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ORIGINAL ARTICLE

The outcomes of methicillin-resistant *Staphylococcus aureus* infection after living donor liver transplantation in a Japanese center

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Abstract
Objective The objective of this study is to present results from our review of methicillin-resistant *Staphylococcus aureus* (MRSA) infection in living-donor liver transplant (LDLT) recipients.
Methods Seventy patients with primary LDLT between August 1997 and May 2007 were retrospectively reviewed.
Results Overall, 9 patients (12.9%) encountered various kinds of MRSA infection after transplantation [peritonitis (6), bacteremia (6), pneumonia (3), wound infection (3), cholangitis (1)]; 4 of these 9 patients died. Of these 4 expired patients, 3 were highly urgent cases with very poor pretransplant status under ventilator support. In one patient, linezolid was effective after teicoplanin failure for severe systemic MRSA infections (bacteremia, peritonitis, cholangitis, pneumonia, and enteritis). Of the 4 patients in whom MRSA was isolated only in a nasal swab before transplantation, none developed MRSA infection after transplantation with a 3-day course of mupirocin prophylaxis.
Conclusions MRSA infection was a contributing factor in death after transplantation in cases with poor pretransplant status. Linezolid was effective even for treating systemic MRSA infection after LDLT. A short course of mupirocin prophylaxis seemed to be effective and did not have any adverse effects.

Keywords Liver transplantation · Living donor · Methicillin-resistant *Staphylococcus aureus*

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Introduction

Since methicillin-resistant *S. aureus* (MRSA) was identified in the UK in 1961 [1], it has been recognized as one of the most troublesome bacteria to manage, including after general abdominal surgery [2, 3]. In liver transplantation, the risk and severity of MRSA infection might be more serious because of additional immunosuppression. Although MRSA infection has actually been recognized as the leading cause of fatal bacterial infection in liver transplant recipients [4, 5], an appropriate strategy to manage it has not yet been established. Especially for nasal carriers, the efficacy of prophylactic treatment is not clear. Bert et al. [6] reported that the nasal carriage of MRSA is an independent risk factor for post-transplant MRSA infection, which indicates that mupirocin prophylaxis would be a reasonable treatment; however, its efficacy in deceased donor liver transplantation is under debate [7]. However, in comparison to deceased donor liver transplantation, living donor liver transplantation (LDLT) has an advantage in that we can plan pretransplant elective prophylaxis in the majority of cases. With regard to the treatment of infection after surgery, there are currently several options, including not only glycopeptides, but also linezolid, daptomycin, and tigecycline. The aim of this study was to show the outcomes of MRSA infection in our LDLT recipients and to propose an appropriate strategy for treatment.

Patients and methods

Patients

This is a retrospective observational study to describe the outcomes of MRSA infection and/or colonization among

patients who underwent LDLT in a Japanese hospital. Seventy sequential patients with primary LDLT between August 1997 and May 2007 in a Japanese hospital (Nagasaki University hospital, 869 beds in total) were enrolled in this study. The medical records of these 70 patients were retrospectively reviewed. During preoperative evaluation, several samples, including nasal swab, urine, and stool samples, were routinely obtained for culture study of the recipients. This screening was not routinely performed in the living donors. In any patients who were MRSA-positive only in the nasal swab before elective LDLT, mupirocin calcium ointment was applied twice daily for 3 days before transplantation without rechecking the nasal culture. For the patients who were MRSA-positive in the stool or urine, oral or intravenous vancomycin was administered until we confirmed the eradication of MRSA before the surgery. In some highly urgent cases, the patients underwent LDLT without checking the results of the culture studies, after clinical signs of infection had been ruled out carefully. Basic immunosuppression after transplantation consisted of tacrolimus and steroids. Tacrolimus was begun orally at 0.05 mg/kg twice a day from the day after transplantation. The target trough level was from 10 to 15 ng/ml until 1 month after surgery, and around 10 ng/ml or less thereafter. Regarding steroids, methylprednisolone was administered intravenously at 10 mg/kg for pediatric cases and 1 g for adult cases just after reperfusion during surgery. In the postoperative period, we administered a 0.5 mg/kg i.v. four times a day for the first 3 postoperative days, followed by 0.5 mg/kg twice a day for the next 3 days. Thereafter, we switched to oral prednisolone at 0.5 mg/kg once a day at 7 days after transplantation, and the steroid was discontinued by 6 months when the liver function was stable after a staged reduction. In selected cases, additional mycophenolate mofetil or azathioprine was used. For patients with hepatitis C-related cirrhosis, tacrolimus was converted to microemulsified cyclosporine when the patients started receiving anti-viral therapy after transplantation. Post-transplant antibiotic prophylaxis consisted of cefazolin and ampicillin at 1 g each, four times a day, for 3 days. After surgery, samples were obtained from the nares, respiratory secretions, urine, stool, ascites, and bile (when applicable) for the surveillance culture weekly for each patient after transplantation.

Definition of MRSA infection

Each sample was plated onto mannitol-salt agar. After incubation at 37°C for 24–48 h, *S. aureus* was identified by microscopic and growth characteristics, the coagulase test, and DNA hydrolysis. Methicillin resistance was determined by the disk diffusion method on Mueller-Hinton agar plates (Becton–Dickinson Co.) containing 5 μg of oxacillin, incubated at 30°C for 24–48 h. The medical records were retrospectively reviewed, and MRSA infections were defined as follows. Bacteremia was defined as the isolation of MRSA in at least one blood culture with obvious clinical signs of infection [high fever and/or elevation of serum level of C-reactive protein (CRP)]. Pneumonia was defined as a new pulmonary infiltrate developed on radiographic studies in conjunction with clinical signs (high fever and/or elevation of serum level of CRP, with purulent sputum), and MRSA was isolated from a respiratory secretion. Cholangitis was defined as the elevation of serum bilirubin, and the isolation of MRSA in bile obtained from biliary drainage (if applicable) with clinical signs of infection (high fever and/or elevation of CRP). Peritonitis was diagnosed if MRSA was isolated from ascites obtained intra- or postoperatively with clinical signs of infection (abdominal pain, high fever, and/or elevation of CRP). Wound infection was defined as the isolation of MRSA from a purulent fluid drained from the wound. Even if MRSA was isolated from various kinds of specimens, it was defined as the carrier when there were no clinical signs of infection.

Results

The characteristics of the patients

The 70 patients with primary LDLT during the study period consisted of 40 males and 30 females, with a median age of 52 (range, 0.5–67). The original diagnoses included hepatitis C-related cirrhosis in 20 patients (14 with hepatocellular carcinoma), hepatitis B-related cirrhosis in 15 (11 with hepatocellular carcinoma), acute liver failure in 10, biliary atresia in 9, primary biliary cirrhosis in 5, cryptogenic cirrhosis in 5, alcoholic cirrhosis in 2, and other diagnoses in 4. With regard to preoperative status, the median model for end-stage liver disease (MELD) score was 18 (range, 7–41) in the adult cases older than 12 years.

Isolation of MRSA before LDLT

The characteristics of the MRSA infections are shown in Table 1.

MRSA was isolated in 10 samples from 8 patients (11.4%) before transplantation (nasal swab [6], respiratory secretion [2], and stool [2]). The 2 patients with MRSA in respiratory secretions were highly urgent cases under mechanical ventilation, and we finally performed transplantation after clinical signs of infection had been ruled out carefully. Of these 8 patients, 3 (37.5%) developed MRSA infection after transplantation, which was a contributing factor in the death of 2 of the patients. Both of

Table 1 Characteristics of MRSA infection

Case no.	Gender/ age	Diagnosis	MELD	Isolation of MRSA before Tx	Isolation of MRSA after Tx	Definition	Treatment	Outcome
3	M/5	BA	NA		Nasal swab	Carrier	None	Alive
17	M/45	PBC	17		Bile	Carrier	None	Alive
20	M/58	C-LC	23		Respiratory secretion	Carrier	None	Alive
21	M/57	C-LC/HCC	40		Blood, pleural fluid, ascites	Bacteremia, pneumonia, peritonitis	VCM	Died
28	F/0	BA	NA	Respiratory secretion	Ascites	peritonitis	None	Died
32	M/65	B-LC	36	Nasal swab, respiratory secretion	Blood, ascites, wound	Bacteremia, peritonitis, wound infection	TEIC	Died
39	F/60	C-LC/HCC	11	Nasal swab, stool	Nasal swab, blood, ascites, bile, pleural fluid, stool	Bacteremia, peritonitis, cholangitis, pneumonia	Linezolid	Alive
44	M/59	B-LC/HCC	19		Blood, ascites	Bacteremia, peritonitis	TEIC	Alive
45	M/53	B-LC	24	Stool		Carrier	None	Alive
47	F/11	BA	NA		Wound	Wound infection	None	Alive
51	F/55	C-LC/HCC	8	Nasal swab		Carrier	None	Alive
52	M/57	C-LC/HCC	22		Nasal swab, blood, ascites, respiratory secretion	Bacteremia, peritonitis, pneumonia	TEIC[a]	Died
55	F/62	C-LC	14	Nasal swab		Carrier	None	Alive
56	M/52	FHF	23		Ascites, wound	Peritonitis, wound infection	Linezolid	Alive
60	M/68	B-LC/HCC	25	Nasal swab		Carrier	None	Alive
61	M/37	Cryptogenic-LC	16	Nasal swab		Carrier	None	Alive
68	M/58	B-LC/HCC	13		Respiratory secretion	Carrier	None	Alive
69	M/63	B-LC/HCC	9		Blood	Bacteremia	Linezolid	Alive

M male, *F* female, *BA* biliary atresia, *PBC* primary biliary cirrhosis, *C-LC* hepatitis C virus-related liver cirrhosis, *HCC* hepatocellular carcinoma, *B-LC* hepatitis B virus-related liver cirrhosis, *FHF* fulminat hepatic failure, *NA* not applicable, *VCM* vancomycin, *TEIC* teicoplanin

[a] Linezolid was discontinued because of severe thrombocytopenia due to hemophagocytic syndrome

these patients encountered septic shock with MRSA peritonitis followed by multiorgan failure.

