean score, 2.02) was the highest, followed by those of AZT + AMK (mean score, 1.68) and AZT + GM (mean score, 1.38) in all 47 strains. The combined effect of AZT + ABK was significantly higher than that of AZT + GM ($P < 0.0001$) (Fig. 2). The combination effects on 41 MBL-positive strains were highest for AZT + ABK (mean score, 2.05), followed by AZT + AMK (mean score, 1.56) and

antibiotic B (aminoglycosides)			
	intermediate	—	—
	susceptible	—	—
Score 4		susceptible	intermediate
		antibiotic A (aztreonam)	

antibiotic B (aminoglycosides)			
	intermediate	—	—
	susceptible	+	—
Score 3		susceptible	intermediate
		antibiotic A (aztreonam)	

antibiotic B (aminoglycosides)			
	intermediate	—	—
	susceptible	+	+
Score 2		susceptible	intermediate
		antibiotic A (aztreonam)	

antibiotic B (aminoglycosides)			
	intermediate	+	—
	susceptible	+	—
Score 2		susceptible	intermediate
		antibiotic A (aztreonam)	

antibiotic B (aminoglycosides)			
	intermediate	+	—
	susceptible	+	+
Score 1		susceptible	intermediate
		antibiotic A (aztreonam)	

antibiotic B (aminoglycosides)			
	intermediate	+	+
	susceptible	+	+
Score 0		susceptible	intermediate
		antibiotic A (aztreonam)	

Fig. 1. Combination effects were scored and evaluated. Minus signs indicate each drug combination concentration that inhibited bacterial growth. Scoring was performed using a scale from 0 to 4 in this study. Score 4 (inhibited bacterial growth in the following combination, aztreonam [AZT]: susceptible + aminoglycoside: susceptible) indicated the most promising combined effect, while score 0 (bacterial growth not inhibited even by the following combination, AZT: intermediate + aminoglycoside: intermediate) indicated that there was no combination effect.

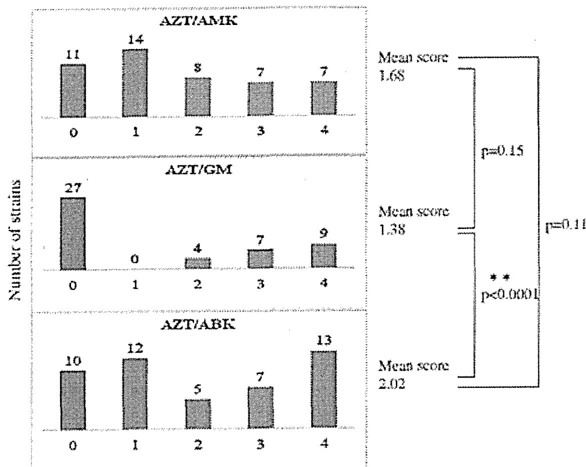

Fig. 2. Scoring of combination effects for each drug combination against all 47 multidrug-resistant (MDR) *Pseudomonas aeruginosa* strains. AZT, aztreonam; AMK, amikacin; GM, gentamicin; ABK, arbekacin.

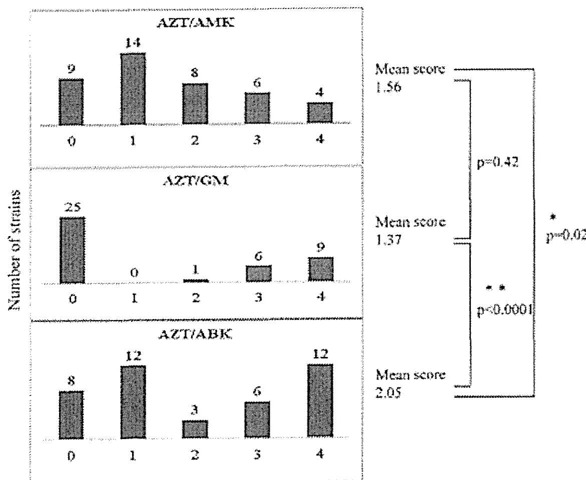

Fig. 3. Scoring of combination effects for each drug combination against 41 metallo-β-lactamase (MBL)-positive MDR *P. aeruginosa* strains.

AZT + GM (mean score, 1.37). The combined effect of AZT + ABK was significantly higher than that of AZT + GM ($P < 0.0001$) and AZT + AMK ($P = 0.02$) (Fig. 3).

In Japan, the production of MBL is often implicated in the high-level resistance of *P. aeruginosa*. It has been reported that IMP is encoded by the bla_{IMP} gene on an integron (2). In this study, out of the 47 MDR *P. aeruginosa* strains, 41 were MBL positive (18 strains for IMP-1, 10 for IMP-7, 12 for IMP-10, and 1 for VIM-2); 6 strains were MBL negative, and most MBL-producing MDR *P. aeruginosa* strains were the IMP type, as previously reported (2). PCR detection of various MBLs was performed by Hiroshi Kataoka, and this information was received as a personal communication.

Strains producing MBLs often remain susceptible to monobactams. However, AZT as a single agent achieved a clinical effect in only 4% of the strains (2 of 47 strains). AZT and aminoglycosides in combination were associated with a high probability of achieving a clinical effect. In addition, there was no antagonism. Therefore, the combination of AZT and aminoglycosides seems to be promising. This regimen may provide an effective second line of therapy for patients in whom intravenous colistin cannot be used. Our study was conducted to evaluate which aminoglycoside was appropriate to be used in combination with AZT. With respect to the combination effects, AZT + ABK showed the highest scores, followed by AZT + AMK and AZT + GM. Statistical analysis indicated that the combined effect of AZT + ABK was significantly higher than that of AZT + GM in all 47 strains.

In Japan, studies have shown that the mechanism most frequently underlying resistance to aminoglycosides was inactivation of the antibacterial agent by aminoglycoside-modifying enzymes (8,9). Other known mechanisms include the methylation of 16S rRNA (10) and increased expression of drug-efflux pumps (11). ABK, the semisynthetic aminoglycoside used in clinical settings in Japan, is effective against Gram-negative bacilli, including *P. aeruginosa*, as well as methicillin-resistant *Staphylococcus aureus* (MRSA) (12). ABK is stable against most aminoglycoside-modifying enzymes, and only the bifunctional enzyme AAC(6′)/APH(2″) is known to have low or moderate resistance to ABK (13,14). Therefore, ABK, similar to AMK, is regarded a strong candidate for combination use with AZT. Further studies are needed to analyze MDR *P. aeruginosa* strains from the perspective of aminoglycoside-modifying enzymes.

In conclusion, our study showed that AZT + ABK was the most promising combination regimen against MDR *P. aeruginosa* strains; the other promising regimens were AZT + AMK and AZT + GM.

Acknowledgments This work was supported in part by a Research Grant from Okinaka Memorial Institute for Medical Research, Tokyo, Japan.

This work was presented in part at the 50th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Boston, USA, September 12–15, 2010.

Conflict of interest None to declare.

