presence of nosocomial infection and other pathogenic organisms were absent, MRSA infection was diagnosed.

Management of precaution for transmission of MRSA

0.2% benzalkonium chloride ethanol solution (Welpas, Maruishi Pharmaceutical Corporation, Oosaka, Japan) were used for hand hygiene of patients, medical, and non-medical staffs in contact with patients. Contact precautions were taken in cases with MRSA colonization and/or infection. Eradication therapy such as intranasal mupirocin was not routinely performed. The screening of medical staffs for detection of MRSA was not also performed during the study period.

Background and clinical data collection

Background and clinical data collected for each patient included:

1) preoperative variables (age, gender, etiology of the underlying liver disease, presence of hepatocellular carcinoma, Child-Pugh score, MELD score, presence of ascites, use of diuretics, presence of encephalopathy, the international normalized ratio of prothrombin time level, serum bilirubin level [mg/dl], serum albumin level [g/dl], serum creatinine level [mg/dl], use of steroid, use of antimicrobials during the month before LDLT, presence of diabetes mellitus, history of hospital stay during the 6 months before LDLT, and methicillin-susceptible *Staphylococcus aureus* colonization;

2) surgical variables (operation time [hours], estimated blood loss [ml], blood transfusion [ml], graft volume/standard liver volume ratio [%], and application of duct to duct biliary reconstruction;

3) postoperative variables (length of urinary catheter insertion [days], length of arterial catheter insertion [days], length of central venous catheter insertion [days], length of endotracheal tube insertion [days], necessity for reoperation, acute rejection, cytomegalovirus infection, fungal infection, and postoperative use of antimicrobials other than the routine perioperative prophylaxis); and

4) pre- and postoperative variables (length of intensive care unit stay [days], and application of dialysis and/or apheresis).

Statistical analysis

Quantitative variables are presented as median and range. Categorical variables are presented as absolute counts. Univariate analysis was used to identify associations between each of the variables recorded and postoperative acquisition of MRSA. Wilcoxon rank sum test was used to compare the quantitative variables. Chi-square test or Fisher's exact test was used to compare the categorical data.

For multivariate analysis, only variables with a p value of less than 0.25 in the univariate analysis were entered into a logistic regression model by the backward-elimination procedure. The final regression model included covariates associated with a likelihood ratio of p less than 0.15. The results of the logistic regression were reported as odds ratios with 95% confidence intervals. A p value of less than 0.05 was considered statistically significant. All statistical analyses were performed using the JMP5.1 software package (SAS Institute Inc., Cary, NC).

Results

Acquisition of MRSA after LDLT

The median number of screening samples for each patient and the compliances with surveillance culture for nares, pharynx, sputum, urine, and stool were 9 (range, 1–25), 9 (range, 0–25), 5 (range, 0–25), 9 (range, 1–24), and 6 (range, 0–22) samples, and 82%, 82%, 50%, 80%, and 60%, respectively. Data on the detection of postoperative acquisition of MRSA are summarized in Figure 1. Postoperative acquisition of MRSA was detected in 35 of 158 patients (22%) during the study period. The median period of time between LDLT and detection of MRSA was postoperative day 18 (range, 1–89 days). In 8 of 35 patients, MRSA was detected during the intensive care unit stay. Median length of hospital stay after LDLT were 45 (range, 6–90) days in patients without MRSA acquisition and 59 (range, 33–90) in those with MRSA acquisition, respectively (p = 0.0006). Eleven of 158 (7%) patients developed MRSA infection during the study period: deep incisional surgical site infection (SSI) in 5, organ/space SSI in 2, intraabdominal infection in 2, lower respiratory infection in 1, and primary bloodstream infection in 1 patient, respectively. MRSA infections were eventually

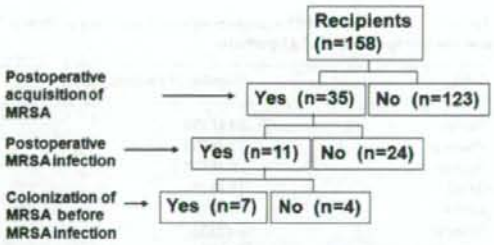

Figure 1
The patient profile of postoperative MRSA colonization and infection Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; LDLT, living donor liver transplantation.

diagnosed in 7 of 31 patients (23%) who were colonized with MRSA while only 4 of 127 subjects (3%) with negative surveillance cultures developed MRSA infection ($p = 0.01$). Table 1 shows the frequency of detection of MRSA from different clinical and surveillance specimens. In 30 subjects (86%) MRSA was isolated in more than 2 sites.

Risk factors for the Acquisition of MRSA after LDLT

The results of the univariate analyses assessing the association between the acquisition of MRSA and clinical covariates are shown in an additional file 1. Age of at least 60 years ($p = 0.01$), presence of an endotracheal tube for at least 3 days ($p = 0.03$), and perioperative dialysis and/or apheresis ($p = 0.008$) were significant factors affecting the acquisition of MRSA. In the multivariate analyses (Table 2), 10 risk factors with a univariate p value of less than 0.25 were entered into a logistic regression model by the backward-elimination procedure. In the final model, age of at least 60 years and perioperative dialysis and/or apheresis predicted the postoperative acquisition of MRSA. In contrast, postoperative use of fluoroquinolone was negatively associated with acquisition of MRSA.

Discussion

To date, this is the largest series study of the presence of MRSA after LDLT in adults. Of 158 patients, 35 (22%) presented a positive culture for MRSA by median postoperative day 18. The rate in the present study was higher than that in the recently published prospective study in DDLT [7], in which 9 of 60 (15%) patients acquired nasal MRSA colonization by median postoperative day 24. Patients who acquired MRSA were significantly associated with an increased length of hospital stay in the present study. Similary, Singh et al. reported that an increased length of hospital stay was associated with new Staphylococcus aureus carriage acquisition in DDLT[15]. Longer hospital stay, which is one of a marker for greater severity of illness, also might have identified high risk candidates requiring more intensive care, which could lead an increasing chance of MRSA transmission. Although the anterior nares is the most frequent carriage site for *Staphylococcus aureus* [16], other extra-nasal sites such as skin, perineum, pharynx, gastrointestinal tract, vagina, and axillae can harbor the organism [5,16]. MRSA from the nares was detected in 24 of 158 (15%) patients in the present study, which was comparable to the result of the previous report [7]. Of the 31 patients with MRSA-positive cultures, 7 (23%) subsequently developed MRSA infection. In addition, 7 of 11 (64%) patients who developed MRSA infection were colonized with MRSA prior to infection while only 4 of the 127 subjects (3%) with negative surveillance cultures developed MRSA infection ($p = 0.001$). This is in line with the findings of other authors, which indicated that interventions aimed at curtailing the transmission of MRSA may have a beneficial impact on the incidence of MRSA infection [17,18]. Furthermore, this is useful information in that it allows for earlier administration of a more appropriate antibiotic such as vancomycin in patients suspected of having MRSA infection.

Table 1: Frequency of MRSA detection in different surveillance and clinical specimens of 35 patients

Sites	Number of patients (%)
Nares	24 (69%)
Pharynx	21 (60%)
Sputum	18 (51%)
Stool	18 (51%)
Urine	11 (31%)
Wound	8 (23%)
Intraabdominal drain	5 (14%)
Bile	3 (9%)
Intravascular catheter	2 (6%)
Ascites	2 (6%)
Pleural effusion	2 (6%)
Blood	1 (3%)

Thirty of 35 patients had MRSA detected from multiple sites.

The present study indicated that age of at least 60 years increased the risk of postoperative acquisition of MRSA by multivariate analysis. As a large number of variables (N = 39) were included in the analyses in the present study, we must recognize the possibility that statistical association might have occurred by chance. The exact reason why older patients acquire MRSA more frequently after LDLT is unclear. Some previous studies [19,20] reported that older age was a risk factor for MRSA acquisition during hospitalization, although the interpretation was not described.

Another risk factor indicated by the present study, dialysis and/or apheresis, requires indwelling devices such as intravascular catheters. Invasive procedures are "entrance gates" for microorganisms, and potential hand contamination of personnel who perform these procedures might increase the risk of MRSA transmission [19]. On the other hand, perioperative dialysis and/or apheresis might merely be suggestive of the intensity of care required for patients in the present study. Perioperative dialysis and/or apheresis, mostly indicated in cases of deteriorated liver dysfunction in the present study, might suggest the deteriorated general conditions of patients, making them more prone to infectious diseases. Intensity of care required can be considered a surrogate marker for a number of manipulations that are major risk factors for MRSA transmission [21].