MRSA infection after LDLT

Overall, 9 patients (12.9%) encountered various kinds of MRSA infection after transplantation (peritonitis in 6 patients, bacteremia in 6, pneumonia in 3, wound infection in 3, cholangitis in 1); 4 of these 9 patients died. Of the 4 expired patients, 3 were highly urgent cases with very poor pretransplant status under ventilator support, including the 2 patients mentioned above. Another patient had systemic MRSA infections (bacteremia, peritonitis and pneumonia) followed by hemophagocytic syndrome possibly due to cytomegalovirus viremia; this patient died 48 days after transplantation. In this patient, linezolid was started, but discontinued and replaced by teicoplanin because of severe thrombocytopenia (nadir platelet count, 4000/mm^3) due to hemophagocytic syndrome. One patient, a 60-year-old female, also developed severe systemic MRSA infections (bacteremia, peritonitis, cholangitis, pneumonia), which were promptly resolved by linezolid after ineffective treatment with teicoplanin. In this case, the trough level of teicoplanin was maintained in the therapeutic range, around 10 μg/ml or greater. As shown in Table 1, the patients who were defined as carriers did not require any treatment; this was true not only for nasal carriers, but also for the patients with MRSA isolated in respiratory secretions, stool, or bile. The median MELD score in adult cases (older than 12 years) was not significantly different between the groups with or without MRSA infection [19 (range, 8–40) vs. 18 (range, 7–41), Mann-Whitney test]. Although there were no

statistical differences in MELD score between the patients who died or survived after MRSA infection, 2 of the 4 expired patients revealed a high MELD score greater than 30 (40 and 36, respectively; Table 1).

Discussion

Although MRSA infection is well recognized as a life-threatening complication after liver transplantation, the criteria for transplant indication and appropriate prophylaxis have not yet been established. Several studies have indicated that nasal carriers are at a high risk of post-transplant MRSA infection [3, 4], which means that mupirocin prophylaxis is reasonable, but Paterson and colleagues showed the lack of efficacy of this strategy in a deceased donor liver transplantation series [7]. However, in deceased donor liver transplantation, appropriate elective prophylaxis is difficult, and mupirocin resistance is of great concern with the prolonged or repeated use of an elective prophylaxis [8]. In LDLT, an elective protocol can be established, and a short course of mupirocin prophylaxis was adapted in our series. In our study, there were 4 nasal carriers in whom MRSA was isolated in the nasal swab only, but none of them had any MRSA infection after transplantation. Accordingly, we adopted a 3-day course of mupirocin prophylaxis for elective cases because this course can be undergone safely, without any adverse effects. Although a randomized and controlled study is needed to show the efficacy of mupirocin prophylaxis in LDLT, we propose it as one possible effective strategy.

Currently, there are several prophylaxis and treatment options for MRSA infection, including mupirocin, glycopeptides (vancomycin, teicoplanin), linezolid, and more recently, daptomycin and tigecycline [9]. Glycopeptides are widely used, but their current use to treat MRSA infections has been the subject of much debate because they have a modest effect despite showing in vitro sensitivity, especially in pulmonary infection [10]. Based on our experience of successful salvage therapy with linezolid after teicoplanin failure for systemic severe MRSA infection, our current policy is to adopt linezolid as the rescue treatment for MRSA infection. In principle, glycopeptides should still be the first-line for MRSA infection, because the majority of the cases could be controlled by these drugs as shown in this study. Linezolid can be an alternative for glycopeptides, but indications should be considered carefully, because several studies demonstrated treatment failure [11] and severe adverse effects such as myelosuppression [12]. However, we recommend using linezolid as a second-line treatment in liver transplant recipients with MRSA infection, who easily tend to fall into critical condition because of immunosuppression.

Of the 4 expired patients in this study, 3 had a very poor preoperative status under mechanical ventilation, and 2 revealed high MELD score greater than 30. It is well recognized that preoperative status affects the outcome of transplantation. The leading cause of death is infection, which means that liver transplantation might be contraindicated even for carriers when a patient's status is poor. In regard to the risk factors of MRSA infection after LDLT, Hashimoto et al. [13, 14] showed that independent predictive factors included preoperative MRSA colonization, preoperative use of antimicrobials, prolonged operation time, and postoperative apheresis, none of which were seen as predictive factors in our study (data not shown), possibly because of our smaller number of cases. In regard to preoperative MRSA colonization, none of the patients in whom MRSA was detected only in a nasal swab during preoperative evaluation developed MRSA infection after transplantation. Mupirocin prophylaxis might be effective in these cases, but we have to follow such cases carefully after transplantation in order to monitor them for signs of MRSA infection.

It is unclear whether we should treat carriers in whom MRSA is isolated without any clinical signs of infection after transplantation. In our series, not only nasal carriers, but also patients with MRSA isolated in respiratory secretions, bile, or stool did well and did not require any treatment. Although such isolated MRSA might lead to subsequent severe infection in an immunosuppressive state, it seems that we can safely follow such patients with close observation. Another concern is that of possible MRSA transmission from the living donors [15]. Although such cases are probably rare, routine MRSA screening in the living donors might be recommended.

In conclusion, MRSA infection is life threatening in LDLT recipients, especially for patients with a poor pretransplant clinical condition. Linezolid is an effective option for reversing even critical infections, and we therefore recommend it as the second line of treatment for MRSA infection after LDLT. A short course of mupirocin prophylaxis seemed to be effective for elective cases, although a prospective and randomized study is needed to fully determine its efficacy.

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Case Report

Two-Staged Living Donor Liver Transplantation for Fulminant Hepatic Failure

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KEY WORDS:
Two-staged; Liver transplantation; Living-related; Hepatectomy

ABBREVIATIONS:
Fulminant Hepatic Failure (FHF); Living Donor Liver Transplantation (LDLT); Continuous Hemodiafiltration (CHDF)

SUMMARY

We reported a first successful and life-saving two-staged living-related liver transplantation for a patient with imminent brain death due to fulminant hepatic failure that otherwise had to be performed after a pre-treated and scheduled blood-type incompatible liver transplantation. The patient was anhepatic for 6 hr 34 min, and continuous hemodiafiltration was given throughout the operation. The patient recovered quickly and was extubated within 24 hr after transplant. This two-staged procedure is useful for emergency living-related liver transplantation that needs to be performed when the operating room is busy with other emergency or scheduled surgical procedures, and may allow clearance of toxic metabolites during the anhepatic period.

INTRODUCTION

For fulminant hepatic failure (FHF), liver transplantation is an established and effective therapy (1). Nevertheless, the therapeutic window is narrow, and the procedure needs to be performed as an emergency operation. If the allograft is to be obtained from a live donor, two emergency operations need to be performed simultaneously and in a coordinated fashion at a single institution (2). Such a circumstance is demanding not only for the surgeons but also for the anesthesiologists, operating room personnel and facility as well as for the blood bank. We were confronted with an instance in which we had to perform two living-related liver transplants at the same time, i.e., an emergency living donor liver transplantation (LDLT) for a patient with imminent brain-death due to FHF and a scheduled blood-type incompatible LDLT after full preparatory treatment with repeated plasma exchange to reduce serum isoagglutinin titer. We report our experience with a two-staged LDLT, i.e., total hepatectomy and temporary portocaval shunting which was followed by allograft implantation to prevent brain stem herniation for a patient with FHF in such a difficult situation.

CASE REPORT

A 34-year-old woman suffered from fulminant hepatic failure due to hepatitis B and was transferred to our hospital on May 7, 2001. In spite of plasma exchange and continuous hemodiafiltration (CHDF), she developed grade IV coma (responsive only to painful stimuli) with the prothrombin time below 12% and serum ammonia of 176 microgm/ml. She was judged to require emergency liver transplantation in the evening of May 10, 2001, when her father, 67-years-old and blood type identical, volunteered to be a donor. His preoperative work-up and informed consent was completed by the end of the same day.

In the mean time, an 11 month-old-girl with biliary atresia, status-post Kasai's operation was scheduled and being prepared for blood-type incompatible (A to O) LDLT from her mother the next morning. She had undergone two courses of preoperative plasma exchange under general anesthesia to reduce the serum anti-A isoagglutinin titer. The family of the infant was extremely reluctant to postpone their transplant and became nervous about any negative influence on their transplant from the emergency liver transplantation for FHF. We therefore considered performing emergency LDLT immediately after the elective LDLT. Nevertheless, the woman with FHF started to exhibit decerebrate posture in spite of CHDF and plasma exchange, and computed tomography revealed brain edema, which suggested imminent brain death. We confronted with the need to perform two LDLT at the same time.