REFERENCES

1. Tateda, K., Ishii, Y., Matsumoto, T., et al. (2006): 'Break-point Checkerboard Plate' for screening of appropriate antibiotic combinations against multidrug-resistant *Pseudomonas aeruginosa*. Scand. J. Infect. Dis., 38, 268–272.
2. Sekiguchi, J., Asagi, T., Miyoshi-Akiyama, T., et al. (2007): Outbreaks of multidrug-resistant *Pseudomonas aeruginosa* in community hospitals in Japan. J. Clin. Microbiol., 45, 979–989.
3. Docquier, J.D., Riccio, M.L., Mugnaioli, C., et al. (2003): IMP-12, a new plasmid-encoded metallo-β-lactamase from a *Pseudomonas putida* clinical isolate. Antimicrob. Agents Chemother., 47, 1522–1528.
4. Docquier, J.D., Lamotte-Brasseur, J., Galleni, M., et al. (2003): On functional and structural heterogeneity of VIM-type metallo-β-lactamases. J. Antimicrob. Chemother., 51, 257–266.
5. Araoka, H., Baba, M., Takagi, S., et al. (2010): Monobactam and aminoglycoside combination therapy against metallo-β-lactamase-producing multidrug-resistant *Pseudomonas aeruginosa* screened using a 'break-point checkerboard plate'. Scand. J. Infect. Dis., 42, 231–233.
6. Clinical and Laboratory Standard Institute (2009): Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard–8th Edition. vol. M07-

A8. Clinical and Laboratory Standard Institute, Wayne, Pa.
7. Clinical and Laboratory Standard Institute (2009): Performance Standards for Antimicrobial Susceptibility Testing; 19th Informational Supplement. vol. M100-S19. Clinical and Laboratory Standard Institute, Wayne, Pa.
8. Sekiguchi, J., Asagi, T., Miyoshi-Akiyama, T., et al. (2005): Multidrug-resistant *Pseudomonas aeruginosa* strain that caused an outbreak in a neurosurgery ward and its aac(6')-*Iae* gene cassette encoding a novel aminoglycoside acetyltransferase. Antimicrob. Agents Chemother., 49, 3734-3742.
9. Shimizu, K., Kumada, T., Hsieh, W.C., et al. (1985): Comparison of aminoglycoside resistance patterns in Japan, Formosa, and Korea, Chile, and the United States. Antimicrob. Agents Chemother., 28, 282-288.
10. Yokoyama, K., Doi, Y., Yamane, K., et al. (2003): Acquisition of 16S rRNA methylase gene in *Pseudomonas aeruginosa*. Lancet, 362, 1888-1893.
11. Masuda, N., Sakagawa, E., Ohya, S., et al. (2000): Substrate specificities of MexAB-OprM, MexCD-OprJ, and MexXY-oprM efflux pumps in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother., 44, 3322-3327.
12. Kobayashi, Y., Uchida, H. and Kawakami, Y. (1992): Synergy with aztreonam and arbekacin or tobramycin against *Pseudomonas aeruginosa* isolated from blood. J. Antimicrob. Chemother., 30, 871-872.
13. Kondo, S. and Hotta, K. (1999): Semisynthetic aminoglycoside antibiotics: development and enzymatic modifications. J. Infect. Chemother., 5, 1-9.
14. Zhu, C.B., Sunada, A., Ishikawa, J., et al. (1999): Role of aminoglycoside 6'-acetyltransferase in a novel multiple aminoglycoside resistance of an actinomycete strain #8: inactivation of aminoglycosides with 6'-amino group except arbekacin and neomycin. J. Antibiot., 52, 889-894.

INFECTION CONTROL AND HOSPITAL EPIDEMIOLOGY JANUARY 2012, VOL. 33, NO. 1

ORIGINAL ARTICLE

Pandemic 2009 Influenza A (H1N1) Virus among Japanese Healthcare Workers: Seroprevalence and Risk Factors

Yoko Nukui, MD, PhD;[1] Shuji Hatakeyama, MD, PhD;[2] Takatoshi Kitazawa, MD, PhD;[1] Tamami Mahira, RN;[3] Yoshizumi Shintani, MD;[1] Kyoji Moriya, MD, PhD[1]

OBJECTIVE. To evaluate the seroprevalence and risk factors for 2009 influenza A (H1N1) virus infection among healthcare personnel.

DESIGN. Observational cross-sectional study.

PATIENTS AND SETTING. Healthcare workers (HCWs) in an acute care hospital.

METHODS. Between September 14 and October 4, 2009, before 2009 H1N1 vaccination, we collected serological samples from 461 healthy HCWs. Hemagglutination-inhibition antibody assays were conducted. To evaluate the risk factors of seropositivity for 2009 H1N1 virus, gender, age, profession, work department, usage of personal protective equipment, and seasonal influenza vaccination status data were gathered via questionnaires.

RESULTS. Our survey showed that doctors and nurses were at highest risk of seropositivity for the 2009 H1N1 virus (odds ratio [OR], 5.25 [95% confidence interval {CI}, 1.21–22.7]). An increased risk of seropositivity was observed among pediatric, emergency room, and internal medicine staff (adjusted OR, 1.98 [95% CI, 1.07–3.65]). Risk was also higher among HCWs who had high titers of antibodies against the seasonal H1N1 virus (adjusted OR, 1.59 [95% CI, 1.02–2.48]).

CONCLUSIONS. Seropositivity for the 2009 H1N1 virus was associated with occupational risk factors among HCWs.

Infect Control Hosp Epidemiol 2012;33(1):58-62

Pandemic 2009 influenza A (H1N1) virus infections were first reported during April in Mexico and the United States.[1] A few weeks later, the virus had spread worldwide. On June 11, 2009, the World Health Organization announced a phase-6 pandemic alert as the 2009 H1N1 outbreak achieved pandemic status.[2] In Japan, the incidence of 2009 H1N1 infection began to increase in August 2009, that is, 3–4 months prior to the usual influenza season. The indigenous transmission of 2009 H1N1 infection in Japan, as well as in many other countries, largely involved children.[3]

Because of their constant close interaction with infected patients, healthcare workers (HCWs) are most likely to be at greater risk for contracting influenza than the general public.[4] Patient-to-HCW transmission and infection with 2009 H1N1 virus have been reported in the United States[5] and in Germany.[6] It is not clear, however, which HCWs were most affected by the 2009 H1N1 virus. Moreover, in hospitals, the prevention of 2009 H1N1 outbreaks was an important issue. In particular, concerns were raised by the possibility that even mild disease could result in staff absenteeism, with subsequent reduction of staff strength at a time of increased demand for health services. Nevertheless, cases of patient-to-HCW transmission may have occurred due to inadequate use of personal protective equipment (PPE). The objective of this study was, therefore, to identify which HCWs were at highest risk for 2009 H1N1 virus infection in an acute-care hospital setting.

METHODS

Subjects and Study Procedures

A cross-sectional study comprising different divisions of HCWs was performed at the University of Tokyo Hospital, an acute-care hospital with 1,250 beds and a medical team of approximately 2,000 workers. HCWs who were scheduled to receive the 2009 H1N1 vaccine and agreed to participate in the study were enrolled. From August 2009 onward, HCWs who received a diagnosis of or were highly suspected of having influenza A were excluded from vaccination because of the vaccine shortage faced in that year.

We especially focused on doctors and nurses for precise analysis. On the basis of the frequency of exposure to patients with influenza-like illness, recruited doctors ($n = 262$) and nurses ($n = 176$) were subdivided into 4 groups: pediatric staff (group 1, $n = 147$), emergency room staff (group 2,

Affiliations: 1. Department of Infection Control and Prevention, University of Tokyo Hospital, Tokyo, Japan; 2. Department of Infectious Diseases, University of Tokyo Hospital, Tokyo, Japan; 3. Department of Nursing, University of Tokyo Hospital, Tokyo, Japan.

Received June 27, 2011; accepted September 13, 2011; electronically published November 21, 2011.

© 2011 by The Society for Healthcare Epidemiology of America. All rights reserved. 0899-823X/2012/3301-0010$15.00. DOI: 10.1086/663208

This content downloaded on Tue, 19 Feb 2013 05:30:44 AM
All use subject to JSTOR Terms and Conditions

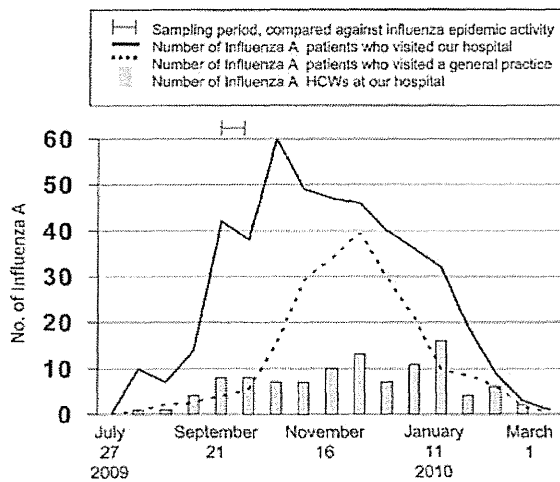

FIGURE 1. Epidemic curves constructed from the number of patients with influenza A and the number of healthcare workers (HCWs) at the University of Tokyo Hospital.