It might be better to adopt additional strategies for patients with these risk factors of MRSA acquisition. Singh et al. [15] reported an impact of an aggressive infection control strategy on *Staphylococcus aureus* infection in liver transplant recipients, including use of surveillance cul-

Table 2: Multivariate analysis of risk factors for the acquisition of MRSA after LDLT

Variable	Odds Ratio (95% Confidence interval)	p Value
Age >= 60	3.33(1.17–9.58)	0.03
Duct to duct biliary reconstruction	3.18(0.92–15.22)	0.07
Endotracheal tube (day) >= 3	2.26(0.87–5.84)	0.09
Postoperative use of beta lactam	0.49(0.20–1.23)	0.13
Postoperative use of fluroquinolone	0.14(0.007–0.88)	0.03
Perioperative dialysis and/or apheresis	2.92(1.16–7.39)	0.02

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; LDLT, living donor liver transplantation.

tures to detect nasal and rectal colonization, use of cohort and contact isolation precautions, decolonization with intranasal mupirocin therapy, and educating patients and visitors about hand hygiene and MRSA transmission. In that study, the rate of new acquisition of *Staphylococcus aureus* decreased from 46% during the pre-intervention period to 10% during the post-intervention period, and the rate of *Staphylococcus aureus* infection decreased from 40% to 4%, respectively.

The intensity of the use of antimicrobials, measured by the presence of preoperative antibiotic use during the month before LDLT did not correlate with the acquisition of MRSA after transplantation in the present study. Furthermore, postoperative use of fluoroquinolone was negatively associated with acquisition of MRSA, which was contrary to our expectations. It was difficult to analyze whether the postoperative frequency of use of antimicrobials increased a risk of MRSA acquisition in the present study. Only the antimicrobials used before the first date of detection of MRSA were included in the analysis, which caused the difference of observation period for exposure to antimicrobials between patients with and without acquisition of MRSA. The median period of time between LDLT and detection of MRSA was postoperative day 18, and 17 of 35 (49%) patients acquired MRSA within 2 weeks after the operation. Although there is little doubt that widespread use of antimicrobials provides multidrug-resistant strains of MRSA with a selective survival advantage [22], the relation between MRSA and antimicrobials seems more complex in the current series. Some studies [23-29] failed to show such an association by multivariate analysis. In other studies [30-33], exposure to specific antimicrobials, such as third generation cephalosporins, amoxicillin with clavulanic acid, quinolones, and other broad-spectrum antibiotics, increased the risk of MRSA infection or colonization. Crowcroft et al. [33] found no association between total antimicrobial use and MRSA colonization or infection and suggested that the problem was the inappropriate use of antimicrobials, not excessive use. This discrepancy is probably due to the fact that in the present study all the patients received long courses of multiple antimicrobials resulting in broad coverage, as perioperative prophylaxis per protocol, and it is therefore difficult to detect the effect of a specific antimicrobial. It might also be better for reducing the acquisition of MRSA to shorten prophylactic use of antimicrobials to a maximum of 48 hours as used in other transplant centers [7,34].

One limitation of the present study is that the MRSA carriage pattern was not analyzed. Longitudinal studies have distinguished three *Staphylococcus aureus* carriage patterns in healthy individuals [15,35]. This distinction is important because persistent carriers have higher *Staphylococcus aureus* loads and a higher risk of acquiring *Staphylococcus aureus* infection [36].

Another limitation of the present study is that we could not differentiate the specific MRSA strains. Pulsed-field gel electrophoresis analysis was not accessible and the data were not obtained. Therefore, we could not analyze the impact of MRSA transmission, such as patient-to-patient transmission by transient carriage on the hands of the medical staff in detail. Our observation remains speculative on this point. Similarly, it was impossible to know whether infection was due to the same strain as that of the colonization or to a newly acquired strain. Chang et al. [4] analyzed the isolates from infected sites and from the anterior nares in seven patients with MRSA infection, and reported that the same isolates were detected. Such detailed analyses might yield further information to prevent the spread of MRSA following LDLT.

Conclusion

There is a high incidence of MRSA early after LDLT in adults. Surveillance cultures should be performed periodically after LDLT to identify and prevent the transmission of MRSA.

Abbreviations

LDLT: living donor liver transplantation; DDLT: deceased donor liver transplantation; MELD: model for end stage liver diseases; MRSA: methicillin-resistant *Staphylococcus*

aureus; MSSA: methicillin-susceptible *Staphylococcus aureus*.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MH, YS, and MM designed the Research project and gave a critical view of manuscript writing. JK, YM, JT, KM, and KK helped in collecting the specimens, and the microbiologic and medical records. MH, YS, and ST wrote the manuscript. All the authors have read and approved the final manuscript.

Additional material

Additional file 1

An additional table. Association between postoperative acquisition of MRSA and perioperative variables by the univariate analysis.
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2334-8-155-S1.doc]

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Methicillin-resistant *Staphylococcus aureus* infection after living-donor liver transplantation in adults

M. Hashimoto, Y. Sugawara, S. Tamura, J. Kaneko, Y. Matsui, K. Moriya, K. Koike, M. Makuuchi. Methicillin-resistant *Staphylococcus aureus* infection after living-donor liver transplantation in adults. Transpl Infect Dis 2008: **10**: 110–116. All rights reserved

Abstract: *Background.* Methicillin-resistant *Staphylococcus aureus* (MRSA) infection frequently complicates the postoperative course in deceased-donor liver transplantation. The incidence and risk factors of MRSA infection after Living-donor Liver transplantation (LDLT), however, are unclear.

Methods. We retrospectively reviewed the data from 242 adult patients who underwent LDLT at the University of Tokyo Hospital. The microbiologic and medical records of the patients from admission to 3 months after LDLT were reviewed. Uni- and multivariate analyses were performed to identify the independent risk factors for postoperative MRSA infection.

Results. Postoperative MRSA infection occurred in 25 of 242 patients by median postoperative day 23. Preoperative MRSA colonization, preoperative use of antimicrobials, operation time ($\geq$ 16 h), and postoperative apheresis independently predicted postoperative MRSA infection.

Conclusion. Surveillance culture should be checked periodically after admission to identify patients at high risk for MRSA infection and to administer appropriate antimicrobials for perioperative infection. Postoperative apheresis, suggesting postoperative liver dysfunction, predisposed patients to MRSA infection.

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Key words: living-donor liver transplantation; methicillin-resistant *Staphylococcus aureus*; risk factor

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Living-donor liver transplantation (LDLT) is currently the most effective alternative for patients with end-stage liver disease to overcome the problem of the cadaveric organ shortage for adults (1). Although bacterial infections after deceased-donor liver transplantation (DDLT) are the most common infectious complications (2, 3), there are few large series studies of bacterial infections after LDLT (4).

Staphylococcus aureus is a major cause of bacterial infection after DDLT (3, 5). In one recent report, 63 of 323 patients (20%) developed *S. aureus* infection within 1 month after liver transplantation (6). Preoperative infection with methicillin-susceptible *S. aureus* (MSSA) or methicillin-resistant *S. aureus* (MRSA), alcoholic cirrhosis, and decreased prothrombin ratio were independent risk factors for early-onset *S. aureus* infection (6).

MRSA infection frequently complicates the postoperative course of DDLT recipients (5, 7–10). In some centers, 91% (45 of 49 isolates) of all *S. aureus* infections after DDLT were caused by MRSA (7). Long-term use of a urinary catheter, postoperative bleeding at the surgical site, and preoperative use of fluoroquinolones independently increased the risk of MRSA colonization after liver transplantation (11). The clinical outcome of MRSA infection differs from that of MRSA colonization (12) however, and therefore MRSA infection after liver transplantation also should be studied.

MRSA infection after DDLT has been well described in previous reports (5, 6, 8–11); however, LDLT is different from DDLT in some respects. First, in LDLT, small-for-size grafts (13) are often transplanted in adults because the

Abbreviations: DDLT, deceased-donor liver transplantation; LDLT, living-donor liver transplantation; MELD, model for end-stage liver diseases; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*

available liver volume from the living donor is limited. Small-for-size grafts lead to a clinical syndrome characterized by postoperative liver dysfunction with prolonged cholestasis and coagulopathy, portal hypertension, and severe ascites (14). The continuing liver dysfunction predisposes to further complications, including sepsis (15). Second, some complications such as biliary leakage from cut surface of the graft are unique to LDLT. Under such circumstances, we think previous reports about MRSA infection in DDLT cannot be applied in LDLT without analysis. The aim of the present study was to assess the incidence of MRSA infection after LDLT for adults and to analyze the risk factors using multivariate analysis.