For this difficult clinical situation, we decided to perform after donor operation for the blood-type incompatible transplant a total hepatectomy and end-to-side portocaval shunting for the patient with FHF, and then to implant the allograft after the first elective blood-type incompatible LRLT was

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finished (Figure 1). This modification would prevent the development of brain stem herniation or hemodynmic instability, while allowing the already set LDLT as scheduled.

After the native total hepatectomy with preservation of the inferior vena cava, the patient was anhepatic for 6 hr 34 min, during which she was placed on an end-to-side portocaval shunt. The shunt flow was 462ml/min by Doppler ultrasound, and splanchnic decompression was adequate with no signs of mesenteric petechiae or intestinal edema. CHDF was given throughout the operation, and her blood ammonia and lactate levels were lowered even during the anhepatic period (Figure 1). The donor right lobe weighed 750gram, with the graft weight/estimated liver volume of 66.3%. The explant liver weighed 520gram and exhibited massive necrosis. The operation for the recipient took 19hr 8min, and the estimated blood loss was 2,300gram.

For postoperative immunosuppression, tacrolimus and steroids were given. The patient's postoperative course was uneventful, and she woke up on the first postoperative day, when she was extubated. She recovered without neurological deficits and was transferred to the medical service on postoperative day 30 with normal liver function and stable blood tacrolimus level. At 12 months posttransplant, she is on tacrolimus 1mg b.i.d. only and remains well with normal liver function and without any restriction as a housewife. The child who received a blood-type incompatible liver from her mother was discharged 39 days after transplant and remains well with normal liver function without immunosuppression.

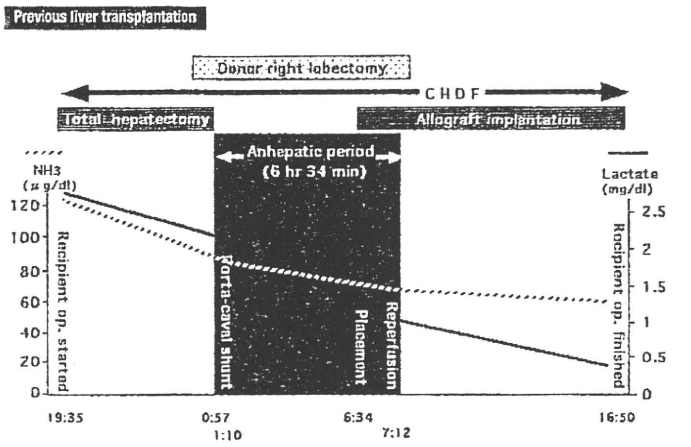

FIGURE 1 Operative course of the two-staged living-related liver transplantation for a patient with fulminant hepatic failure.

DISCUSSION

Keeping a patient with FHF alive and as a liver transplant candidate can be a challenging problem because of the narrow therapeutic window. The concept of two-staged liver transplantation was first reported as a desperate attempt by Ringe *et al.* (3) in 1988 for patients with primary graft non-function of the liver allograft or for intractable hemorrhage during hepatic resection. They later added severe hepatic trauma and FHF as indications for such a procedure (2, 3). The rationale for such a procedure for FHF is based on a clinical observation that the presence of a necrotic liver causes cardiovascular instability and renal as well as respiratory insufficiency which is described as 'toxic liver syndrome'. Husberg *et al.* (6) in 1991 described hepatic devascularization rather than total hepatectomy for three patients with FHF and noted improvement in the acidosis with diuresis after isolation of the failing liver. Rozga *et al.* (7) in 1993 reported combination of hypothermia, plasma exchange, and extracorporeal liver support with total hepatectomy. Their patient was anhepatic for 14 hr but recovered completely after two liver transplants. In our patient, we used CHDF throughout the anhepatic period. As compared to other 5 patients with FHF in our institution, our patient woke up much faster after LDLT. This may be in part due to the clearance of toxic metabolite during the anhepatic period by CHDF. In this regard, intentional anhepatic preconditioning by early total hepatectomy of the failing liver in combination with CHDF or artificial liver support before allograft implantation may facilitate recovery from acute liver failure.

A controversial issue in performing the two-staged liver transplantation for FHF has been the uncertainty with the availability of liver allografts and rather poor outcome (8). In LDLT, however, a viable graft can certainly be obtained at any given time, provided that the donor is willing, medically suitable for donation, and mentally supported.

Furthermore, although liver transplantation for FHF itself is a life-saving procedure, the influence of this procedure on the surgical practice at a hospital level is significant, especially if donor operation is also performed at the same time. We believe that for low-volume transplant centers, the two-stage LDLT for FHF can be performed in combination with emergency surgical procedures.

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ORIGINAL ARTICLE

Regeneration of Graft Livers and Limited Contribution of Extrahepatic Cells After Partial Liver Transplantation in Humans

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Abstract *Background* Liver regeneration is still not fully understood. Partial liver transplantation (LT) can provide the opportunity to investigate the mechanisms of liver regeneration, including the contribution of extrahepatic cells to liver regeneration. *Methods* Of 61 patients transplanted with partial liver graft between August 1997 and October 2006, 56 patients were studied, including 49 adults and 7 children. Sequential computed tomography volumetric analysis was performed for volume measurement, while proliferating cell nuclear antigen (PCNA) labeling index was investigated for liver cell proliferation in nonprotocol liver biopsy specimens. In addition, 15 male recipients who had female liver grafts were investigated in order to detect Y chromosomes as extrahepatic cells in nonprotocol liver biopsy specimens. *Results* Graft volume per standard liver volume was markedly increased after adult-to-adult living-donor (LD) LT. In pediatric transplants, there was no volume increase over time. PCNA labeling index was vigorous in adult-to-adult LDLT in the early period after LDLT. No Y chromosome was evident in hepatocytes from female-donor male-recipient grafts during or after liver regeneration. However, in the cases of failing grafts of this type, many Y-chromosome-positive cells were observed in the graft liver. The character of those cells was CD34(−), CK9(−), hepatocyte-specific antigen(−), and CD68(+/−). *Conclusion* In adult-to-adult LDLT, vigorous liver regeneration occurs in the graft liver, demonstrated by not only volumetric but cell kinetic analysis. Involvement of extrahepatic cells in normal liver regeneration seems limited.

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Keywords Living-donor liver transplantation · Liver regeneration · Extrahepatic cells

Introduction

The mechanism of liver regeneration is still not fully understood. Although vigorous liver regeneration after living-donor liver transplantation (LDLT) has been reported by us and others [1–3], it has been assessed by imaging studies such as computed axial tomography (CAT) scan, not hepatocyte cell division. In the present study, we took the opportunity to use liver biopsy specimens to verify liver regeneration in partial liver recipients during various periods after LDLT.

In addition, during liver regeneration it has been reported that extrahepatic cells, especially bone marrow (BM)-derived cells, are mobilized and involved [4–6]. However, details regarding how extrahepatic cells are involved and how much they contribute to normal liver regeneration have not been fully elucidated [7–10]. Therefore, we investigated liver biopsy specimens from female-donor male-recipient grafts, in which only XX cells should be present in the graft liver. We used fluorescent in situ hybridization (FISH) to detect Y chromosomes in the liver to identify extrahepatic cells in the liver upon liver regeneration.

Materials and Methods

Patients

Of 61 patients who underwent LDLT between August 1997 and October 2006 at Nagasaki University Hospital, 56 Japanese patients with survival times of more than 3 months

were included for volumetric analysis. For adult recipients, right lobe grafts were transplanted in 40 recipients, while left-side grafts (8 extended left lobe graft, 1 left lobe graft) were performed in 9 recipients. Seven pediatric cases with left lateral lobe graft also underwent volumetric study. Adult patients were defined as those over 16 years old. When liver function test was deranged, total 93 liver biopsies were carried out, consisting of 83 in adult cases and 10 in pediatric cases, and were prepared for proliferative cell nuclear antigen (PCNA) staining. Within these, a total of 24 liver biopsies were performed in 15 recipients on indication from a pool of 19 male recipients (XY) who were transplanted with female livers (XX).

Methods of LDLT

All partial liver grafts were preserved in University of Wisconsin solution and implanted using a piggyback technique. In general, graft selection was based on the results of volumetric studies using CAT scans to obtain ratios of graft volume to standard liver volume of more than 35% in the recipients.

A dual or triple immunosuppressive regimen was used, which included tacrolimus or cyclosporine A, steroid, and mycophenolate mofetil. Patients with compromised renal function were given induction therapy with interleukin-2 antibodies. Biopsy-proven rejections were treated if clinical and laboratory signs mandated steroid bolus treatment. Steroid-resistant rejections were treated with OKT3.

Investigation for Liver Regeneration

Incremental growth of the liver in volume was measured by serial CAT scans using Flexi Trace software (Tree Star, Inc., U.S.A.) at 0, 1–2 weeks, and 3 months after LDLT [1]. In liver biopsy specimens, expression of PCNA (DACO, Carpinteria, CA) was analyzed for intrahepatic proliferation [11].