$n = 66$), internal medicine staff (group 3, $n = 142$), and other medical division staff (group 4, $n = 83$). In addition, for comparative purposes, a group of comedical staff ($n = 23$) with no direct contact with patients was also included. Personal data, including gender, age, profession, work department, usage of PPE, and seasonal influenza vaccination status, were gathered with appropriate questionnaires. Compliance with PPE rules (ie, use of surgical masks and gloves) was assessed as the frequency of PPE use during a 4-month period beginning in May 2009, through a 4-point scale: 1, always; 2, most of the time; 3, sometimes; 4, never. We defined appropriate PPE use as categories 1 and 2.

The numbers of patients and HCWs who received a diagnosis of influenza A were reported daily to our infection control team. In addition, we obtained data on Japanese influenza epidemic activity from the national sentinel surveillance.

Sample Collection and Laboratory Procedures

Serum samples were collected before vaccination, between September 14 and October 4, 2009. After collection, serum samples were pretreated with receptor-destroying enzyme (RDE; RDE II, Denka Seiken, Tokyo, Japan) for 18 hours at 37°C to inactivate nonspecific inhibitors. After incubation for 30 minutes at 56°C to inactivate the RDE, the samples were further treated by absorption onto chicken red blood cells for 1 hour at room temperature to remove nonspecific agglutinins. After centrifugation, supernatants were tested with 1 : 2 serial dilutions at an initial dilution of 1 : 10 for the hemagglutination-inhibition (HI) antibody assays. The serum samples were tested against the 2009 H1N1 virus (A/California/7/2009) and seasonal H1N1 virus (2008–2009 seasonal vaccine strain A/Brisbane/59/2007). The tests were performed at Mitsubishi Chemical Medience, Japan.

Data Analysis

Geometric mean titers (GMTs) were estimated by assigning the value of 1 : 5 for titers lower than 1 : 10. Seropositivity against 2009 H1N1 was defined as titers above 1 : 10 on the basis of previous seroepidemiologic studies.[7] Regarding seasonal H1N1, seropositivitity was defined as titers above 1 : 40.[8]

Univariate and multivariate logistic-regression analyses were performed, using the following variables: age, gender, profession, work department, contact with patients, and seasonal H1N1 influenza vaccination status. Multivariate analysis was based on a stepwise approach in which variables that did not improve the model fit at $P < .2$ were discarded. Odds ratios (ORs) with asymptotic Wald 95% confidence intervals (CIs) and 2-sided P values were reported. Statistical significance was set at $P \leq .05$. All statistical analyses were performed using SPSS Statistics 18 (IBM).

Ethics Review

Written informed consent forms were obtained from all participants. The ethics review board of the University of Tokyo approved the study.

RESULTS

Figure 1 shows that at our hospital, the influenza A epidemic curves peaked earlier than did those in other general-practice hospitals. This is because our hospital was designated as specialized in the treatment of 2009 H1N1 influenza. Between

TABLE 1. Cohort Characteristics

	Doctor	Nurse	Comedical staff
No. (%) of participants	262 (57)	176 (38)	23 (5)
Median (range) age, years	32 (24–56)	30 (22–58)	36 (25–59)
Age in years, no. (%)			
20–29	119 (45)	94 (54)	6 (26)
30–39	98 (37)	66 (37)	12 (52)
40–59	44 (17)	16 (9)	5 (22)
No. (%) of men	179 (68)	13 (7)	7 (30)
No. (%) of seasonal influenza vaccine recipients	159 (60)	145 (83)	16 (70)

This content downloaded on Tue, 19 Feb 2013 05:30:44 AM
All use subject to JSTOR Terms and Conditions

TABLE 2. Participants' Characteristics and Distribution of Antibody Titer against 2009 Influenza A (H1N1) Virus before Vaccination

	No. (%) of participants	Distribution of antibody titer, no. (%) of patients			GMT (95% CI)	P
		<10	10–20	≥40		
Department						
Group 1 (pediatric staff)	147 (34)	90 (61)	44 (30)	13 (9)	13.2 (8.5–17.9)	.005[a]
Group 2 (emergency workers)	66 (15)	39 (59)	21 (32)	6 (9)	10.8 (7.8–13.9)	.006[a]
Group 3 (internal medicine staff)	142 (32)	96 (68)	38 (27)	8 (5)	10.1 (8.0–12.2)	.001[a]
Group 4 (other medical division staff)	83 (19)	67 (81)	16 (19)	0 (0)	6.3 (5.6–7.0)	
Contact with patients with influenza-like illness						
Yes, without PPE	62 (14)	39 (63)	15 (24)	8 (13)	17.2 (6.6–27.7)	.17[b]
Yes, with PPE	179 (41)	119 (66)	51 (28)	9 (5)	9.9 (8.2–11.7)	.87[b]
No	197 (45)	134 (68)	53 (27)	10 (5)	9.8 (8.0–11.5)	
Age groups						
20–29	213 (49)	150 (70)	51 (24)	12 (6)	10.4 (7.2–13.6)	
30–39	164 (37)	102 (62)	51 (31)	11 (7)	11.1 (9.1–13.1)	.72[c]
40–59	61 (14)	40 (66)	17 (28)	4 (6)	12.0 (7.6–16.3)	.57[c]
Seasonal influenza vaccination in the past 3 years						
≥2 times	375 (88)	245 (65)	107 (27)	23 (8)	10.0 (8.4–11.6)	.04
<2 times	63 (12)	47 (75)	12 (19)	4 (6)	7.3 (5.2–9.4)	
Antibody titer of seasonal H1N1 virus						
≥40	288 (66)	181 (63)	85 (29)	22 (8)	12.2 (9.6–14.8)	.01
<40	150 (34)	111 (74)	34 (23)	5 (3)	8.4 (6.8–10.1)	

NOTE. CI, confidence interval; GMT, geometric mean antibody titers; PPE, personal protective equipment.
[a] χ^2 test comparing groups 1–3 and group 4.
[b] χ^2 test comparing contact and noncontact groups.
[c] χ^2 test comparing the respective age group and the 20–29 years age group.

the beginning of the pandemic and the sampling period, fewer than 10 HCWs in our hospital were affected by influenza A.

A total of 461 HCWs were included in this study. The cohort characteristics are provided in Table 1. The median age of participants varied between 30 (nurses) and 36 (comedical staff) years. Doctors were predominantly men (68%), and the nurses and other comedical staff were predominantly women (93% and 70%, respectively). Only 60% of the doctors and 83% of the nurses had received the seasonal H1N1 influenza vaccination in 2009.

GMTs for 2009 H1N1 virus antibodies of doctors (GMT, 11.7 [95% CI, 8.9–14.6]) and nurses (GMT, 8.8 [95% CI, 7.4–10.2]) were higher than those of the comedical staff (GMT, 5.4 [95% CI, 4.8–6.1]). Higher seroprevalence was observed in doctors and nurses compared with comedical staff (OR, 5.25 [95% CI, 1.21–22.7]). Doctors and nurses ($n = 438$) were thus subjected to further analysis. GMTs for HI antibodies against the 2009 H1N1 virus were significantly higher ($P < .01$) in groups 1–3 (by approximately 1.6–2.1-fold) than in group 4 (Table 2). Second, doctors and nurses who had received seasonal influenza vaccines more than twice within the previous 3 years showed significantly higher ($P = .04$) GMT values than did the others. In addition, GMT values were also significantly higher ($P = .05$) in doctors and nurses who had high seasonal H1N1 virus antibody titers than in those who had low seasonal H1N1 antibody titers (Table 2).