Patients and methods

We retrospectively reviewed the data from 242 patients (132 males and 110 females) who underwent LDLT at the University of Tokyo Hospital, a 1150-bed teaching hospital, between January 1996 and November 2004. The median patient age was 51 years (range, 18–67). The indications for LDLT in these patients included primary biliary cirrhosis ($n = 52$), fulminant hepatitis ($n = 27$), hepatitis C ($n = 66$), hepatitis B ($n = 35$), autoimmune hepatitis ($n = 9$), biliary atresia ($n = 14$), primary sclerosing cholangitis ($n = 9$), alcoholic cirrhosis ($n = 4$), metabolic disease ($n = 9$), cryptogenic cirrhosis ($n = 6$), and others ($n = 11$). Of the 242 patients, 68 had hepatocellular carcinoma. The median Child–Pugh score and model for end-stage liver disease (MELD) score of those patients were 10 (range, 5–10) and 13 (range, −3 to 48), respectively. The microbiologic and medical records of the patients from admission to 3 months after LDLT were reviewed.

Indication for liver transplantation

Donors were selected from the patients' relatives. Age, blood type, graft size, and liver function were also taken into consideration. ABO blood groups were required to be identical or compatible to that of the recipients. The graft type was determined according to the estimated graft volume to the recipient's standard liver volume ratio (16, 17). Our surgical technique for recipient and donor surgery is described elsewhere (18).

Perioperative management protocol

Antimicrobial prophylaxis consisted of intravenous cefotaxim (1.0 g just before surgery, followed by 1.0 g every 6 h intraoperatively and thereafter), ampicillin/sulbactam (1.0 g just before surgery, followed by 1.5 g every 12 h intraoperatively and thereafter), and gentamicin (60 mg every 12 h after surgery) for 5 days. Vancomycin (0.5 g just before surgery, followed by 0.5 g every 12 h intraoperatively and thereafter) was also administered for 5 days after surgery in cases with preoperative MRSA colonization.

To prevent fungal infection, fluconazole (200 mg every 24 h) was administered intravenously for 7 days after surgery. All the patients received the same immunosuppressive regimens using tacrolimus (Prograf, Fujisawa Pharmaceutical Corporation, Tokyo, Japan) and methylprednisolone (Solu-Medrol, Pfizer Inc., New York, New York, USA). The regimen details are reported elsewhere (19).

Indications for perioperative apheresis were as follows. Hemodialysis or continuous hemodiafiltration were indicated when the serum creatinine level exceeded 3.0 mg/dL or when there was a 10% increase in body weight compared with the preoperative baseline. Plasma exchange combined with hemodialysis was indicated for fulminant hepatitis, hyperbilirubinemia (serum bilirubin $\geq$ 20 mg/dL), or postoperative graft dysfunction with or without multi-organ failure.

Microbiologic data collection

All the patients were screened preoperatively for *S. aureus* after admission for LDLT. Follow-up specimens were collected twice a week during the month after LDLT, and thereafter once a week during the hospital stay. Screened specimens consisted of swabs of the anterior nares, pharynx, sputum, urine, stool, swabs of wound or skin lesions, bile, and discharge from the abdominal cavity. A catheter or blood sample was also submitted when infection was suspected.

Specimens were taken and plated onto mannitol–salt agar or sheep blood agar. *S. aureus* was identified by means of standard microbiologic methods.

Methicillin resistance was determined using a disk diffusion test performed on Mueller–Hinton agar after incubation for 24–48 h at 30°C. The strains with an oxacillin minimum inhibitory concentration value of at least 4 μg/mL were defined as MRSA. Patients colonized with *S. aureus* at any site, and at any time during the hospital stay, were considered carriers, and contact precautions were taken in cases with MRSA. Decolonization with intranasal mupirocin (3 times/day for 3 days) was prescribed preoperatively for the patients who carried MRSA only at the nares from September 1996.

Background and clinical data collection

Background and clinical data collected for each patient included: 1) preoperative variables (age, gender, etiology of the underlying liver disease, presence of hepatocellular carcinoma, MELD score, presence of ascites, use of diuretics,

presence of encephalopathy, hemodialysis, the international normalized ratio of prothrombin time level, serum bilirubin level [mg/dL], serum albumin level [mg/dL], serum creatinine level [mg/dL], steroid use, use of antimicrobials during 1 month before LDLT, history of abdominal surgery, presence of diabetes mellitus, MSSA colonization, and MRSA colonization); 2) surgical variables (operation time [h], blood loss [mL], graft volume/standard liver volume ratio [%]) and application of duct-to-duct biliary reconstruction; 3) postoperative variables (length of intensive care unit stay [days], apheresis, reoperation, acute rejection, incidence of MRSA infection, onset of MRSA infection, and details of MRSA infection).

Definition of MRSA infection

The medical and microbiologic records of the patients were reviewed for the occurrence of MRSA infection for 3 months following LDLT. Only the first MRSA infection was recorded for each patient.

Nosocomial infections were defined according to the reports from the Centers for Disease Control and Prevention in 1988 and in 1992, as described elsewhere (20, 21). Surgical site infection included superficial incisional, deep incisional, and organ/space infections that occurred within 30 days after surgery. Infection at wounds or intra-abdominal cavity that occurred over 1 month after the operation was defined as gastrointestinal system infection. When an organism isolated from blood culture was compatible with a related nosocomial infection at another site, the bloodstream infection was classified as a secondary bloodstream infection. When MRSA was isolated from culture samples in the presence of nosocomial infection including surgical site infection, and other pathogenic organisms were absent, MRSA infection was diagnosed. When MRSA culture samples without causing clinical symptoms were detected, it was diagnosed as MRSA colonization.

Statistical analysis

Quantitative variables are presented as median and range. Categorical variables are presented as absolute counts. Univariate analysis was used to identify associations between each of the variables recorded and postoperative MRSA infection. Chi-square test or Fisher exact test was used to compare the categorical data.

For multivariate analysis, only variables with a P value of <0.25 in the univariate analysis were entered into a logistic regression model by the backward-elimination procedure. The final regression model included covariates associated with a likelihood ratio of $P<0.1$. The results of the logistic regression were reported as odds ratios (ORs) with 95% confidence intervals. A P value of <0.05 was considered statistically significant. All statistical analyses were performed using the JMP5.1 software package (SAS Institute Inc., Cary, North Carolina, USA).

Results

Preoperative colonization of *S. aureus* (MSSA and MRSA)

S. aureus was detected in 133 patients. MSSA was detected in 116 patients, among whom the isolated organism changed from MSSA to MRSA in 6 patients preoperatively. Consequently, MRSA was detected in 23 patients preoperatively.

Pre- and postoperative MRSA colonization and infection

The patient profiles of pre- and postoperative MRSA colonization and infection are shown in Figure 1. MRSA was detected in 70 patients during the study period. Preoperative and both pre- and postoperative colonization were found in 6 and 17 patients, respectively. A total of 38 patients were new MRSA carriers postoperatively, among whom 28 patients carried MSSA preoperatively.

Postoperative MRSA infection occurred in 25 patients: 7 patients were both pre- and postoperative MRSA carriers; 9 patients were new MRSA carriers postoperatively; and 9 patients were MRSA non-carriers until the onset of infection.

The detailed preoperative MRSA colonization sites of MRSA are shown in Table 1. Nasal colonization, detected in 18 patients, was most common. Of the 5 other preoperative MRSA carriers, 3 patients had stool as well as nasal colonization. Therefore, nasal or stool MRSA colonization was detected in 21 (91%) patients preoperatively. There were at least 2 colonization sites in 13 of 23 (57%) patients. Among multisite MRSA carriers, nasal colonization was detected in 12 of 13 (92%) patients.

Six patients who carried MRSA only preoperatively did not develop postoperative MRSA infection. Among the 6, 3

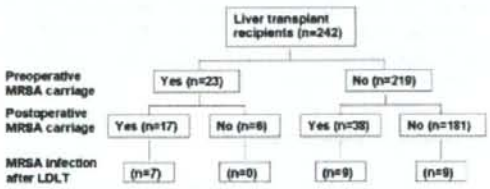

Fig. 1. The number of patients with pre- and postoperative MRSA colonization and infection. MRSA, methicillin-resistant *Staphylococcus aureus*; LDLT, living-donor liver transplantation.