Four-micrometer liver sections were deparaffinized in xylene and hydrated in graded ethanol. After deparaffinization, rehydration, and heating in 95°C buffer, sections were incubated with each antibody and subsequently with Histofine Simple Stain MAX-PO (MULTI) (Nichirei, Japan). Incubation was performed overnight at 4°C and followed by a wash in three changes of phosphate buffered saline (PBS) for 5 min. For all stainings, the reaction product was developed with the use of 3-diaminobenzidine tetrahydrochloride and H_2O_2. The sections were counterstained with Meyer hematoxylin–eosin.

For hepatocyte staining, the goat anti-human hepatocyte-specific antigen Ab (R&D system, Minneapolis, MN), and 2nd Ab biotinylated rabbit anti-goat Ig (DAKO, Carpinteria, CA) were used. For the staining of CK7 (bile duct marker), CD68 (macrophage marker) and CD34 (hematopoietic cells) were used, respectively, according to the manufacturer's protocol.

Fluorescent In Situ Hybridization (FISH)

FISH was performed in our reference laboratory (SRL, Nagasaki, Japan). Sections from paraffin-embedded biopsied liver tissues were placed on silane-coated glass slides. The slides were deparaffinized immediately in two rinses of 1,000 g/l xylene for 10 min each. Each slide was rehydrated in an ethanol series for 5 min. The slides were then treated with 0.2 mol/l HCl for 20 min, followed by 2 × SSC (0.3 mol/l sodium chloride and 0.03 mol/l sodium citrate) for 20 min at 80°C, treated with 0.05 mg/ml proteinase K in TEN [0.05 mol/l Tris–HCl, pH 7.8, 0.01 mol/l ethylenediamine tetraacetic acid (EDTA), and 0.01 mol/l sodium chloride] for 10 min at 37°C, and placed in 40 g/l formaldehyde in PBS for 10 min. Both FISH probes and target DNA were denatured simultaneously for 10 min at 90°C, and the slides were incubated overnight at 42°C, placed in 2 × SSC for 10 min at 42°C, washed twice in 2 × SSC/500 g/l formaldehyde formamide for 5 min each at 42°C, washed 2 × SSC for 5 min at 42°C, and counterstained in 2 × SSC/0.03 μg/ml 4',6-diamidino-2-phenylindole (DAPI).

Statistical Analysis

For the data, Mann–Whitney *U* test was used. Differences were considered statistically significant for *P*-value less than 0.05.

Results

Liver Volume

Graft volume per standard liver volume at 0, 1, 3, and 6 months after adult-to-adult LDLT was 53.2%, 95.9%, 98.5%, and 101.2% in right lobe grafts and 41.1%, 81.9%, 92.7%, and 102.4% in left-sided grafts, respectively (Fig. 1). Since volume changes in pediatric LDLT were not evident, they are not included in the figure.

DNA Synthesis in the Liver

PCNA labeling index was vigorous in adult-to-adult LDLT in the early period after LDLT, while it was not evident in pediatric LDLT (Fig. 2).

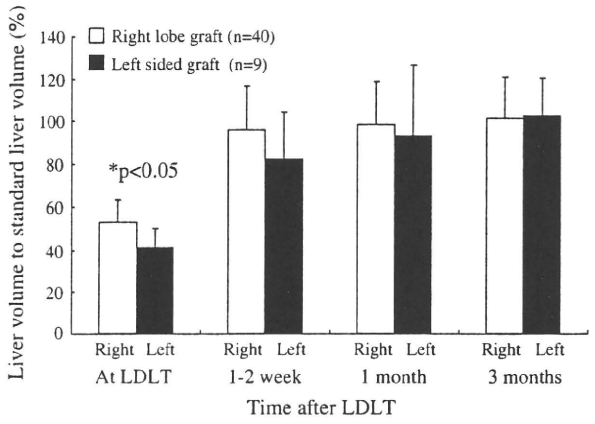

Fig. 1 Liver regeneration of right lobe or left lobe graft liver after adult-to-adult LDLT using volumetric analysis using CAT scan. *LDLT* living-donor liver transplantation, *CAT* computed axial tomography

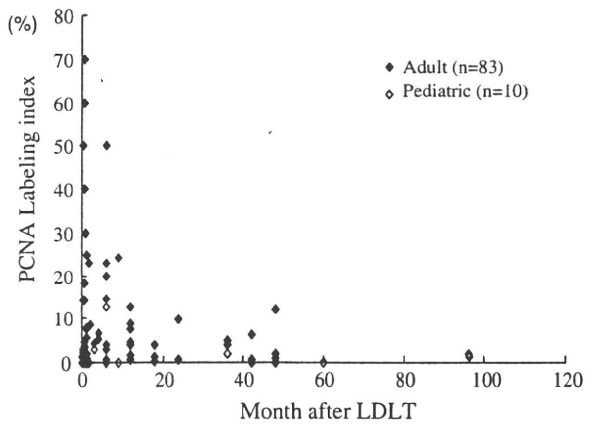

Fig. 2 PCNA labeling index after LDLT using immunohistochemical staining. *PCNA* proliferating cell nuclear antigen, *LDLT* living-donor liver transplantation

FISH and Immunohistochemical Staining for Y-Positive Cells

Y chromosome was not evident in hepatocytes of female-donor male-recipient grafts after normal liver regeneration in adult-to-adult LDLT recipients (Fig. 3, case 1). As seen in this case, when graft livers did not receive any damage and underwent normal liver regeneration, existence of Y-chromosome-positive cells was limited with FISH examination. However, in the case of failing graft, such as in cases 11–13, many Y-chromosome-positive cells were observed in zone 1 of the graft liver (Fig. 3, case 11).

For these cases, immunohistochemical staining was performed in the area with Y chromosomes. CD34(−), CK9(−), hepatocyte Ag(−), and CD68(+/−) were observed using immunohistochemical staining (Fig. 4, case 11). In the case of chronic liver damage (Fig. 5, case 15) after LDLT due to biliary complication, a few Y-positive cells were also detected with nonspecific staining for CD34, CK9, hepatocyte Ag, and CD68. Results of immunohistochemical staining are summarized in Tables 1 and 2.

Discussion

In this report, we showed liver regenerative response after partial LT using not only volumetric CAT scan study but also PCNA labeling of biopsy specimens. Previously, we reported vigorous liver regenerative response after partial liver regeneration and investigated liver regenerative growth factors after liver regeneration [11]. Herein, we showed a clear difference in proliferation of graft liver according to recipient body size and blood flow due to the difference in responses when transplanted in adults and children with different standard liver volumes. We did not carry out statistical analysis on PCNA index since it exhibited wide deviation. Liver regeneration remains an unsolved phenomenon, but our results show that it could be related to factors in recipients, as we reported previously [1]. Since protocol biopsy tends to be avoided because of risk of hemorrhage etc., further investigation is needed to assess cell proliferation noninvasively aside from CAT scan. Also since liver biopsy was not done on protocol, rejection or inflammation could have affected the data of PCNA staining. Although it would be interesting to investigate the difference in liver regeneration between patients after liver resection and those after partial liver transplantation, biopsy specimen from patients after liver resection cannot be obtained because of risk of complications. Therefore this also remains for further investigation. Our liver specimens from liver transplant recipients were obtained because of on-demand liver biopsy.

In addition, for combinations of female donor (XX) and male recipient (XY), the Y chromosome was investigated in the biopsy specimen of the female liver (XX) in order to investigate the contribution of extrahepatic cells to liver regeneration. Previously, in an in vivo experiment conducted in 2000, it was reported that hepatocytes could be derived from BM cells [12]. Subsequently, in 2001, Baccarani et al. [13] reported that, in human recipients, replacement of a female liver venous endothelium with male BM showed the possibility of involvement of BM cells in liver rearrangement. Fujii et al. [4] reported that BM cells participated in liver regeneration after hepatectomy, whereas the majority of cells were committed to sinusoidal endothelial cells. Very recently, Conzelmann et al. [5], using their reduced-size LT model, reported that recipient-derived progenitor cells were present and might contribute to liver regeneration in mice. However, in 2005 Di Campli et al. [7] reported no evidence of hematopoietic

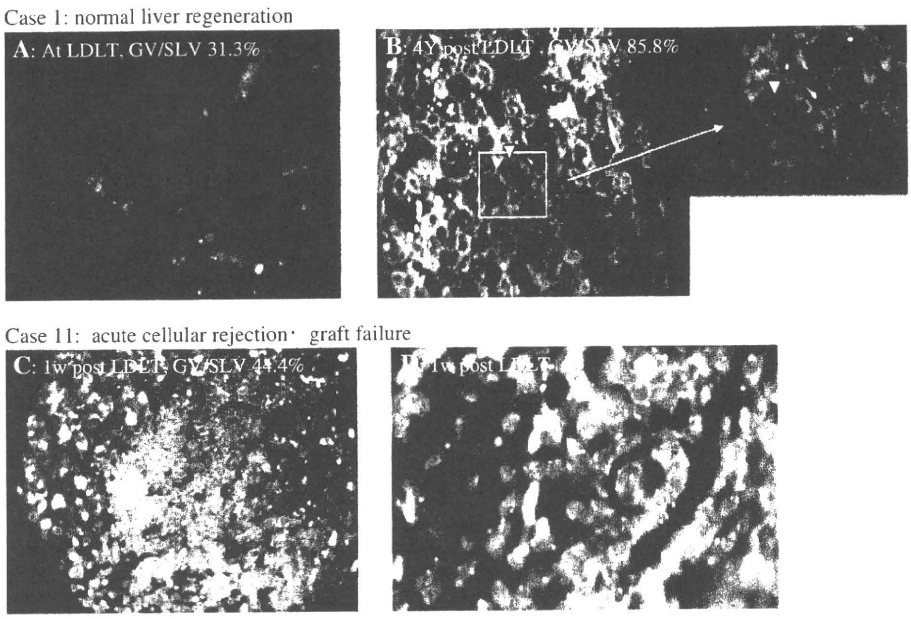

Fig. 3 FISH for Y chromosome in liver biopsy specimens. Case 1 showed normal liver regeneration after LDLT. **a** At the time of LDLT, few Y-chromosome-positive cells were seen. **b** With time, although GV/SLV increased, a few Y-chromosome-positive cells were seen only in the sinusoid. **c** Case 11 had severe acute rejection at 1 week after LDLT. **d** In the biopsy specimen, massive accumulation of Y-chromosome-positive cells was seen, mimicking hepatic structure. *FISH* fluorescent in situ hybridization, *GV/SLV* graft volume versus standard liver volume ratio