Regarding the use of personal protective equipment, GMT values of doctors and nurses who had not used proper PPE (eg, surgical masks or gloves) while in contact with patients who had influenza-like illness tended to be slightly higher than those of workers who had no contact with patients who had influenza-like illness (Table 2). However, these differences were not statistically significant. Additionally, the GMT values of doctors and nurses who had used PPE while in contact with patients who had influenza-like illness did not differ from the values observed in the no-contact group.

The results of the univariate and multivariate analyses of doctors and nurses who were seropositive for 2009 H1N1 virus are presented in Table 3. An increased risk for seropositivity was found for doctors and nurses belonging to groups 1–3 compared with medical staff belonging to group 4 (adjusted OR, 1.98 [95% CI, 1.07–3.65]).

DISCUSSION

Our study shows that a higher seroprevalence was observed in doctors and nurses compared with comedical staff. The seroprevalences for doctors and nurses were 7% and 5%, respectively. One possible explanation for these low values is

This content downloaded on Tue, 19 Feb 2013 05:30:44 AM
All use subject to JSTOR Terms and Conditions

TABLE 3. Univariate and Multivariate Analysis of Factors Associated with Seropositivity for the 2009 Influenza A (H1N1) Virus

	Proportion of seropositivity, %	Crude OR (95% CI)	P	Adjusted OR (95% CI)	P
Department (pediatric, emergency, internal medicine staff)	36.6	2.42 (1.35–4.35)	.003	1.98 (1.07–3.65)	.03
Contact with patients with influenza-like illness without PPE	37.1	1.18 (0.68–2.06)	.56	NA	NA
Seasonal influenza vaccination ≥2 times in the past 3 years	34.7	1.56 (0.85–2.86)	.15	0.97 (0.63–1.51)	.9
Antibody titer of seasonal H1N1 virus ≥40	37.2	1.68 (1.09–2.60)	.02	1.59 (1.02–2.48)	.04

NOTE. CI, confidence interval; NA, not applicable; OR, odds ratio; PPE, personal protective equipment.

that our study was conducted during the initial period of the epidemic. In this study, HCWs who worked as pediatric staff, emergency workers, or internal medicine staff also showed significantly higher seroprevalences than did other medical division staff. No significant differences between these 2 groups were found either for the history of seasonal H1N1 infection or for vaccination. This may suggest that occupational exposure is an important factor associated with preexisting antibodies. Our results are in agreement with the findings of other studies, which reported that HCWs in the emergency room had the highest risk of infection.[9,10]

Additionally, our cross-sectional study demonstrates that HCWs with high baseline seasonal H1N1 titers showed high seropositivity for the 2009 H1N1 virus. This is in agreement with the finding that elevated preexposure antibody titers to the 2009 H1N1 virus was associated with previous infection with the seasonal H1N1 virus occurring in 2007.[11] These findings are further supported by the evidence that memory cytotoxic T lymphocytes established by seasonal influenza A viruses may show cross-reactivity against the 2009 H1N1 virus.[12] On the other hand, others reported that there is no significant relationship between the 2009 H1N1 virus antibodies and the presence or absence of antibodies against the seasonal H1N1 virus.[13,14] It remains unclear whether the seasonal influenza vaccination provides cross-reactive antibody response to the pandemic H1N1 influenza. Thus, further studies are needed to determine whether these different results are due to exposure to the seasonal influenza virus, seasonal influenza vaccination, or community-specific factors.

For infection control, surgical masks and gloves may have helped to prevent the transmission of 2009 H1N1 influenza. Nevertheless, in our study, the specific effects of these measures are difficult to ascertain because statistically significant differences in GMT were not observed in workers using PPE as compared with their colleagues, and multivariate analysis did not identify this as a risk factor. In contrast, Cheng et al[15] reported that the failure to comply with the standard precautions such as wearing a surgical mask during contact with suspected influenza patients was associated with an increased risk of 2009 H1N1 infection.

Several limitations of our study may, however, have contributed to the results obtained. First, seropositivity in our study did not include data regarding HCWs who received a diagnosis of or were highly suspected of having influenza A from May 2009 to just before vaccination (ie, at the time of blood sample collection); these HCWs were excluded from the 2009 H1N1 vaccination because of vaccine shortage. This may have affected seroprevalence and risk analysis in our study. However, it is worthy of note that between the beginning of the pandemic and the sampling period, the number of HCWs affected by influenza A in our hospital was less than 10. Furthermore, these were evenly distributed by the job categories considered herein. Therefore, we believe that the effect of excluding the symptomatic HCWs from this study was limited. Second, the low seroconversion rates in our cohort reduced the power of the study to investigate exposures more weakly associated with the effect of PPE. Moreover, we did not collect detailed data on whether there was strict adherence to the use of PPE.

In the process of establishing vaccination policies and identifying vaccination priority groups, it is imperative to determine the relative risk of contracting the disease that the various groups face. HCWs were considered a priority group for vaccination against the 2009 H1N1 virus because they were at high risk of contracting the disease. Additionally, HCWs may serve as a target group for prevaccination surveillance in whom early seroepidemiological changes indicating novel, emergent infectious diseases may be detected and vaccine efficacy evaluated.

ACKNOWLEDGMENTS

We thank Yuko Tago and Rie Suzuki of the Department of Infection Control and Prevention, University of Tokyo Hospital, for their contributions to this study.

Financial support. This work was funded by a grant from the Japanese Ministry of Health, Labor, and Welfare.

Potential conflicts of interest. All authors report no conflicts of interest relevant to this article. All authors submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and the conflicts that the editors consider relevant to this article are disclosed here.

Address correspondence to Kyoji Moriya, MD, Professor and Chairman,

This content downloaded on Tue, 19 Feb 2013 05:30:44 AM
All use subject to JSTOR Terms and Conditions

Department of Infection Control and Prevention, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan (moriya-tky@umin.ac.jp).

Presented in part: SHEA 2011 Annual Scientific Meeting; Dallas, Texas; April 2010.

REFERENCES

1. Jain R, Goldman RD. Novel influenza A (H1N1): clinical presentation, diagnosis, and management. *Pediatr Emerg Care* 2009; 25:791–796.
2. Chan PK. Lancet conference: influenza in the Asia-Pacific and the international scientific symposium on influenza pandemic response and preparedness. *Expert Rev Anti Infect Ther* 2009;7: 1165–1166.
3. Wada K, Nishiura H, Kawana A. An epidemiological analysis of severe cases of the influenza A (H1N1) 2009 virus infection in Japan. *Influ Other Resp Viruses* 2010;4:179–186.
4. Bernard H, Fischer R, Mikolajczyk RT, Kretzschmar M, Wildner M. Nurses' contacts and potential for infectious disease transmission. *Emerg Infect Dis* 2009;15:1438–1444.
5. Novel influenza A (H1N1) virus infections among healthcare personnel: United States, April–May 2009. *MMWR Morb Mortal Wkly Rep* 2009;58:641–645.
6. Melzl H, Wenzel JJ, Kochanowski B, et al. First sequence-confirmed case of infection with the new influenza A (H1N1) strain in Germany. *Eurosurveillance* 2009;14:19203.
7. Jackson C, Vynnucky E, Mangtani P. Estimates of the transmissibility of the 1968 (Hong Kong) influenza pandemic: evidence of increased transmissibility between successive waves. *Am J Epidemiol* 2010;171:465–478.
8. Carrat F, Vergu E, Ferguson NM, et al. Time lines of infection and disease in human influenza: a review of volunteer challenge studies. *Am J Epidemiol* 2008;167:775–785.
9. Zhou Y, Ng DMW, Seto WH, et al. Seroprevalence of antibody to pandemic influenza A (H1N1) 2009 among healthcare workers after the first wave in Hong Kong. *J Hosp Infect* 78:308–311.
10. Lau LL, Cowling BJ, Fang VJ, et al. Viral shedding and clinical illness in naturally acquired influenza virus infections. *J Infect Dis* 2010;201:1509–1516.
11. Lemaitre M, Leruez-Ville M, Lamballerie XN, et al. Seasonal H1N1 2007 influenza virus infection is associated with elevated pre-exposure antibody titers to the 2009 pandemic influenza A(H1N1) virus. *Clin Microbiol Infect* 2011;17:732–737.
12. Tu W, Mao H, Zheng J, et al. Cytotoxic T lymphocytes established by seasonal human influenza cross-react against 2009 pandemic H1N1 influenza virus. *J Virol* 2010;84:6527–6535.
13. Gilbert G, Cretikos MA, Hueston L, et al. Influenza A (H1N1) 2009 antibodies in residents of New South Wales, Australia, after the first pandemic wave in the 2009 Southern Hemisphere winter. *PLoS One* 2010;5:e12562.
14. Skowronski DM, De Serres G, Crowcroft NS, et al. Association between the 2008–09 seasonal influenza vaccine and pandemic H1N1 illness during spring-summer 2009: four observational studies from Canada. *PLoS Med* 2010;7:e1000258.
15. Cheng VC, Tai JW, Wong LM, et al. Prevention of nosocomial transmission of swine-origin pandemic influenza A/H1N1 by infection control bundle. *J Hosp Infect* 2010;74:271–277.