Preoperative colonized sites of MRSA in 23 patients

Sites	Preoperative colonized sites, *n* (%)
Nares	18 (78)
Pharynx	10 (43)
Sputum	7 (30)
Urine	4 (20)
Stool	8 (35)

Several patients had multiple sites colonized.
MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 1

carried MRSA only at nares. Whereas, among the 17 patients who carried MRSA both pre- and postoperatively and developed postoperative MRSA infection, 3 carried MRSA only at nares. In total, among the 6 patients who carried MRSA only at nares preoperatively, 3 had postoperative MRSA infection as a complication.

Details of postoperative MRSA infection

The median day of onset of MRSA infection was postoperative day 23 (range, 7–64). During the study period, median length of hospital stay after LDLT was 50 (range, 6–90) days in patients without MRSA infection and 59 (range, 26–90) days in those with MRSA infection, respectively. MRSA infection occurred during hospitalization in 24 patients and after discharge in only 1 patient.

Surgical site infection was detected in 12 patients (deep incisional infection in 7 and organ/space infection in 5), pneumonia in 1, lower respiratory infection in 5, gastrointestinal system infection in 6 (all of which were intra-abdominal infection), and primary bloodstream infection in 1. Two patients with organ/space surgical site infection and 1 patient with gastrointestinal system infection had secondary bloodstream infection. We treated MRSA infection by reoperation and intravenous vancomycin in 15, only intravenous vancomycin in 5, only by reoperation in 2, lavage of intra-abdominal cavity through surgical drain in 2, and debridement of the wound in 1 patient.

Two of the 25 patients with MRSA infection died during 3 months after LDLT, among whom 1 patient with organ/space surgical site infection and secondary bloodstream infection died of MRSA infection.

Risk factors for postoperative MRSA infection

The results of the univariate analyses assessing the association between postoperative MRSA infection and covariates are shown in Table 2. Postoperative MRSA infection was significantly associated with serum creatinine level ($\geq$ 1.5 mg/dL; $P = 0.02$), preoperative MRSA colonization ($P = 0.004$), preoperative use of antimicrobials ($P = 0.01$), operation time ($\geq$ 16 h; $P = 0.0003$), postoperative intensive care unit stay ($\geq$ 10 days; $P = 0.01$), and postoperative apheresis ($P = 0.003$).

In the multivariate analyses (Table 3), 10 risk factors with P values of <0.25 were entered into a logistic regression model by the backward-elimination procedure. In the final model, preoperative MRSA colonization (OR, 3.47), preoperative use of antimicrobials (OR, 2.78), operation time ($\geq$ 16 h; OR, 2.80), and postoperative apheresis (OR, 3.19) independently predicted postoperative MRSA infection.

Discussion

To our knowledge, this is the first large series study of MRSA infection after LDLT in adults. MRSA infection occurred in 25 of 242 patients (10%) on median postoperative day 23. This finding is consistent with the results of previous studies among DDLT recipients (5, 8–10). Although only 1 of 242 patients (0.4%) died of MRSA infection during 3 months after LDLT in the present study, it was difficult in patients who died after liver transplantation to attribute death just to 1 factor. A similar result was reported by Desai et al. (10), wherein MRSA infection occurred in 22 of 157 patients and only 1 patient died due to MRSA. The present study indicated that preoperative MRSA carriage, preoperative antimicrobials use, prolonged operation time, and postoperative apheresis independently increased the risk for postoperative MRSA infection.

Preoperative surveillance culture is important because MRSA carriage increases the risk of MRSA infection (5, 8–10). In our series, MRSA infection occurred in 7 of 23 preoperative MRSA carriers (30%) and in 18 of 219 preoperative MRSA non-carriers (8%). The anterior nares is the most frequent carriage site for *S. aureus* (22) and most studies investigated only nasal colonization (5, 6, 8, 9). Other extra-nasal sites, such as skin, perineum, pharynx, gastrointestinal tract, vagina, and axillae, can harbor the organism (10, 22). Preoperative extra-nasal sites of MRSA colonization were detected in 5 patients, among whom 2 patients developed MRSA infection. Periodic surveillance culture should be continued postoperatively. The presence of MRSA was detected before the onset of infection in 16 of 25 (64%) patients and among these 16 patients, 9 were newly colonized postoperatively.

Preoperative antimicrobials use increased the risk for MRSA infection in the present study, as reported in previous studies (23–26). Widespread use of antimicrobials

Association between postoperative MRSA infection and preoperative, surgical, and postoperative variables

Variables	MRSA infection (−) (n = 217)	MRSA infection (+) (n = 25)	P value
Preoperative variables			
Age (years)	51 (18–67)	50 (24–62)	
≥ 50	120	13	0.83
Gender, male/female	114/103	18/7	0.09
Underlying liver disease			
Primary biliary cirrhosis	48	4	
Fulminant hepatitis	23	4	
Hepatitis C	58	8	
Hepatitis B	33	2	
Autoimmune hepatitis	8	1	
Biliary atresia	12	2	
Primary sclerosing cholangitis	8	1	
Alcoholic cirrhosis	2	2	
Metabolic disease	9	0	
Cryptogenic cirrhosis	6	0	
Others	10	1	
Hepatocellular carcinoma	62	6	0.81
Child–Pugh score	10 (5–14)	10 (5–14)	
≥ 10	112	17	0.14
MELD score	13.1 (−3.4–48.2)	14.3 (2.4–34.8)	
≥ 20	40	6	0.60
Ascites	101	15	0.21
Use of diuretics	116	15	0.67
Encephalopathy	36	6	0.40
Preoperative apheresis	47	6	0.80
PT-INR	1.61 (0.89–7.48)	1.62 (1.12–2.73)	
≥ 1.7	86	9	0.83
Serum bilirubin (mg/dL)	4.2 (0.3–40.0)	7.6 (0.6–32.4)	
≥ 3.0	147	18	0.82
Serum albumin (mg/dL)	2.9 (1.5–4.4)	2.8 (1.8–4.4)	
≥ 2.8	142	13	0.19
Serum creatinine (mg/dL)	0.7 (0.2–7.7)	0.66 (0.4–4.4)	
≥ 1.5	12	5	0.02
Use of steroid	26	3	1.0
Use of antimicrobials	49	12	0.01
β lactam	39	9	0.06
Glycopeptide	4	1	0.42
Fluoroquinolone	14	3	0.40
Aminoglycoside	5	1	0.48
Others	2	0	1.0
History of abdominal surgery	101	12	1.0
Diabetes mellitus	26	3	1.0
MSSA colonization	106	10	0.53
MRSA colonization	16	7	0.004
Surgical variables			
Operation time (h)	15 (10.7–33.2)	17 (12.2–40.1)	
≥ 16	73	18	0.0003
Blood loss (mL)	5150 (830–53,835)	5155 (2590–55,165)	
≥ 5000	113	13	1.0
Blood transfusion (mL)	7120 (900–42,890)	6800 (4240–46,120)	
≥ 5000	89	11	0.83
GV/SLV ratio (%)	46 (25–88)	42 (31–66)	
≥ 40	172	20	1.0
Duct-to-duct biliary reconstruction	151	18	1.0
Postoperative variables			
ICU stay (days)	5 (3–46)	6 (3–26)	
≥ 10	20	7	0.01
Apheresis	25	9	0.003
Reoperation	74	6	0.37
Acute rejection	60	8	0.64

PT-INR, the international normalized ratio of prothrombin time; MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; GV, graft volume; SLV, standard liver volume; ICU, intensive care unit; MELD, model for end-stage liver diseases.

Table 2

might provide multidrug-resistant strains of MRSA with a selective survival advantage (27); the relationship between MRSA and the antimicrobials used is complicated. Some studies fail to show such an association as assessed by multivariate analysis (28, 29). Other studies found an association only with specific antimicrobials, such as third-generation cephalosporins, amoxicillin with clavulanic acid, quinolones, and broad-spectrum antimicrobials (23, 25,

Multivariate analysis of risk factors for MRSA infection after LDLT

Variable	Odds ratio (95% confidence interval)	*P* value
Preoperative MRSA colonization	3.47 (1.10–10.41)	0.04
Preoperative use of antimicrobials	2.78 (1.10–7.06)	0.03
Operation time (h) ≥ 16	2.80 (1.04–8.03)	0.04
Postoperative apheresis	3.19 (1.12–8.78)	0.03

MRSA, methicillin-resistant *Staphylococcus aureus*; LDLT, living-donor liver transplant.