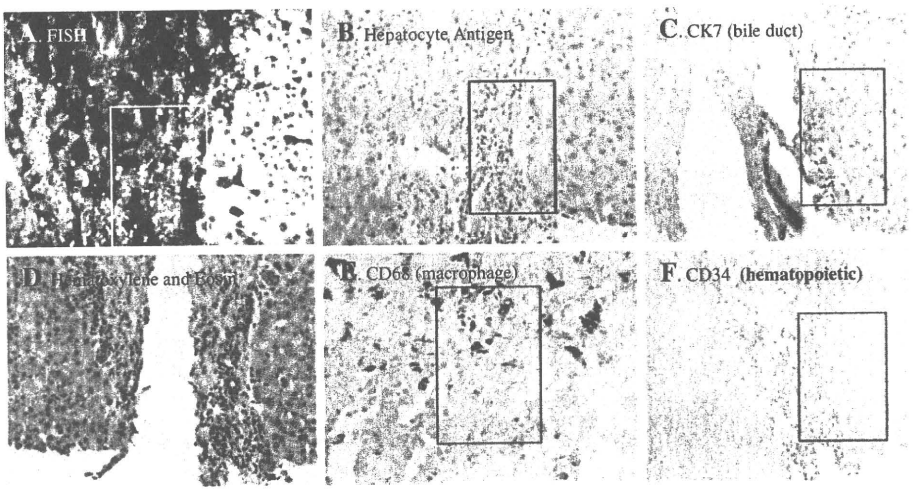

Fig. 4 Immunohistochemical stainings in case 11. Characterization of Y-chromosome-positive cells was attempted in corresponding area. **a** FISH showing Y-chromosome-positive cells (white square), **b** hepatocyte antigen was not positive in the black square, **c** CK7 (cytokeratin 7, bile duct) was not positive in the black square, **d** hematoxylin and eosin staining, **e** CD68 (macrophage) was partially positive in the black square, **f** CD34 (hematopoietic cell) was not positive in the black square

stem cell mobilization in patients who underwent hepatectomy or in patients with acute liver failure. Similarly, in 2006, Moritoki et al. [8], using green fluorescent protein transgenic mice, demonstrated that BM cell transfer seemed not to contribute to the differentiation of cholangiocytes in a chronic cholestasis model. In 2007, Tomiyama [6] reported the limited contribution of cells originating from intact extrahepatic tissue in hepatocyte regeneration in transplanted rat livers. Thus, it is still unknown whether extrahepatic cells such as BM cells could contribute to liver regeneration or liver repair, especially in humans.

In our study, we did not find many Y-chromosome-positive cells after liver transplantation with normal liver regeneration. If extrahepatic cells had been involved and integrated into normal liver regeneration, they should have stayed and been found in the liver biopsied a long time after LDLT. This is indirect evidence that would seem to rule out extrahepatic cell contribution to normal liver regeneration in humans, in contrast to previous reports [12, 13]. On the other hand, when failing livers were biopsied, many Y-chromosome-positive cells were present. Although we could not clearly show the origin of those Y-positive cells, circulating macrophages were candidate sources

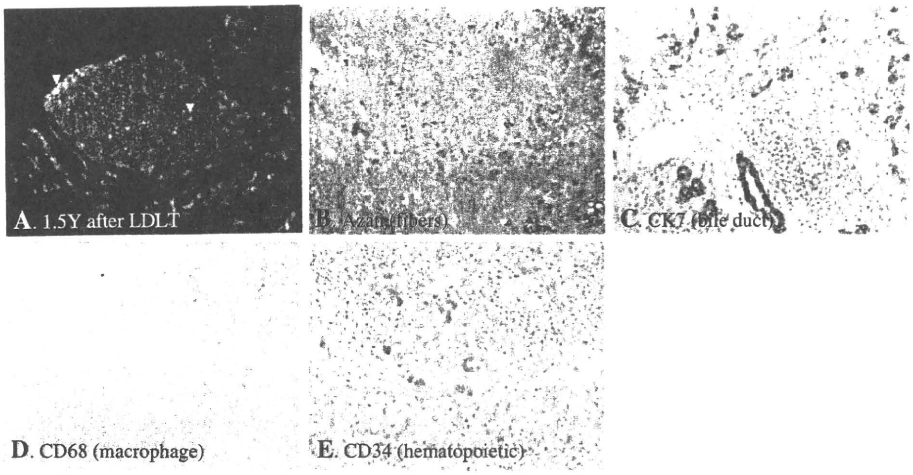

Fig. 5 Immunohistochemical stainings in case 15, secondary biliary cirrhosis after LDLT. **a** FISH showing Y-chromosome-positive cells (white square), **b** Azan staining was positive, showing the presence of liver fibrosis, **c** CK7 (cytokeratin 7, bile duct) was not positive in the black square, **d** CD68 (macrophage) was partially positive in the black square, **e** CD34 (hematopoietic cell) was not positive in the black square. *LDLT* living-donor liver transplantation

Table 1 Demographics of male recipients with female donors

Case no.	Age	Gender	Etiology	Donor	Blood type match	Graph type	Biopsy period after LDLT	Comments	Outcome
1	16	M	FHF	Mother	Identical	L	3d, 4Y	None	Survived
2	5	M	BA	Mother	Identical	LL	2M, 8Y	Cholestasis	Survived
3	56	M	LC-B/HCC	Sister	Identical	R	1.5M, 1.8Y, 2Y mild ACR	None	Survived
4	20	M	FHF	Aunt	Identical	R	1M, 5M, 2Y	Cholestasis	Survived
5	58	M	LC-C	Sister	Identical	R	2M	Hepatitis	Survived
6	56	M	LC-B/HCC	Daughter	Identical	R	8M	Vanishing BD	Survived
7	56	M	LC-B/HCC	Daughter	Identical	R	9M (Re-LDLT)	Poor quality	Survived
8	56	M	LC-B/HCC	Wife	Identical	R	6M	Mild ACR	Survived
9	58	M	LC-C/HCC	Daughter	Incompatible	R	3W	Hepatitis	Survived
10	62	M	LC-C	Sister	Compatible	L	1.5M	Moderate ACR	Survived
11	41	M	PBC	Wife	Identical	R	1W, 1M (autopsy)	Severe ACR	Died (2M)
12	50	M	LC-B	Wife	Identical	R	10d (graft failure)	Malcirculation	Died (1M)
13	57	M	LC-C/HCC	Wife	Identical	R	10d, 2M (graft failure)	Moderate ACR	Died (2M)
14	47	M	LC-Al	Sister	Identical	R	3.8Y (liver cirrhosis)	Poor quality	Died (3.8Y)
15	51	M	LC-C	Sister	Identical	R	2.5Y (chronic liver failure)	Biliary cirrhosis	Died (2.5Y)

FHF fulminant hepatic failure, *BA* biliary atrasia, *ACR* acute cellular rejection, *LC-B* liver cirrhosis due to hepatitis B, *LC-C* liver cirrhosis due to hepatitis C, *LC-Al* liver cirrhosis due to alcohol hepatitis, *HCC* hepatocellular carcinoma, *PBC* primary biliary cirrhosis, *d* days, *M* months, *Y* years, *LDLT* living-donor liver transplantation

Table 2 Summary of results

	Normal regeneration	Acute graft failure	Chronic graft failure
Y chromosome	−	++	+
Hepatocyte antigen	−	−	−
CK7 (bile duct)	−	−	−
CD68 (macrophage)	−	Partial +	−
CD34 (hematopoietic)	−	+	+

because some cells were positive for CD68, which we used to identify macrophages. However, CD34, used for hematopoietic cells, was negative, which indicated that those Y-positive cells did not have hematopoietic origins. In addition, there may be significant sampling variability in liver biopsy specimens from a single liver biopsy, which may not necessarily be representative of the entire liver. In liver chronically damaged by biliary complication, Y-chromosome-positive cells were not as numerous as seen in the case of acute graft failure. In addition, despite the information about expression of progenitor cell markers such as c-kit and Thy-1, we did not investigate this in this study; this awaits further investigation. With regard to CD68(+) Y chromosome(+) cells, we presume that they are regular macrophages from recipient side to dispose of damaged cells in failing liver, not special multipotent stem cells expressing CD68.