This content downloaded on Tue, 19 Feb 2013 05:30:44 AM
All use subject to JSTOR Terms and Conditions

Oncology

Oncology 2011;81(suppl 1):11–17
DOI: 10.1159/000333253

Published online: December 22, 2011

Hepatocarcinogenesis in Hepatitis C: HCV Shrewdly Exacerbates Oxidative Stress by Modulating both Production and Scavenging of Reactive Oxygen Species

Hidetake Fujinaga Takeya Tsutsumi Hiroshi Yotsuyanagi Kyoji Moriya
Kazuhiko Koike

Department of Internal Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Key Words
Hepatitis C · Hepatocellular carcinoma · Oxidative stress · Transgenic mouse · Core protein

Abstract
Persistent infection with hepatitis C virus (HCV) is a major risk for the development of hepatocellular carcinoma (HCC). One of the characteristics of HCV infection is the unusual augmentation of oxidative stress, which is exacerbated by iron accumulation in the liver, as observed frequently in hepatitis C patients. Using a transgenic mouse model, in which HCC develops late in life after the preneoplastic steatosis stage, the core protein of HCV was shown to induce the overproduction of reactive oxygen species (ROS) in the liver. In excessive generation of ROS, HCV affects the steady-state levels of a mitochondrial protein chaperone, i.e. prohibitin, leading to an impaired function of the mitochondrial respiratory chain with the overproduction of ROS. Insulin resistance and hepatic steatosis, which frequently accompany HCV infection, exacerbate ROS production. On the other hand, HCV compromises some of the antioxidant systems, including heme oxygenase-1 and NADH dehydrogenase quinone 1, resulting in the provocation of oxidative stress, together with ROS overproduction, in the liver with HCV infection. Thus, HCV infection not only induces ROS but also hampers the antioxidant system in the liver, thereby exacerbating oxidative stress that would facilitate hepatocarcinogenesis. Combination with the other activated pathway, including an alteration in the intracellular signaling cascade of MAP kinase, along with HCV-associated disturbances in lipid and glucose metabolism would lead to the unusual mode of hepatocarcinogenesis, i.e. very frequent and multicentric development of HCC, in persistent HCV infection.

Copyright © 2011 S. Karger AG, Basel

Introduction

Approximately 200 million people are infected with hepatitis C virus (HCV) worldwide. More than two thirds of those with acute HCV infection suffer from persistent infection causing active or inactive chronic hepatitis, and approximately 30% of patients with chronic hepatitis are assumed to develop cirrhosis within their lifetime. Once HCV infection develops into cirrhosis, hepatocellular carcinoma (HCC) develops at an annual rate of 7% [1]. The strong association of oxidative stress with HCV infection has been demonstrated and can explain at least part of the clinical progression of the disease. The patho-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2011 S. Karger AG, Basel
0030–2414/11/0817–0011$38.00/0

Accessible online at:
www.karger.com/ocl

Kazuhiko Koike, MD, PhD
Department of Gastroenterology, Graduate School of Medicine
The University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655 (Japan)
Tel. +81 3 5800 8800, E-Mail kkoike-tky@umin.ac.jp

genesis of chronic hepatitis C is not merely ascribed to inflammation caused by viral infection; the role of viral proteins in the pathogenesis has also been reported [2]. Of the proteins constituting HCV, the core protein in particular has various functions with respect to host cells and is closely related to oxidative stress. In this article, the relationship between HCV infection and oxidative stress is analyzed focusing on the pathological effect of the core protein of HCV, and the significance of oxidative stress in the pathogenesis of liver disease is discussed.

HCV Infection and Hepatocarcinogenesis

The mechanism underlying hepatocarcinogenesis in HCV infection is not fully understood yet. Inflammation induced by an immune response to HCV should be considered, of course, in a study on hepatocarcinogenesis in hepatitis viral infection: necrosis of hepatocytes due to chronic inflammation followed by regeneration enhances genetic aberrations in host cells, the accumulation of which culminates in HCC. This theory presupposes an indirect involvement of hepatitis viruses in HCC via hepatic inflammation. However, this context leaves us with a serious question: can inflammation alone result in the development of HCC in HCV infection with such a high incidence (90% in 15 years) or in a multicentric fashion? The other role of HCV would have to be weighed against a rare occurrence of HCC, even after the development of cirrhosis, in patients with autoimmune hepatitis in which severe inflammation in the liver persists. These backgrounds and reasonings lead to a possible activity of viral proteins for inducing neoplasia. This possibility has been evaluated by introducing genes of HCV into hepatocytes in culture with little success. One of the difficulties in using cultured cells is the carcinogenic capacity of HCV, if any, which would be weak and would take a long time to manifest itself. Actually, it takes 30–40 years for HCC to develop in individuals infected with HCV. On the basis of these viewpoints, we started to investigate carcinogenesis in chronic hepatitis C in vivo using transgenic mouse technology.

Transgenic Mouse Model for HCV-Related HCC

One of the major issues regarding the pathogenesis of HCV-associated liver lesions is whether the HCV proteins have direct effects on pathological phenotypes. For this purpose, several lines of mice have been established which are transgenic for the HCV cDNA. We have engineered transgenic mouse lines carrying the HCV genome by introducing the genes from the cDNA of the HCV genome of genotype 1b [3, 4]. Four different kinds of transgenic mouse lines are established, and they carry the core gene, envelope genes, the entire nonstructural (NS) genes, or the NS5A gene, respectively, under the same transcriptional regulatory element. Among these mouse lines, only the transgenic mice carrying the core gene developed HCC in two independent lineages [4]. The envelope gene transgenic mice did not develop HCC despite high expression levels of both E1 and E2 proteins [5], and the transgenic mice carrying the entire NS or NS5A gene developed no HCC.

Early in life, core gene transgenic mice develop hepatic steatosis, which is one of the histologic characteristics of chronic hepatitis C, along with lymphoid follicle formation and bile duct damages [6]. Thus, the core gene transgenic mouse model well reproduces the feature of chronic hepatitis C. It is important to note that no significant inflammation is observed in the liver of this animal model. Late in life, these transgenic mice develop HCC. Notably, the development of steatosis and HCC has been reproduced by other HCV transgenic mouse lines, which harbor the structural genes including the core gene [4, 7, 8]. These outcomes indicate that the core protein per se of HCV has an oncogenic potential when expressed in vivo.