Table 3

26, 30). In the present study, there was an association between preoperative antimicrobials use and MRSA infection, whereas there was no association when different antimicrobials were classified. Similarly, Muller et al. (31) reported an association between MRSA acquisition and antimicrobials use by multivariate analysis, only without antimicrobials hierarchization.

Prolonged operation time increased the risk of MRSA infection in the present study. Prolonged surgical duration indicates technically more difficult surgical procedures in which the risk of complication is increased (32). George et al. (33) used multivariate analysis to demonstrate that prolonged duration of surgery increases the risk of bacterial infection in liver transplant recipients. In contrast, Singh et al. (5) reported that there was no association.

The present study identified postoperative apheresis as a risk factor of MRSA infection after LDLT. Previous studies about MRSA infection in DDLT did not find such an association (5, 6, 8–11). Postoperative apheresis was mostly indicated to compensate deteriorated liver function due to postoperative complications such as severe acute rejection, vascular complications, thrombotic microangiopathy, and small-for-size graft syndrome in our series. Although other markers of perioperative liver functions like preoperative bilirubin level or graft volume/standard liver volume ratio could not predict postoperative MRSA infection in the present study, liver dysfunction might promote MRSA infection. Patients with acute hepatic insufficiency are vulnerable to infection (34). The nonspecific defense systems have an important role in host defense against *S. aureus* infection (35). They are suppressed by the reduced hepatic production of complement and acute phase proteins, a reduction in the normal portal filtering capacity, and impairment in Kupffer cell and neutrophil function (36). In the present study, none of the patients who received postoperative apheresis developed primary bloodstream MRSA infection, including catheter-related infection. Thus, it was difficult to attribute the increased risk of MRSA infection to apheresis itself, which requires indwelling devices and components including blood products, which are potential vehicles of infection.

One of the limitations of the present study was that it was difficult to analyze the impact of carriage patterns on MRSA infection. Longitudinal studies distinguish 3 *S. aureus* carriage patterns in healthy individuals (22, 37). This distinction is important because persistent carriers have higher *S. aureus* loads and a higher risk of acquiring *S. aureus* infection (38). It is difficult to distinguish carriage patterns in the present study because the number of cultures obtained differed among patients.

Conclusions

Surveillance culture should be checked periodically after admission to determine which patients are at high risk for MRSA infection and to administer appropriate antimicrobials for perioperative infection. Postoperative apheresis, suggesting postoperative liver dysfunction, predisposed patients to MRSA infection.

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Comparison of vasculoprotective effects of benidipine and losartan in a rat model of metabolic syndrome

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ABSTRACT

Although antihypertensive drugs confer improvement in endothelial dysfunction and protection from atherogenesis in hypertension, different classes of antihypertensive drugs may elicit different degrees of vasculoprotective effects. We have investigated the effects of a long-acting calcium antagonist, benidipine, and an angiotensin AT_1 receptor antagonist, losartan, on the vascular damage observed in OLETF rats, an animal model of metabolic syndrome. At 34 weeks of age, OLETF rats were treated with either benidipine (3 mg/kg/day, per os) or losartan (25 mg/kg/day, per os) for 8 weeks. The extent of blood pressure reduction, restoration endothelium-dependent aortic relaxation, and elevation of serum nitrite/nitrate concentration did not differ significantly between benidipine- and losartan-treated OLETF rats. Benidipine and losartan also reduced the aortic expression of transforming growth factor-β1 mRNA and thickening of the vascular wall to a similar extent. Increased cardiac fibrosis was also inhibited by both benidipine and losartan. These data suggest that, when used in an antihypertensive dose, benidipine is as effective as losartan in restoring vascular endothelial function and in suppressing of cardiovascular remodeling in an animal model of metabolic syndrome.

1. Introduction

It has been shown that antihypertensive agents that belong to several different classes are effective in ameliorating endothelial dysfunction, an early feature of vascular damage, in the condition of hypertension (Dohi et al., 1994, Perticone et al., 1999, Taddei et al., 2001, Tschudi et al., 1994, Yao et al., 2003, Zhou et al., 2004). Although controlling blood pressure per se is postulated to largely account for cardiovascular outcome (Wang et al., 2007), blockers of the renin-angiotensin system may be more potent in protecting vascular function in the context of diabetes than other classes of antihypertensive drugs (Cheetham et al., 2000, Cheng et al., 2001, Lindholm et al., 2002, Oniki et al., 2006), including calcium channel blockers (Candido et al., 2004). On the other hand, in the Valsartan Antihypertensive Long-term Use Evaluation (VALUE) trial, it was found that the outcome of cardiac morbidity and mortality did not differ between a treatment group given an angiotensin AT_1 receptor antagonist and one given a calcium channel blocker-based treatment groups in the subgroup of hypertensive patients with diabetes (Zanchetti et al., 2006). This finding supports the concept that intensive control of blood pressure is the most important factor for the reduction of cardiovascular morbidity and mortality even in diabetic patients, regardless of the class of antihypertensive drugs administered (Messerli et al., 2001).

The Otsuka Long-Evans Tokushima Fatty (OLETF) rat is characterized as having increased insulin resistance, diabetes, obesity, hypertension, and dyslipidemia; in other words, metabolic syndrome. In the current study, we aimed to compare the effectiveness of two classes of antihypertensive agents, a long-acting dihydropyridine-calcium channel blocker (benidipine) and an angiotensin AT_1 receptor antagonist (losartan), on the restoration of vascular function and vessel morphology in this rat model of metabolic syndrome.

2. Materials and methods

2.1. Animal models

The experiments were performed in accordance with the guidelines for animal experimentation approved by the Animal Center for Biomedical Research, Faculty of Medicine, University of Tokyo. Male OLETF and age-matched Long-Evans Tokushima Otsuka (LETO) rats were obtained from the Tokushima Research Institute, Otsuka Pharmaceutical (Tokushima, Japan) and maintained under constant temperature and lighting conditions with free access to food and water. At 34 weeks of age, some OLETF rats were given benidipine at a dose of 3 mg/kg/day or losartan at a dose of 25 mg/kg/day per os, which was

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continued for 8 weeks. One day before the sacrifice, rats were kept in the metabolic cage, and urine was collected for 24 h under fasting condition. Systolic blood pressure and heart rate were measured in conscious rats by tail-cuff plethysmography (BP-98A, Softron, Tokyo, Japan).

2.2. Isolated vascular ring experiments

Ring segments (5 mm in length) of the thoracic aorta were suspended in individual organ chambers filled with Krebs buffer of the following composition (mmol/L): NaCl, 118.3; KCl, 4.7; CaCl2, 2.5; MgSO4, 1.2; KH2PO4, 1.2; NaHCO3, 25.0; and glucose, 10, pH 7.4. The solution was continuously aerated with a 95% O2, 5% CO2 mixture which was maintained at 37°C. Isometric tension was recorded by using an isometric force displacement transducer (NIHON KOHDEN, Tokyo, Japan) connected to a data acquisition system (Power Lab Chart 5, ADInstruments Japan Inc., Nagoya, Japan). The vessels were then precontracted with phenylephrine (3×10^{-6} mol/l). After a stable contraction plateau was reached, the rings were exposed to either acetylcholine or sodium nitroprusside (SNP).

2.3. Real time polymerase chain reaction

Real time reverse transcription polymerase chain reaction (RT-PCR) with gene-specific hybriprobes was performed by LightCycler (Roche Diagnostics, Basel, Switzerland) as described previously (Saito et al., 2005). We examined the three isozymes nitric oxide synthase (NOS). The following primers and probes were used: for transforming growth factor (TGF) -β1: forward 5′ GCAACAACGCAATCTATGAC 3′, reverse 5′ CCTGTATTCCGTCTCCTT 3′ (Nihon Gene Research Lab's Inc., Sendai, Japan); for endothelial NOS (eNOS): forward 5′ CTGGCAAGACCGATTAC-AC 3′, reverse 5′ GTCCAAACACTCCACGCT 3′ (Nihon Gene Research Lab's Inc.); for inducible NOS (iNOS): forward 5′ ACCCAAGGTCTACGTTCAAG 3′, reverse 5′ AAGACCGCACCGAAGATATC 3′ (Nihon Gene Research Lab's Inc.); and for neuronal NOS (nNOS): forward 5′ TGAGCTTTGCGGACAG-GA 3′, reverse 5′ TACGTGAGGCGGAACTTGT 3′. After normalization to the expression of GAPDH mRNA levels, eNOS expression was presented as the percentages of the data from aortas of OLETF rats without benidipine treatment.