In conclusion, in adult-to-adult LDLT, vigorous liver regeneration occurs in graft livers. Involvement of extrahepatic cells in normal adult-to-adult liver regeneration seems limited.

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Research Article

Noncultured Autologous Adipose-Derived Stem Cells Therapy for Chronic Radiation Injury

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Increasing concern on chronic radiation injuries should be treated properly for life-saving improvement of wound management and quality of life. Recently, regenerative surgical modalities should be attempted with the use of noncultured autologous adipose-derived stem cells (ADSCs) with temporal artificial dermis impregnated and sprayed with local angiogenic factor such as basic fibroblast growth factor, and secondary reconstruction can be a candidate for demarcation and saving the donor morbidity. Autologous adipose-derived stem cells, together with angiogenic and mitogenic factor of basic fibroblast growth factor and an artificial dermis, were applied over the excised irradiated skin defect and tested for Patients who were uneventfully healed with minimal donor-site morbidity, which lasts more than 1.5 years.

1. Introduction

There is an increasing worry on radiation injuries probably caused by nuclear power plant (NPP) reactor accidents, therapeutic irradiation for malignancy, and interventional radiology (IRV) of unexpectedly prolonged fluoroscopic procedures for cardiovascular diseases such as arrhythmia, ischemic heart diseases, or nuclear medicine of overdose intake of the radioactive for nuclear medicine of internal radiation therapy. The problems are concerning chronic radiation injury as well as how to heal such local and systemic injures acutely. Local chronic radiation injury is resistant to conventional therapeutic modalities such as flap coverage or skin grafting because the deteriorated margins are sometimes indistinguishable from normal intact tissue, and thus sufficient enough debridements are not obtained with surgeons' naked eyes.

These conditions should be treated properly for the sake of life saving and improvement of local wound healing [1]. However, data of total evidence-based clinical analysis were not established yet. Authors' institute, Nagasaki University, is selected as a global strategic center for radiation health risk control by the Japan's Ministry of Education, Culture, Sports and Technology from FY 2007 to 2011 and exploring to establish such therapeutic regimens, to prevent the radiation injuries, and possibly to regenerate medical and surgical therapy for radiation injuries by using patients' own adipose tissue-derived stem cell therapy.

Often seen chronic radiation injuries are well handled by sufficient enough blood supply to the radiated tissues, especially in the cartilage, bare bone, and hardened scar tissues. For this purpose, local, distant, and microsurgical vascularized flaps are applied. Recent development of microvasculature of the skin and soft tissues including the connective tissues plays major roles in attributing to accelerate local wound healing. Also, externally administered angiogenic growth factor such as basic fibroblast growth factor (bFGF) together with temporal wound coverage of artificial skin substitute is very effective for those patients with severe injuries, patients with comorbidities, who are

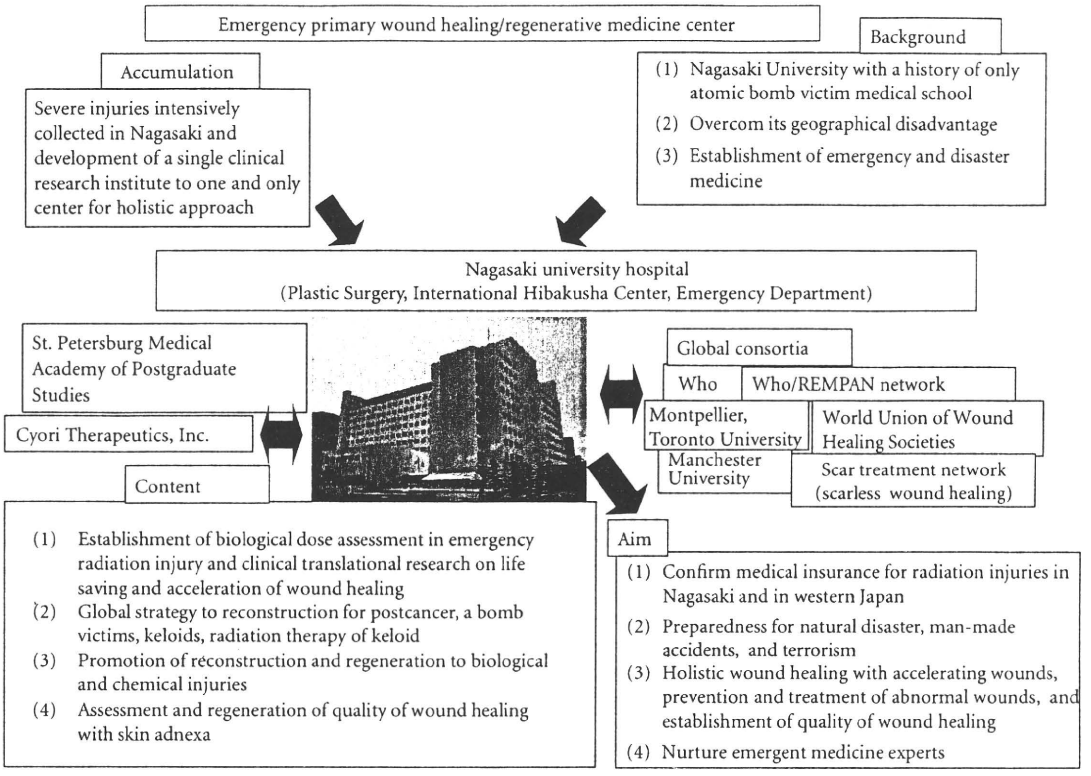

FIGURE 1: Strategy of emergency radiation injury. Collaborative work with highly established international centers and organ is proposed.

intolerant to the extensive and long surgeries [2]. Here, chronic radiation-injured wounds are tested with non-cultured autologous adipose-derived stem cells and clinical implications are discussed.

2. Materials and Methods

2.1. Treatment of Chronic Local Radiation Injury with Conventional Methods and Stem Cells. Often experienced in radiation therapy for malignancy, cardiovascular modalities should be categorized as difficult wounding with poor vasculature or less healing potentials.

From January 1990 to April 2007, 10 (8 females and 2 male) patients who demonstrated chronic radiation injuries such as telangiectasia, xerosis, epidermal atrophy karatoses, and fibrosis as well as deep ulcers in the costal ribs and sternum by adjuvant radiation therapy after mastectomy and prolonged fluoroscopic procedures for cardiovascular diseases were surgically treated.

Other selective clinical cases used angiogenic growth factor namely human recombinant basic fibroblast growth factor (rh-bFGF), which is clinically approved and widely used for clinical wounds in Japan with skin substitutes, which are also clinically available not only in Japan but also in many other nations including USA, the majority of EU nations, and several Asian counties, and the effectiveness of using the artificial skin substitutes in the chronic radiation injuries is temporal coverage and sustainability of both internal and external cells and growth factors. Therefore, combined use of bFGF and artificial skin substitute leads to improved quality of wounds (scar tissue) as well as facilitated wound healing [3].

One case was treated with non-cultured autologous adipose-derived stem cell (ADSC) for chronic sacro-coccygeal radiation ulcer in 2008, which was caused by a therapeutic radiation at fractionate 50 Gy at 40 years previously.

2.2. Methods. This study was approved by the Ethics Committee of the Nagasaki University Hospital, and written informed consent was obtained from all patients (approved no. 08070296) and partly supported by the Global COE (Center of Excellence) Program E08, Global Strategic Center for Radiation Health Risk Control, and it was funded by the Japan Society for the Promotion of Science. This national research grant enables us to investigate 3 main themes related to radiation health risk: (1) atomic-bomb disease followup cohort research with over 60-year continuous research history, (2) radiation basic science, and (3) international radiation health research. Especially, this radiation regeneration research was involved in further international collaboration framework under international organizations such as WHO (World Health Organization) and IAEA (International Atomic Energy Agency) (Figure 1).

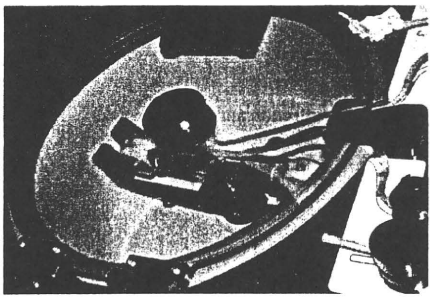

FIGURE 2: The Adipose-derived stem cells are processed in a closed-circuit machine within 1.5 hours.

2.3. Harvesting of Adipose Tissue by Liposuction and Isolation of ADSCs. 3–5 mm incisions, two incisions in the abdomen, four incisions in the thigh, and two incisions in the gluteal region, were made on the abdominal region, the thigh, and the gluteal region. The subcutaneous layer was infiltrated with a lactated Ringer's solution with addition of 0.5 mL of epinephrine and 25 mL of 1% lidocaine per 500 mL. Adipose tissue was suctioned using an 18-G Becker cannula with a 50 mL syringe. Total 250 gram-fat tissues, 120 grams from the abdominal region, 80 grams from the gluteal regions, and 50 grams from the thighs were harvested.