Augmentation of Oxidative Stress in Hepatitis C

There is a notable feature in the localization of the core protein in hepatocytes; while the core protein predominantly exists in the cytoplasm associated with lipid droplets, it is also present in the mitochondria and nuclei [4]. On the basis of this finding, the pathways related to these two organelles, the mitochondria and nuclei, were thoroughly investigated.

One effect of the core protein is an increased production of oxidative stress in the liver. We would like to draw particular attention to the fact that the production of oxidative stress is increased in the core gene transgenic mouse model in the absence of inflammation in the liver [4]. The overproduction of oxidative stress results in the generation of deletions in the mitochondrial and nuclear DNA, an indicator of genetic damage [2].

Augmentation of oxidative stress is implicated in the pathogenesis of liver disease in HCV infection as shown by a number of clinical and basic studies [2, 9]. Reactive

oxygen species (ROS) are endogenous oxygen-containing molecules formed as normal products during aerobic metabolism. ROS can induce genetic mutations as well as chromosomal alterations and thus contribute to cancer development in multistep carcinogenesis [10, 11]. Recent studies have shown that oxidative stress is more augmented in hepatitis C than in other types of hepatitis such as hepatitis B [9].

Thus, a major role in the pathogenesis of HCV-associated liver disease has been attributed to oxidative stress augmentation, but little is known regarding the mechanism of increased oxidative stress in HCV infection. Hence, it is important to understand the mechanism of oxidative stress augmentation, in terms of both generation and scavenging of ROS, which may allow us to develop new tools of therapies for chronic hepatitis C.

Oxidative Stress and the Liver

Oxidative Stress and Reactive Oxygen

The main source of ROS in hepatocytes is the mitochondria. Outside of hepatocytes, ROS also originate from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase in Kupffer cells and inflammatory cells. A large percentage of consumed oxygen is constantly converted into ROS in the mitochondria accompanied by oxygen consumption in the electron transport system (ETS). Hepatocytes contain many mitochondria and therefore have a high ROS production. Generated ROS are very unstable and highly reactive and attack biomolecules such as DNA, lipids, and proteins. The liver not only produces much ROS but is also the center of the antioxidative effect in the form of protein synthesis. Oxidative stress refers to the oxidation-reaction-dominant state of the living body induced by an imbalance between the oxidation reaction caused by ROS and the antioxidation reaction. Main ROS include superoxide ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($HO\cdot$). ROS are mainly produced from $\cdot O_2^-$ and converted into stable H_2O_2 through a dismutation reaction. H_2O_2 is converted into highly reactive $HO\cdot$ in the presence of a transition metal.

The Antioxidant System and Oxidative Stress Markers

Antioxidants include glutathione (GSH), thioredoxin (TRX), vitamin E, vitamin C, and β-carotene. Reactive oxygen elimination enzymes include superoxide dismutase (SOD), GSH peroxidase, heme oxygenase (HO)-1, and catalase. SOD is induced by oxidative stress and dismutates $\cdot O_2^-$ to H_2O_2 and oxygen. Catalase in peroxisomes also decomposes H_2O_2 to water and oxygen. TRX is also a protein induced by oxidative stress and is reduced via S-S binding of the substrate protein by two SH groups in TRX and acts on the H_2O_2 elimination system via peroxiredoxins. HO-1 is an inducible cytoprotective enzyme that catalyzes the initial and rate-limiting reaction in heme catabolism and cleaves prooxidant heme to form biliverdin with the release of carbon monoxide. Biliverdin is converted into bilirubin in mammals; both of these have been known to have very strong antioxidant activities.

ROS cause various forms of cellular damage. 4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA) are the peroxidation reaction products of lipids, and 8-hydroxydeoxyguanosine (8-OHdG) is the product of DNA base modification. These products serve as oxidative stress markers.

The Origin of ROS Production in HCV Infection

Then, where is the place for oxidative stress overproduction in the liver of hepatitis C patients? The core protein is mostly localized to the endoplasmic reticulum, but we and other groups have shown its localization to the mitochondria in cultured cells and transgenic mice [12]. In addition, the double structure of mitochondrial membranes is disrupted in hepatocytes of core gene transgenic mice. Evidence suggests that the core protein modulates some mitochondrial functions, including fatty acid β-oxidation, the impairment of which may induce lipid abnormalities and hepatic steatosis. In addition, the mitochondrion is an important source of ROS. In livers of transgenic mice harboring the core gene, increased ROS production has been observed [2]. A recent study found, via proteomic profiling of biopsy specimens, that impairment of key mitochondrial processes including fatty acid oxidation and oxidative phosphorylation and of the response to oxidative stress occurs in HCV-infected human liver with advanced fibrosis [13]. Therefore, it is probable that the HCV core protein affects mitochondrial functions since such pathogenesis is observed in both HCV core-transgenic mice and HCV-infected patients.

The recent progress in proteomics has opened new avenues for disease-related biomarker discovery. We performed a two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) of mitochondria isolated from HepG2 cells stably expressing the HCV core protein and

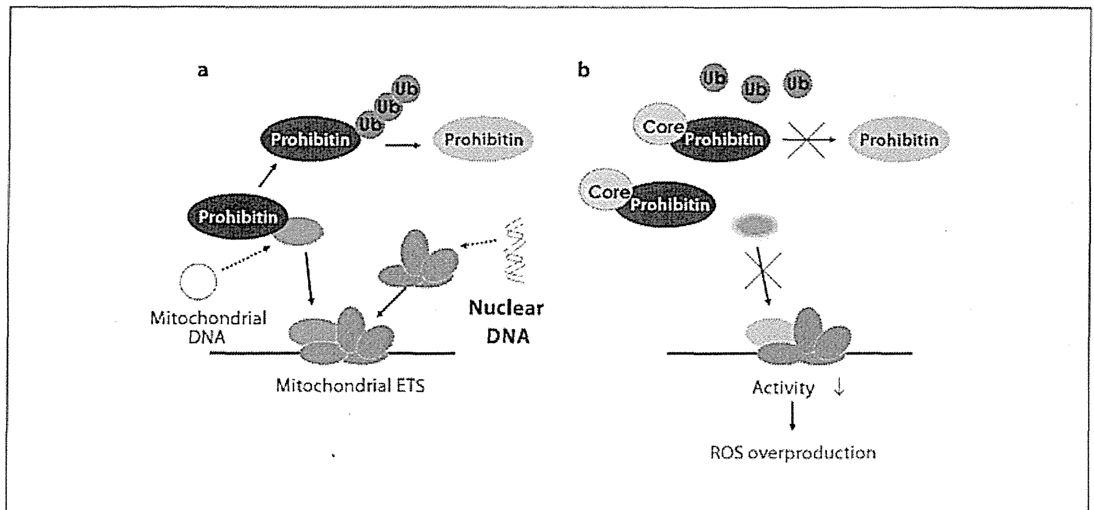

Fig. 1. The HCV core protein binds prohibitin and impairs its chaperone function leading to ROS overproduction. **a** Mitochondrial proteins consist of nuclear DNA-encoded proteins as well as mitochondrial DNA-encoded ones. Prohibitin acts as a protein chaperone for the mitochondrial proteins that are encoded by mitochondrial DNA by stabilizing newly synthesized mitochondrial translation products through direct interaction. **b** The HCV core interacts with prohibitin, disturbing its molecular chaperone function, and leads to a decrease in the activity of ETS complex IV, COX. Subunit II of COX is encoded by the mitochondrial DNA, while other subunits are encoded by the nuclear DNA. This is a new mechanism for oxidative stress overproduction in viral infection in that HCV induces mitochondrial ETS dysfunction by inhibiting chaperone function. Ub = Ubiquitin.

identified several proteins of different expressions when compared with control HepG2 cells. Among upregulated proteins in the core-expressing cells, we focused on prohibitin, which functions as a mitochondrial protein chaperone, and found that the core protein interacts with prohibitin and represses the interaction between prohibitin and subunit proteins of cytochrome c oxidase (COX), which may lead to decreases in the expression level of the proteins and in COX activity.