2.4. Remodeling of aortic wall and heart

Remodeling of aortic wall was examined as described previously (Ishizaka et al., 2005). Briefly, the paraffin-embedded specimens of thoracic aorta in the 3 μm of thickness were stained with hematoxylin and eosin and Masson's trichrome staining. Vascular wall thickness and perivascular fibrosis were taken as indicators of structural abnormalities of aorta. Quantification of cardiac fibrous areas was performed on the Masson's trichorme stained samples as described previously (Ishizaka et al., 2002). Histopathology and morphometry were performed by investigators who were unaware of the treatment being administered.

2.5. Measurement of serum nitrite/nitrate

After protein-free filtrate of the serum was prepared with the Centricon YM-10 (Millipore, Billerica, MA), concentrations of nitrite/nitrate were measured by the Griess method with the NO2/NO3 Assay Kit-C II (Dojin Chemical Laboratory, Japan). The absorbance of the solution was determined at 540 nm with a micro plate reader, Biotrak II (GE Healthcare, Buckinghamshire, England).

2.6. Statistical analysis

Data are expressed as the mean ± S.E.M. We used ANOVA followed by a multiple comparison test to compare raw data, before expressing the results as a percentage of the control value using the statistical analysis software Statistica version 5.1 J for Windows (StatSoft Inc, Tulsa, OK). A value of $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Characteristics of experimental animals

OLETF rats aged 42 weeks were significantly heavier than LETO rats of the same age. Neither antihypertensive agent significantly modified the body weight of OLETF rats (Table 1). Systolic blood pressure was significantly higher in OLETF rats than in LETO rats, and treatment of OLETF rats with either benidipine or losartan lowered blood pressure to a range comparable to that in LETO rats. The plasma fasting glucose level was significantly higher in untreated OLETF rats than in LETO rats, and was affected by neither benidipine nor losartan.

3.2. Relaxations of aortic segments

As compared to the aortas from LETO rats, peak relaxation produced by acetylcholine in the aorta from untreated OLETF rats was significantly reduced, whereas that produced by SNP was not significantly different between LETO and OLETF rats (Fig. 1, Table 2). Both benidipine and losartan reversed, albeit partially, the impaired vascular relaxation in response to acetylcholine in OLETF rats. Neither peak relaxation ($P=0.288$) nor the ED50 ($P=0.128$) in response to acetylcholine was significantly different between the aorta of the OLETF/Ben group and that of the OLETF/Los group.

3.3. Remodeling of aortic wall

As compared to LETO rats, the wall-to-lumen ratio and area of perivascular fibrosis were increased in OLETF rats (Fig. 2). As compared to

Table 1
Characteristics of the experimental animals

Variables	LETO	OLETF	P value (vs. LETO)	OLETF/Ben	P value (vs. OLETF)	OLETF/LOS	P value (vs. OLETF)
n	11	11		11		6	
Body weight (g)	445±8	481±13	0.014	476±11	0.394	470±15	0.311
Systolic blood pressure (mm Hg)	136±2	149±4	0.012	138±3	0.027	135±3	0.015
Heart rate (bpm)	406±21	409±16	0.447	418±15	0.359	408±14	0.482
Heart weight (g)	1.46±0.03	1.85±0.05	<0.001	1.69±0.04	0.010	1.62±0.11	0.049
Heart weight (g/100 g BW)	0.33±0.01	0.40±0.02	0.001	0.36±0.01	0.047	0.34±0.01	0.036
Total cholesterol (mg/dl)	78.7±2.6	78.6±3.0	0.487	79.2±4.5	0.461	78.5±4.3	0.495
Triglyceride (mg/dl)	17.2±2.3	41.0±9.1	0.005	28.5±6.1	0.461	22.8±5.0	0.098
Plasma fasting glucose (mg/dl)	159±4	203±22	0.028	184±16	0.238	187±16	0.288

Values are mean ± S.E.M. Both benidipine and losartan lowered the blood pressure of OLETF rats to levels comparable to those of LETO rats. Both hypertensive drugs lowered the heart weight significantly. Levels of serum triglyceride and plasma glucose were also slightly lowered by either antihypertensive drug, although the reduction did not reach statistical significance.

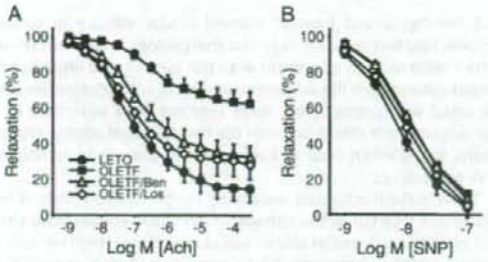

Fig. 1. Endothelium-dependent vascular relaxations in response to acetylcholine (Ach) and endothelium-independent sodium nitroprusside (SNP) in aortic segments from LETO, untreated OLETF, and benidipine and losartan-treated OLETF rats. Vessels were studied as ring segments in organ chambers, and relaxations in response to Ach and SNP were studied after the vessels had been pre-constricted with phenylephrine, 3×10^{-6} mol/l.

LETO rats (100.0±2.9, $n=6$), the luminal area was significantly greater in OLETF rats (138.3±.3, $P<0.001$, $n=6$), OLETF/Ben rats (122.5±5.4, $P<0.001$, $n=6$), and OLETF/Los rats (128.0±5.3, $P<0.001$, $n=6$). Both benidipine and losartan reduced these variables to levels comparable to those in LETO rats. Between the OLETF/Ben and OLETF/Los rats, the difference in the wall-to-lumen ratio ($P=0.336$) and in the area of perivascular fibrosis ($P=0.479$) was not statistically significant. Expression of TGF-β1 mRNA was greater in the aorta of OLETF rats than in that of LETO rats, and both benidipine and losartan reduced the expression of TGF-β1 mRNA in the aorta of OLETF rats (Fig. 3A).

3.4. Serum level of nitrite/nitrate and mRNA expression of NOS isoforms

As compared with the aorta of LETO rats, expression of eNOS and nNOS mRNA was significantly lower, whereas that of iNOS mRNA was significantly higher, in the aorta of OLETF rats (Fig. 3B–D). Both benidipine and losartan reduced the expression eNOS and nNOS mRNA, and increased that of iNOS mRNA, in the aorta of OLETF rats to levels comparable to those in the aorta of LETO rats. The serum nitrite/nitrate level was significantly lower in OLETF rats than in LETO rats; however, it was again increased to a level comparable to that in LETO rats by either benidipine or losartan (Fig. 4).

3.5. Fibrosis of the heart

As compared to the heart of LETO rats, interstitial fibrosis was enhanced in the heart of OLETF rats. Losartan and benidipine suppressed the increase in fibrosis in the heart of OLETF rats to a similar extent (Fig. 5).

4. Discussion

In the current study, we showed that acetylcholine-induced endothelium-dependent vascular relaxation was attenuated in OLETF rats as compared with age-matched LETO rats. Treatment of OLETF rats with an antipressor dose of either losartan or benidipine restored depressed endothelium-dependent vascular relaxation and increased

Table 2
Responses of isolated vessels to acetylcholine and sodium nitroprusside (SNP)

Variables	LETO	OLETF	*P* value (vs. LETO)	OLETF/ Ben	*P* value (vs. OLETF)	OLETF/ LOS	*P* value (vs. OLETF)
n	6	7		7		9	
Ach ED_{50}	7.0±0.3	6.5±0.1	0.037	7.0±0.1	<0.001	7.1±0.2	0.006
Ach peak relaxation	95±4	38±7	<0.001	74±5	0.001	73±7	0.003
SNP ED_{50}	7.6±0.1	7.3±0.1	0.104	7.1±0.0	0.075	7.5±0.2	0.184
SNP peak relaxation	94±8	89±4	0.333	91±2	0.190	95±6	0.169

Values are mean±S.E.M. ED_{50}s are −log[M]. Relaxations are the peak response given as a percentage of the pre-constricted tension. Ach indicates acetylcholine and SNP indicates sodium nitroprusside.