ADSCs were isolated from the suctioned adipose tissue by using the Celution system. (Cytori Therapeutics, Inc., USA). Briefly, the suctioned adipose tissue was introduced into the Celution cell-processing device, which automatically and aseptically extracts and concentrates the mononuclear fraction of adipose tissue and removes unwanted or deleterious cells, cell and matrix fragments such as lipids. By using the Celution system, a 5 mL solution is added to isolated ADSCs in about one and a half hour (Figure 2). The whole procedure is in a closed circuit and this reduces the chance of the contamination.

The small portion of processed ADSCs was used for the ex vivo cell culture and confirmed the proliferation and differentiation potential. The ADSCs-rich fraction was then plated onto collagen type-I-coated plastic culture flasks in a serum-free medium for primate embryonic stem cells (Primate ES medium, RiproCELL, Tokyo), and the cells, clonally expanded, were collected and stored in Liquid Nitrogen as the primary ADSCs. ADSCs were subcultured when they reached to 80% confluence. Cells were treated with trypsin/EDTA solution, neutralized with tripsin-neutralizing solution, and collected by centrifugation for 5 minutes at 1,200 rpm. The pellects were resuspended in a fresh medium; the number of cells was counted, and 3×10^5 cells were plated into T25 flasks (25 cm^2) for subculture while the rest of the cells were stored in liquid nitrogen.

2.4. Adipose-Derived Stem Cell Grafting and Postoperative Management. For the scaffold purpose, we used the artificial dermis (Terudermis, Olympus-Terumo Biomaterials Co., Ltd., Japan) (Figure 3). The Terudermis is composed of two layers: a lower layer of bovine atelocollagen and an upper layer comprising a silicone sheet which protects against infection and dryness from the outside. After minimum debridement, the Terdermis was multilayered and stacked over freshly debrided wounds. The silicone sheets were removed except top Terudermis. The two-thirds of isolated ADSCs alone were injected; around the debrided wounds, at the base of the wounds, and into Terudermis. Another one-third of ADSCs was mixed with the autologous adipose which was rinsed with a lactated Ringer's solution. In the Celution system, after isolating ADSCs, the disposable cell collection plastic case one was again used to mix the suctioned fat, which is rinsed separately in the 50-cc syringe and repeated until the oil droplets are removed. After being mixed, it was injected into a zone of hard fibrotic tissue around the debrided wounds in 2-cm width in all directions.

2.5. Angiogenic Growth Factor and Basic Fibroblast Growth Factor (bFGF). Genetically recombinant human bFGF (Fiblast, Trafermin) was purchased from Kaken Pharmaceutical Co., Inc (Tokyo, Japan). The Freeze-dried bFGF was dissolved in 5 mL of benzalkonium chloride containing solution right before the first use and stored at 4°C for one day, with 300 μL sprayed over 30 cm^2 area from 5 cm distance, and 0.3 mL per day of this solution was applied over the wound. One week after removing the silicone layer, human recombinant fibroblast growth factor (bFGF: Fiblast, Kaken Co., Ltd., Japan) (Figure 4) was sprayed. The wound was covered with nonadherent occlusive foam dressing.

3. Results

3.1. Treatment of Chronic Local Radiation Injury with Conventional Method. All wounds were healed after several surgical modalities. None of the cases was healed with single procedure (2 to 6 surgeries, mean 4.3).

Of our cases, one breast-cancer patient was treated by a standardized Halsted method with major and minor pectoralis muscle, radical neck, and axillary and internal mammary lymph node dissections. This patient has undergone 50-Gy fractionate radiation therapy postoperatively. The radiated area showed chest fistula deep to the pleura with surrounding unhealthy hardened scar tissue and chronic inflammation.

The whole affected area was sequentially excised in 3 reconstructive surgeries, starting with rectus abdominis musculocutaneous flap, then latissimus dorsi musculocutanous flap, and finally with groin-free flap. In the course after each surgery, the margin of the flap was partially dehiscent and necrotized, which required further touchups? The total number of the reconstructive surgery was 6 (Figure 5).

3.2. Treatment of Chronic Local Radiation Injury with Adipose-Derived Stem Cells. Regeneration method with patient's own non-cultured ADSCs was planned for a patient underwent 50-Gy fractionate radiation therapy for uterine cancer 40 years ago. The pigmented sacrococcygeal region appeared with central intractable wound. Necrotized bone and fascia muscle along with malodour were observed. The ADSCs-treated chronic radiation wounds underwent

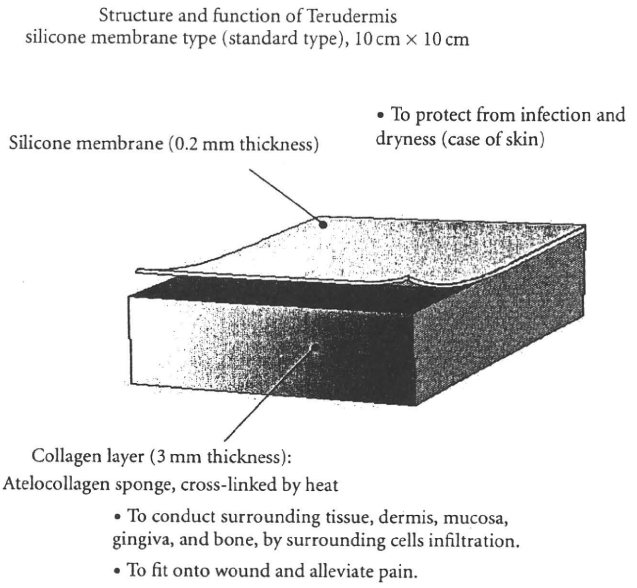

FIGURE 3: Freeze-dried bilayer artificial dermis made of bovine dermis. The outer membrane of silicone layer is easily removed and easily soaked with cell-containing solution.

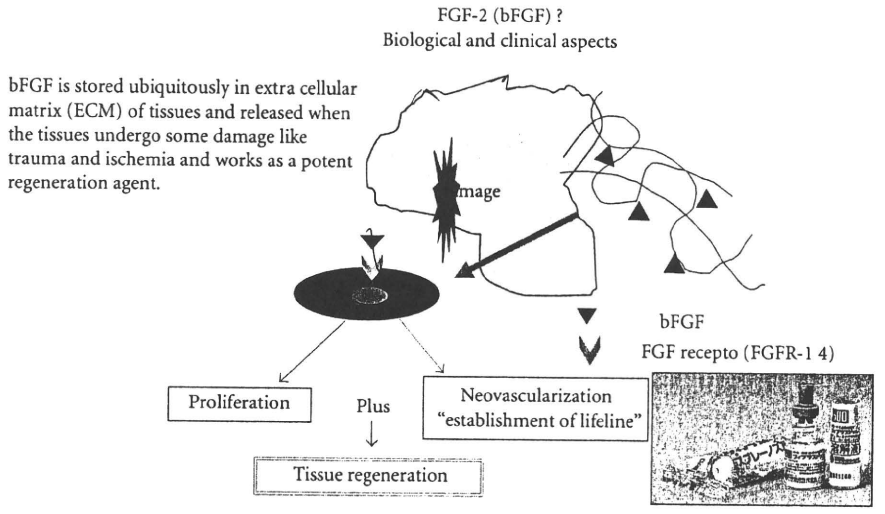

FIGURE 4: Commercially available growth factor and basic fibroblast growth factor (bFGF). Mode of action is explained and the mechanism is proposed.

debridement to remove unhealthy superficial necrotized bone, fascia, periosteum, and muscle. 3.8×10^7 cells in 5-mL of final volume from 250 mL of subcutaneous aspirated fat obtained from nonradiated area were used. Some ADSCs were directly injected in wound bed and margins; others were soaked with the artificial dermis. In a few days postoperatively, the silicone upper layer of the artificial dermis (Terdermis) was removed, and bFGF was sprayed over the regenerated wound for three weeks. There was no significant adverse effect neither in donor site or treated wound. The wound was healed uneventfully by day 82 and no sign of recurrence appeared, but the regenerated tissue developed mature in 1.5 years (Figure 6).