Prohibitin, a mitochondrial protein chaperone, was identified as an upregulated protein in core-expressing cells. Prohibitin is a ubiquitously expressed and highly conserved protein that was originally determined to play a predominant role in inhibiting cell cycle progression and cellular proliferation by attenuating DNA synthesis [14]. It is present in the nucleus and interacts with transcription factors that are important in cell cycle progression. In core-expressing cells, prohibitin was also detected in the nucleus and its expression level was also higher than that in control Hepswx cells or HepG2 cells. Mitochondrial prohibitin acts as a protein chaperone by stabilizing newly synthesized mitochondrial translation products through direct interaction [15]. We examined the interaction between prohibitin and the mitochondrially encoded subunit II of COX and found a suppressed interaction between these proteins in core-expressing cells. In addition, there are several studies that showed the association of prohibitin with the assembly of mitochondrial respiratory complex I as well as complex IV (COX) [15] (fig. 1). Complex I also consists of both nuclear- and mitochondrial-DNA-encoded subunits; therefore, it is probable that the assembly and function of complex I are impaired by the core protein. In respect to the complex I function, we previously found a decreased complex I activity in core-expressing cells. Other groups have also shown that complex I activity is decreased in cultured cells [16]. Based on these findings, the interaction between prohibitin and the core protein may impair the function of complex I as well as complex IV, leading to an increase in ROS production. In fact, the suppression of prohibitin function has been shown to result in an increased production of ROS [17], a phenomenon observed in the core-expressing cells used in this study as well as in the liver of core-gene transgenic mice [2]. Interestingly, Shelly Lu et al. [18] recently reported that the liver-specific deletion of prohibitin resulted in morphological abnormality and HCC.

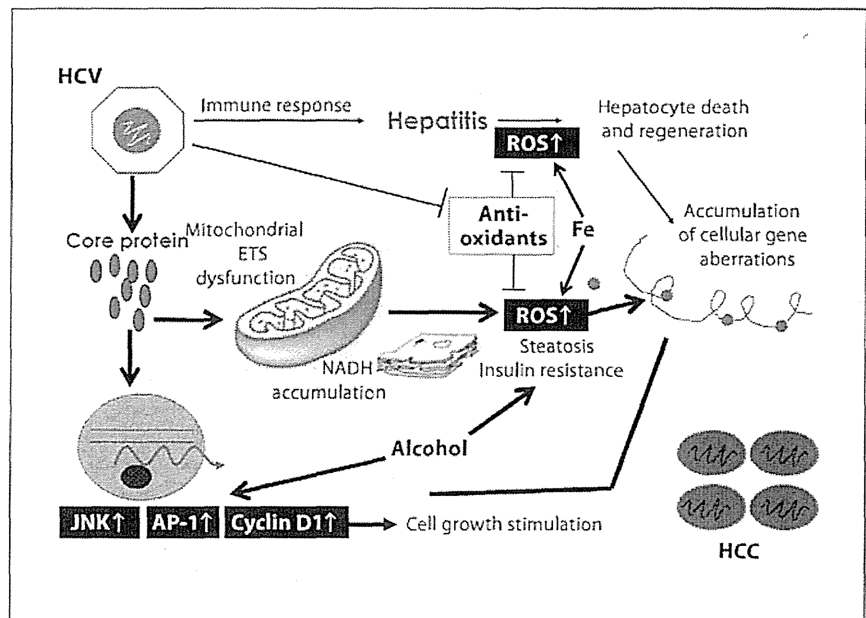

Fig. 2. Molecular pathogenesis of HCC development in HCV infection. Inflammation should contribute to hepatocarcinogenesis by producing genetic aberrations via continual cell death and regeneration. In the case of HCV infection, the virus itself contributes to hepatocarcinogenesis via two pathways. In one pathway, the core protein acts on the function of the mitochondrial ETS, leading to the overproduction of oxidative stress. The core protein also compromises some antioxidants and exacerbates ROS generation. Fe accumulation is an aggravating factor. The presence of steatosis and insulin resistance augments oxidative stress production. The other pathway is the modulation of cellular gene expression and signal transduction including the JNK pathway, which would give a growth advantage to hepatocytes. The combination of these alterations would escalate the development of HCC in HCV infection.

This is a new mechanism for ROS overproduction in viral infection in that HCV induces mitochondrial dysfunction through the inhibition of chaperone function in the mitochondria [19].

HCV Compromises the Antioxidant System

As discussed above, chronic hepatitis C is characterized by its prominent augmentation of oxidative stress. Related to this, iron accumulation in the liver has been shown to aggravate the oxidative stress as shown by the increase in the amount of DNA adducts in the liver [2, 9]. Iron is accumulated in the liver of HCV core gene transgenic mice [20]. The accumulation of iron observed in the liver of the core gene transgenic mice fed with normal chow corroborates the observation in chronic hepatitis C patients [9, 10]. Then, the impact of iron overloading on the oxidant/antioxidant system was examined using this mouse model and cultured cells. Iron overloading caused the induction of ROS as well as antioxidants. However, some of the key antioxidant enzymes, including HO-1 and NADH dehydrogenase quinone 1 (NDQ-1), were not augmented sufficiently by iron overloading, while other antioxidant enzymes such as catalase and GST were augmented more strongly in the iron-overloaded core gene transgenic mice than in the iron-overloaded control or non-iron-overloaded core gene transgenic mice. The attenuation of iron-induced augmentation of HO-1 was also confirmed in HepG2 cells expressing the core protein. HO-1 catalyzes the initial and rate-limiting reaction in heme catabolism and cleaves prooxidant heme to form biliverdin, which is converted into bilirubin in mammals; both of these have been known to have very strong antioxidant activities [21]. In addition, HO-1 has been also suggested to be a central antioxidant in conditions of GSH depletion [22]. Thus, HO-1 is an essential protective endogenous mechanism against oxidative stress, particularly in the case of iron overload. Therefore, it is probable that the attenuation of HO-1 and NQO-1 would hamper the antioxidant system and lead to a robust production of oxidative stress in HCV infection.

Thus, HCV infection not only induces ROS but also hampers antioxidant activation in the liver, thereby exacerbating oxidative stress that would facilitate hepatocarcinogenesis.

Conclusion

Pathways other than oxidative stress provocation in HCV-related hepatocarcinogenesis are alteration of the expression of cellular genes and modulation of intracellular signaling pathways. For example, tumor necrosis factor (TNF)-α and interleukin-1β have been found transcriptionally activated [23]. The mitogen-activated pro-

tein kinase (MAPK) cascade, which is involved in numerous cellular events including cell proliferation, is also activated in the liver of the core gene transgenic mouse model. In the liver prior to HCC development, only the c-Jun N-terminal kinase (JNK) route is activated. Downstream of the JNK activation, transcription factor activating protein (AP)-1 activation is markedly enhanced [23, 24]. Far downstream, both the mRNA and protein levels of cyclin D1 and cyclin-dependent kinase (CDK)4 are increased. Thus, the HCV core protein modulates the intracellular signaling pathways and gives advantage for cell proliferation to hepatocytes. The combination of these pathways that are activated in HCV infection, i.e. ROS overproduction, attenuation of antioxidants, cell growth stimulation via MAPK activation, metabolic disturbances such as hepatic steatosis, and insulin resistance [25], which are all induced by HCV itself, would contribute to hepatocarcinogenesis, together with moderate but long-lasting inflammation in chronic hepatitis C (fig. 2).