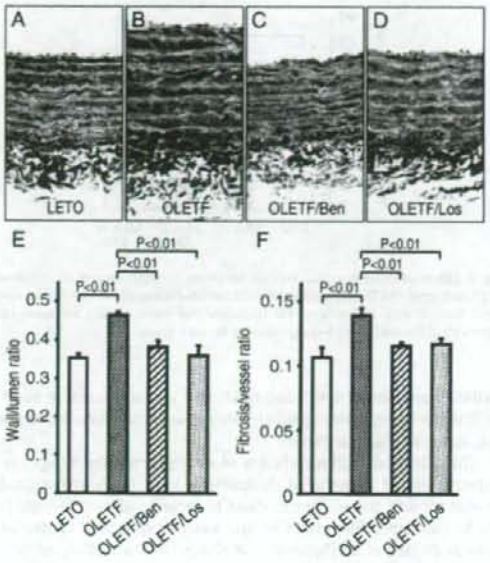

Fig. 2. Effects of benidipine and losartan on vascular remodeling in OLETF rats. (A–D) Masson trichrome staining of the aorta of the LETO rat (A), untreated OLETF rat (B), and benidipine (C) and losartan (D) treated OLETF rat. Both antihypertensive agents significantly reduced the wall/lumen ratio and fibrosis area in the aorta of OLETF rats. Original magnification, ×200. E, F. Values are mean±S.E.M. Summary of the wall-to-lumen ratio (E) and perivascular fibrosis (F) data of the aortas from 4–6 experiments for each group.

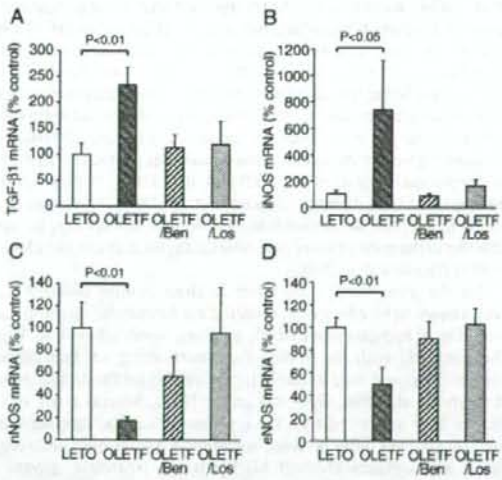

Fig. 3. Effects of benidipine and losartan on mRNA expression of eNOS, iNOS, nNOS, and TGF-β1 Results of quantitative RT-PCR examining the expression of TGF-β1 (A), eNOS (B), iNOS (C), and nNOS (D) mRNA in the aorta of LETO, untreated OLETF, and benidipine and losartan-treated OLETF. Values are mean±S.E.M. Summary of the data from 4–6 experiments for each group.

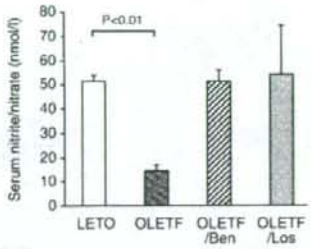

Fig. 4. Effects of benidipine and losartan on serum concentration of nitrite/nitrate in LETO, untreated OLETF, and benidipine and losartan-treated OLETF rats. Serum samples were filtered with a Centricon YM-10 before the assay. Values are mean±S.E.M. Summary of the data from 4–6 experiments for each group.

mRNA expression of eNOS and nNOS to a similar extent. In addition, both of these depressor agents improved aortic and cardiac remodeling, again, to a similar extent.

The effects of various classes of antihypertensive drugs on the preservation of endothelial dysfunction have been investigated in animal models and in human cases of hypertension. Although there might be some differences in the vasculoprotection conferred by various classes of antihypertensive drugs (Bennett et al., 1996), ACE inhibitors, angiotensin AT_1 receptor antagonists (Bennett et al., 1996, Clozel et al., 1990, Dohi et al., 1996), and calcium channel blockers, especially the long-acting ones (Krenek et al., 2001; Tschudi et al., 1994; Wang et al., 2007; Yao et al., 2003; Zhou et al., 2004) are all effective in restoring vascular function in hypertension. In this sense, it is noteworthy that a regiment that based on angiotensin AT_1 receptor antagonist, candesartan and that based on calcium channel blocker, amlodipine, produced no statistical differences in terms of the primary cardiovascular end point in 4728 Japanese hypertensive patients (Ogihara et al., 2008).

In the condition of hypertension with diabetes, however, ACE inhibitors (Baluchnejadmojarad et al., 2004, Oltman et al., 2008) and angiotensin AT_1 receptor antagonists (Cheng et al., 2001; Schafer et al., 2007) may be more effective in cardiovascular protection (Candido et al., 2004). Kagota et al. (2007) reported that an angiotensin AT_1 receptor blocker, telmisartan, but not a calcium channel blocker, amlodipine, ameliorated the impaired endothelium-dependent vasodilatation in spontaneously hypertensive obese rats (Kagota et al., 2007). Considering that certain AT_1 receptor antagonists may potently improve insulin sensitivity, the effectiveness of ACE inhibitors, AT_1 receptor antagonists and calcium channel blockers should further be compared from the viewpoint of cardiovascular protection, as well as renoprotection (Fogari et al., 2007), in the setting of hypertension accompanied with diabetes (Zanchetti et al., 2006) (Kawamori et al., 2006). In addition, an angiotensin AT_1 receptor blocker may be more effective in the prevention of new-onset diabetes than calcium channel blocker (Ogihara et al., 2008).

On the other hand, long-acting calcium channel blockers have been shown to be effective in reducing cardiovascular morbidity and mortality in hypertensive diabetic patients (Tuomilehto et al., 1999). This contrasts with the finding that short-acting, calcium channel blocker treatment may induce a higher rate of cardiovascular events (Borhani et al., 1996; Byington et al., 1998). Several other recent studies have suggested that the outcome of cardiac morbidity and mortality did not differ between angiotensin AT_1 receptor antagonist-based and calcium channel blocker-based treatment groups in patients with both diabetes and hypertension (Zanchetti et al., 2006), supporting the idea that blood pressure control is most important for the reduction of cardiovascular morbidity and mortality in diabetic hypertensive patients regardless of the classes of antihypertensive drugs (Messerli et al., 2001). Therefore, the finding that benidipine and losartan showed similar efficacy in restoring vascular function and cardiovascular morphology in the current study would seem to be in agreement with this idea. On the other hand, as we did not compare the subdepressor dose of antihypertensive drugs, we could not conclude that there may not be a difference in the vasculoprotective effects between the two classes of antihypertensive agents tested when used at a subdepressor dose or in combination with other drugs.

What is the mechanism underlying the preferable effects of benidipine and losartan in the current study? Many studies have shown that eNOS plays a crucial role in vasculoprotection (Forstermann and Munzel, 2006). In the current study, expression of eNOS and nNOS was found to be significantly lower in OLETF rats than in LETO rats; however, both benidipine and losartan preserved eNOS/nNOS expression and increased the serum nitrite/nitrate concentrations. It has also been reported that angiotensin AT_1 receptor antagonist (Yamamoto et al., 2007) and long-acting calcium channel blockers (Ding and Vaziri, 2000; Kobayashi et al., 1999; Toba et al., 2005) can upregulate vascular eNOS expression in hypertension. It has been reported that nNOS is also expressed in vascular cells, especially in certain pathological conditions, such as atherosclerosis and hypertension (Boulanger et al., 1998; Kishi et al., 2003), and nNOS, like eNOS, may also exert important vasculoprotective actions against vascular lesion formation (Channon et al., 1998). It has been reported that ACE inhibitors and angiotensin AT_1 receptor antagonists increase nNOS expression in the adrenal glands (Qadri et al., 2001). In addition, it has been proposed that iNOS induction may be involved in the pathophysiological process leading to inflammation, endothelial dysfunction, and atherosclerosis (Nagareddy et al., 2005, Vane et al., 1994). Therefore, benidipine- and losartan-mediated reduction of iNOS expression, which is enhanced in the aorta of OLETF rats, may be a preferable phenomenon in terms of vasculoprotection. Reduction of iNOS expression might be independent of the antipressor effects of benidipine or losartan, because it has been reported that calcium channel blockers and angiotensin AT_1 receptor antagonists may reduce iNOS expression in cultured cells (Chou et al., 2002, Neri Serneri et al., 2004). This point should be examined in future studies. We also showed that benidipine reduced the cardiac fibrosis in the heart of OLETF rats at 42 weeks of age. This finding is in agreement with a previous report that benidipine is also effective in inhibiting the cardiac remodeling, seen in the pressure-overload model (Liao et al., 2005) and in OELTF rats (Jesmin et al., 2006).

In conclusion, we showed here that, when used in an antihypertensive dose, a long-acting calcium channel blocker, benidipine and an angiotensin AT_1 receptor antagonist, losartan, were equally effective in restoring vascular function and in suppressing cardiovascular remodeling in an animal model of metabolic syndrome. The mechanisms

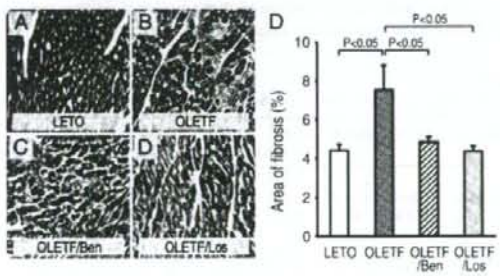

Fig. 5. Effects of benidipine and losartan on cardiac fibrosis. (A–D) Representative image of Masson trichrome staining of the heart of the LETO (A), untreated OLETF (B), and benidipine (C) and losartan (D) treated OLETF rats. (D) Values are mean±S.E.M. Summary of the fibrosis area data from 4–6 experiments for each group.

underlying these preferable effects may include upregulation of vascular expression of eNOS and nNOS, and downregulation of fibrosis-related genes, such as TGF-β1.

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Original Article

Guidelines for the antiviral therapy of hepatitis C virus carriers with normal serum aminotransferase based on platelet counts

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Aim: We aimed to identify the candidates for antiviral therapy, among patients who are hepatitis C virus (HCV) carriers with normal serum aminotransferase (ALT), focused on the inhibition of hepatocellular carcinoma (HCC).

Methods: Four hundred and sixty-four HCV carriers with normal serum ALT and 129 HCV carriers with persistently normal ALT (PNALT) and platelet (PLT) counts ≥150 000/μL who received liver biopsies were enrolled. HCV carriers with normal serum ALT were divided into four groups according to their ALT levels (≤30 U/L or 31–40 U/L) and PLT counts (≥150 000/μL or <150 000/μL).

Results: In 129 HCV carriers with PNALT, the rate of progression of fibrosis stage was 0.05/year and no HCC was detected during the follow up for 10 years. Approximately 20% of patients with ALT ≤40 U/L and PLT counts ≥150 000/μL were at stage F2–3; however, approximately 50% of patients with ALT ≤ 40 U/L and PLT counts <150 000/μL were at stage F2–4. An algorithm for the management of HCV carriers with normal serum ALT was advocated based on ALT and PLT counts.

Conclusion: The combination of ALT and PLT counts is useful for evaluating the fibrosis stage in HCV carriers with normal serum ALT. Most patients with PLT counts <150 000/μL are candidates for antiviral therapy, especially those with ALT levels ≥31 U/L when we focus on the inhibition of the development of HCC.

Key words: antiviral therapy, chronic hepatitis C, hepatitis C virus carriers, normal serum aminotransferase, platelet count

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) caused by hepatitis C virus (HCV) infection usually develops in patients with advanced chronic hepatitis (CH) or liver cirrhosis. The antiviral treatment for chronic hepatitis C (CH-C) is useful for inhibiting hepatic inflammation and progression of hepatic fibrosis, and consequently the development of HCC.[1–6]

Serum aminotransferase (ALT) levels are within the normal ranges in 20–40% of patients with chronic HCV infection,[7–11] defining the upper limit of normal serum ALT as ≤40 U/L. Significant hepatic fibrosis (≥F2 by the METAVIR classification) has been demonstrated in 5–30% of such patients.[9,12–16] We reported previously

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that HCV carriers with persistently normal ALT (PNALT) had histological features ranging from normal to minimal CH[17,18]; they showed slow progression of liver fibrosis and were at very low risk of developing HCC.[18]

The National Institute of Health Consensus Development Conference reported that HCV carriers with normal serum ALT are candidates for antiviral therapy.[19] A controlled study for the treatment of HCV carriers with PNALT with pegylated interferon alpha and ribavirin (PEG-IFN/Riba) for 48 weeks led to the eradication of HCV RNA in 40% of patients with genotype 1 and high viral load,[20] which is similar to the results of CH-C patients with elevated ALT levels.[21,22] However, it remains controversial whether these patients are candidates for antiviral therapy because of the limited efficacy of treatment, post-treatment flare-up, various side-effects, high cost of treatment, and their good prognoses.

In many Western countries, the upper limits of normal serum ALT are below 40 U/L;[23] however, a recent report from Italy demonstrated that the upper limit in healthy individuals was less than 30 U/L for men and 19 U/L for women.[24] We attempted to draft therapeutic guidelines for the treatment of HCV carriers with normal serum ALT. The biochemical and histological analyses were performed in HCV carriers with serum ALT levels below 40 U/L. These patients were divided into two groups based on ALT levels and then further divided into two subgroups according to their platelet (PLT) counts. We proposed an algorithm for the treatment of HCV carriers with normal serum ALT, taking into consideration the risk of progression to cirrhosis and the development of HCC. The present study demonstrated that the ranges of serum ALT and PLT counts are useful for deciding the indication of antiviral therapy for HCV carriers with normal serum ALT.

METHODS

Eligibility and definition

TWELVE HEPATOLOGISTS BELONGING to the Japanese Study Group of the Standard Antiviral Therapy for Viral Hepatitis, supported by the Ministry of Health, Labour and Welfare of Japan, which was settled on April 2004, participated in the study. Hiromitsu Kumada (Toranomon Hospital, Tokyo, Japan) serves as a chief and Takeshi Okanoue served as a researcher responsible for drafting the guidelines for the treatment of HCV carriers with normal serum ALT. In the present study, we tentatively defined the upper limit of the normal serum ALT as ≤40 U/L.

Patients with hepatitis B virus surface antigen, previous IFN treatment, history of heavy alcohol abuse, antinuclear antibody or antismooth muscle antibody, overt diabetes mellitus, or obesity (body mass index; ≥25 kg/m^2) were excluded from the study.

All of the patients underwent liver biopsy (≥2.0 cm in length) within 6 months prior to antiviral therapy, at which time their serum ALT levels were ≤40 U/L. Informed consent was obtained from every patient prior to liver biopsy and antiviral therapy.

Another study was conducted from January 1990 to August 2004 at Kyoto Prefectural University of Medicine (Kyoto, Japan). HCV carriers with PNALT were defined by serum ALT levels ≤30 U/L on at least three different occasions over a 12-month period and PLT counts ≥150 000/μL as reported previously.[18]

Study design

Among the 580 HCV carriers with normal serum ALT (≤40 U/L), 116 patients were excluded from the study because of insufficient data. Thus, 464 patients who received antiviral therapy from 1995 to 2004 were enrolled in this study (Table 1). Formalin-fixed liver specimens were stained with hematoxylin–eosin, and with Masson's trichrome. The liver specimens ($n = 262$) were also stained with Perls' Prussian blue to study hepatic iron loading. The histological findings were scored according to the classification proposed by Desmet *et al.*[25] and Ishak *et al.*[26] Steatosis was defined as fat droplets in >10% of hepatocytes. The degree of iron loading was assessed using a Perls' score of 0–4+, based on the scoring system of MacSween *et al.*[27]

The serum ALT, blood glucose level, immunoreactive insulin (IRI), serum ferritin, PLT count, serum hyaluronic acid, amount of serum HCV RNA, and the HCV genotype were examined. The homeostasis model assessment–insulin resistance was calculated as follows: plasma fasting glucose (mg/dL) × IRI (ng/mL) ÷ 405. The serum HCV RNA levels were determined using an Amplicor GT HCV monitor (Roche Diagnostic Systems, Tokyo, Japan). HCV genotype 1 (G1) and 2 (G2) were determined by a serologic genotyping assay.[28] G1 and G2 in this assay correspond to genotype 1 (1a, 1b) and 2 (2a, 2b) proposed by Simmonds *et al.*[29]

All the patients received IFN monotherapy or IFN/Riba combination therapy for 12–36 weeks. The average