The results of our studies on transgenic mice have indicated a carcinogenic potential of the HCV core protein in vivo; thus, HCV would be directly involved in hepatocarcinogenesis. In research studies of carcinogenesis, the development of colorectal cancer is induced by the accumulation of a complete set of cellular gene mutations [26]. Their theory has been extended to the carcinogenesis of other cancers as well, called 'Vogelstein-type' carcinogenesis. On the basis of the results we obtained for the induction of HCC by the HCV core protein, we would like to introduce a different mechanism for hepatocarcinogenesis in HCV infection. We do allow multistages in the induction of all cancers; it would be mandatory for hepatocarcinogenesis that many mutations accumulate in hepatocytes. Some of these steps, however, may be skipped in the development of HCC in HCV infection to which the core protein would contribute. The overall effect achieved by expression of the viral protein would be the induction of HCC, even in the absence of a complete set of genetic aberrations, required for carcinogenesis.

By considering such a 'non-Vogelstein-type' process for the induction of HCC, a plausible explanation may be given for many unusual events which occur in HCV carriers. It no longer seem so difficult to determine why HCC develops in persistent HCV infection with an outstandingly high incidence. Our theory may also give an account of the multicentric de novo occurrence characteristics of HCC, which would be the result of persistent HCV infection.

Acknowledgements

This work was supported in part by Grant-in-Aid for Scientific Research on Priority Area from the Ministry of Education, Science, Sports and Culture of Japan; Health Sciences Research Grants of The Ministry of Health, Labour and Welfare (Research on Hepatitis).

Disclosure Statement

The authors have nothing to disclose.

References

1 Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Koida I, et al: Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2,215 patients. J Hepatol 1998;28:930–938.

2 Moriya K, Nakagawa K, Santa T, Shintani Y, Fujie H, Miyoshi H, et al: Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. Cancer Res 2001;61:4365–4370.

3 Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, Ishibashi K, Matsuura Y, et al: Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. J Gen Virol 1997;78:1527–1531.

4 Moriya K, Fujie H, Shintani Y, Yotsuyanagi H, Tsutsumi T, Matsuura Y, et al: Hepatitis C virus core protein induces hepatocellular carcinoma in transgenic mice. Nat Med 1998;4:1065–1068.

5 Koike K, Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, Ishibashi K, et al: Sialadenitis resembling Sjögren's syndrome in mice transgenic for hepatitis C virus envelope genes. Proc Natl Acad Sci USA 1997;94:233–236.

6 Bach N, Thung SN, Schaffner F: The histological features of chronic hepatitis C and autoimmune chronic hepatitis: a comparative analysis. Hepatology 1992;15:572–577.

7 Machida K, Cheng KT, Lai CK, Jeng KS, Sung VM, Lai MM: Hepatitis C virus triggers mitochondrial permeability transition with production of reactive oxygen species, leading to DNA damage and STAT3 activation. J Virol 2006;80:7199–7207.

8 Naas T, Ghorbani M, Alvarez-Maya I, Lapner M, Kothary R, De Repentigny Y, et al: Characterization of liver histopathology in a transgenic mouse model expressing genotype 1a hepatitis C virus core and envelope proteins 1 and 2. J Gen Virol 2005;86:2185–2196.

9 Farinati F, Cardin R, De Maria N, Della Libera G, Marafin C, Lecis E, Burra P, Floreani A, Cecchetto A, Naccarato R: Iron storage, lipid peroxidation and glutathione turnover in chronic anti-HCV positive hepatitis. J Hepatol 1995;22:449–456.

10 Fujita N, Sugimoto R, Ma N, Tanaka H, Iwasa M, Kobayashi Y, Kawanishi S, Watanabe S, Kaito M, Takei Y: Comparison of hepatic oxidative DNA damage in patients with chronic hepatitis B and C. J Viral Hepat 2008;15:498–507.

11 Kato J, Kobune M, Nakamura T, Kuroiwa G, Takada K, Takimoto R, Sato Y, Fujikawa K, Takahashi M, Takayama T, Ikeda T, Niitsu Y: Normalization of elevated hepatic 8-hydroxy-2'-deoxyguanosine levels in chronic hepatitis C patients by phlebotomy and low iron diet. Cancer Res 2001;61:8697–8702.

12 Suzuki R, Sakamoto S, Tsutsumi T, Rikimaru A, Tanaka K, Shimoike T, et al: Molecular determinants for subcellular localization of hepatitis C virus core protein. J Virol 2005; 79:1271–1281.

13 Diamond DL, Jacobs JM, Paeper B, Proll SC, Gritsenko MA, Carithers RL Jr, et al: Proteomic profiling of human liver biopsies: hepatitis C virus-induced fibrosis and mitochondrial dysfunction. Hepatology 2007;46: 649–657.

14 Mishra S, Murphy LC, Nyomba BL, Murphy LJ: Prohibitin: a potential target for new therapeutics. Trends Mol Med 2005;11:192–197.

15 Nijtmans LG, de Jong L, Artal Sanz M, Coates PJ, Berden JA, Back JW, et al: Prohibitins act as a membrane-bound chaperone for the stabilization of mitochondrial proteins. EMBO J 2000;19:2444–2451.

16 Piccoli C, Scrima R, Quarato G, D'Aprile A, Ripoli M, Lecce L, et al: Hepatitis C virus protein expression causes calcium-mediated mitochondrial bioenergetic dysfunction and nitro-oxidative stress. Hepatology 2007;46: 58–65.

17 Theiss AL, Idell RD, Srinivasan S, Klapproth JM, Jones DP, Merlin D, et al: Prohibitin protects against oxidative stress in intestinal epithelial cells. FASEB J 2007;21:197–206.

18 Ko KS, Tomasi ML, Iglesias-Ara A, French BA, French SW, Ramani K, Lozano JJ, Oh P, He L, Stiles BL, Li TW, Yang H, Martínez-Chantar ML, Mato JM, Lu SC: Liver-specific deletion of prohibitin 1 results in spontaneous liver injury, fibrosis, and hepatocellular carcinoma in mice. Hepatology 2010;52: 2096–2108.

19 Tsutsumi T, Matsuda M, Aizaki H, Moriya K, Miyoshi H, Fujie H, Shintani Y, Yotsuyanagi H, Miyamura T, Suzuki T, Koike K: Proteomics analysis of mitochondrial proteins reveals overexpression of a mitochondrial protein chaperone, prohibitin, in cells expressing hepatitis C virus core protein. Hepatology 2009;50:378–386.

20 Moriya K, Miyoshi H, Shinzawa S, Tsutsumi T, Fujie H, Goto K, Shintani Y, Yotsuyanagi H, Koike K: Hepatitis C virus core protein compromises iron-induced activation of antioxidants in mice and HepG2 cells. J Med Virol 2010;82:776–792.

21 Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN: Bilirubin is an antioxidant of possible physiological importance. Science 1987;235:1043–1046.

22 Oguro T, Hayashi M, Nakajo S, Numazawa S, Yoshida T: The expression of heme oxygenase-1 gene responded to oxidative stress produced by phorone, a glutathione depletor, in the rat liver; the relevance to activation of c-jun terminal kinase. J Pharmacol Exp Ther 1998;287:773–778.

23 Tsutsumi T, Suzuki T, Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, et al: Intrahepatic cytokine expression and AP-1 activation in mice transgenic for hepatitis C virus core protein. Virology 2002;304:415–424.

24 Tsutsumi T, Suzuki T, Moriya K, Shintani Y, Fujie H, Miyoshi H, et al: Hepatitis C virus core protein activates ERK and p38 MAPK in cooperation with ethanol in transgenic mice. Hepatology 2003;38:820–828.

25 Koike K, Tsutsumi T, Yotsuyanagi H, Moriya K: Lipid metabolism and pathogenesis of liver disease in hepatitis C viral infection. Oncology 2010;78(suppl 1):24–30.

26 Kinzler KW, Vogelstein B: Lessons from hereditary colorectal cancer. Cell 1996;87:159–170.