4. Discussion

Local radiation injuries caused during medical therapy for malignant tumors [4] and heart disease [5] may be accompanied with systemic symptoms of hematologic, neurologic, and gastrointestinal symptoms such as neutropenia, thrombopenia, fatigability, nausea, and diarrhea by contact to the

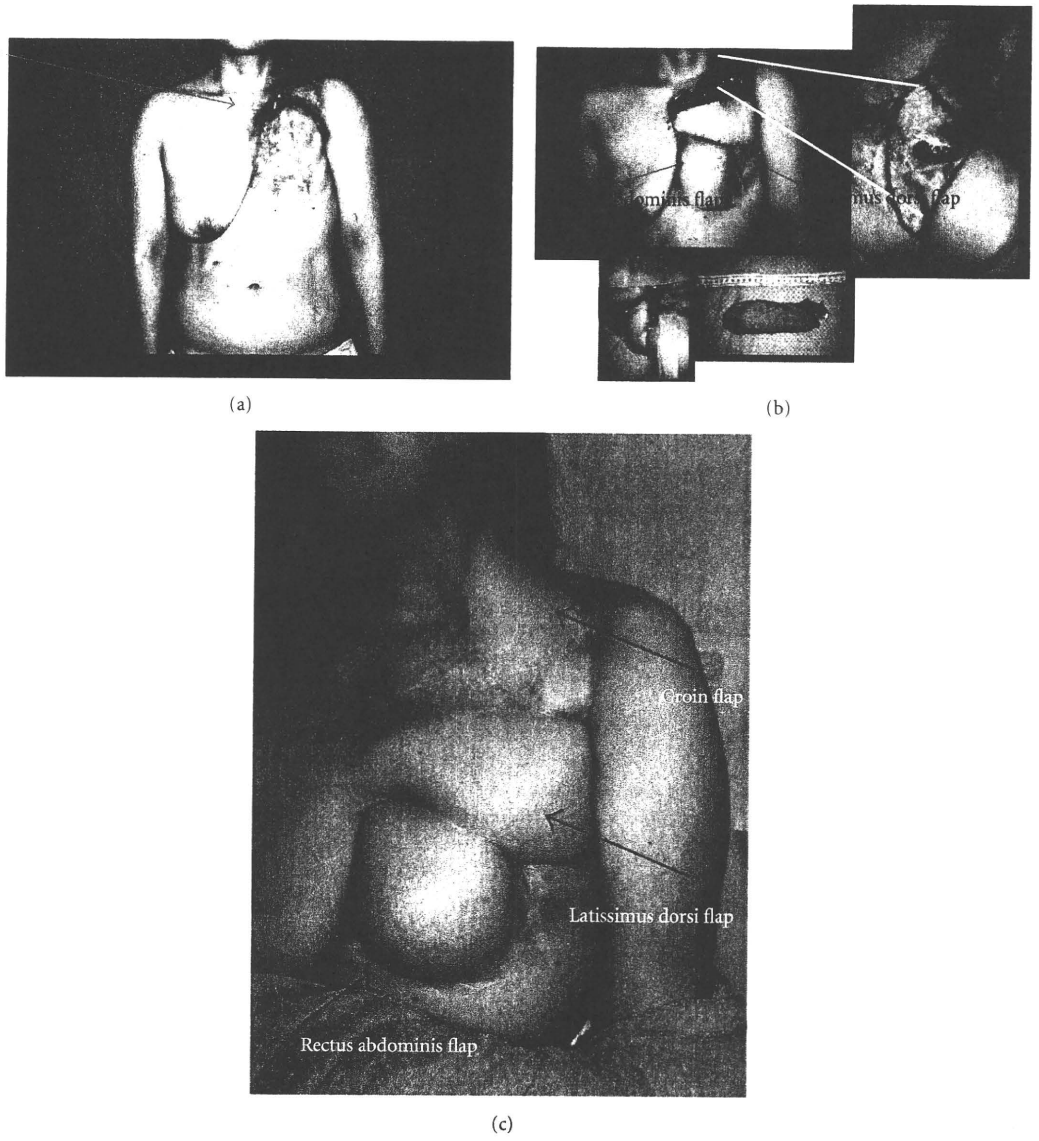

FIGURE 5: 55-year-old woman underwent a left breast cancer surgery by a standardized Halsted methods, followed by 50-Gy fractionate radiation therapy 15 years previously. (a) The chest demonstrates fistula to the costal rib and adjacent to the pleura as the arrow depicts, and the surrounding tissues were firm and various-degree inflammation existed. (b) Sequential three major flaps (rectus abdominis, latissimus dorsi, and free groin flap) are used for total coverage. (c) In 7 years postoperative view. There is irregularity of the scar margins.

scrap yard radioactive wastes without notice [6] or exposure to the radiation accidents [7] by touching gammagraphy radioactive source by mistake [8]. Since locally radiated tissues show decreased or insufficient vascularity and tissue damage, demonstrating erythema, teleangiectasia, pigmentation, or dermal atrophy, once wound is developed, it is often intractable and further leading to tissue necrosis, infection, and later fibrosis in demonstrating chronic radiation injury syndrome [9]. Therefore, radiation-injured wounds tend to persist for a long time, show impaired healing, and be prone to recurrence even by minor trauma. Radiated wounds are treated by adequate debridement both in the depth and in the width and covered with well-vascularized tissues or by cultured bone-derived mesenchymal stem cells [8]; however, the long-term outcome is not warranted, and donor-site morbidity and the duration for treatment are sometimes concerned, especially for the aged patients or patients who somehow have problems in harvesting the donors or being limited due to the coexisting diseases. As seen in our reconstructive cases, the surgical modalities constantly required multiple surgeries partly due to the definitive damage-free margins of the affected tissue.

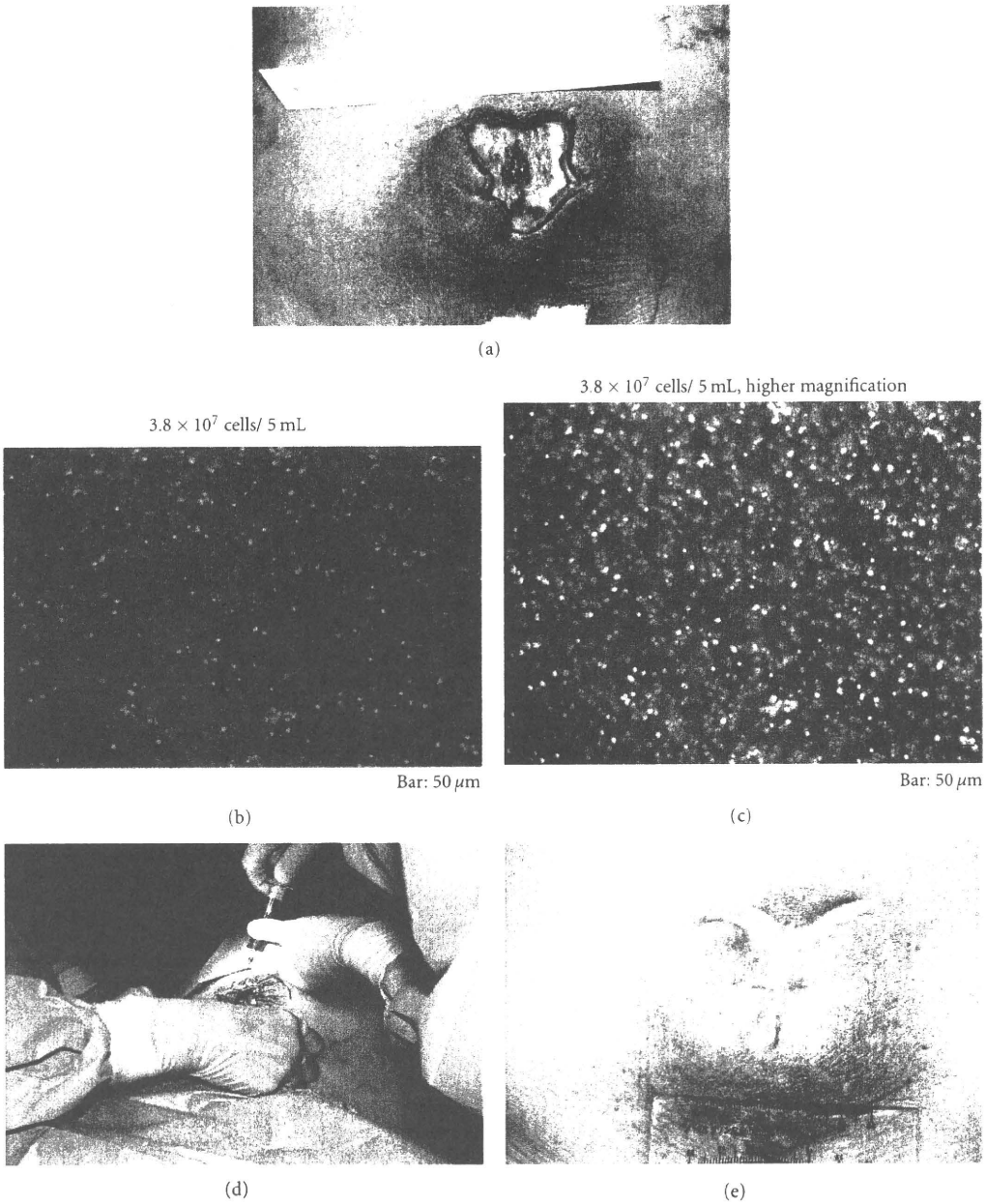

FIGURE 6: 89-year-old woman underwent a uterine cancer surgery followed by 50-Gy fractionate radiation therapy 40 years previously. (a) In 10 × 10 cm area of radiation, 5 × 10 cm area was exposed. Bone, fascia, and muscle as well as skin and fat were affected. (b, c, d) After careful debridement, 3.8×10^7 cells/5 mL were applied over the wound bed and margins and soaked with artificial dermis. In a few days postoperatively, bFGF was sprayed over the peeled-off inner regenerated tissue for 21 days. (e) In 1.5 years postoperative view. The regenerated tissue remained durable, soft, and pliable.

Application of Stem cell therapies for repair and regeneration has recently been investigated at a clinical level in variously defected or injured tissues, among which stem cells and adipose-derived stem cells (ADSCs) can be harvested with a minimally invasive procedure by liposuction procedure through a small incision. Similar to our method but in detail very different, Clinically purified autologous lipoaspirates were used as treatment for radiotherapy tissue damage of consecutive 20 patients. Indirectly, induced ADRCs have potential in cell therapy for radiation injury due to increasing neovascularization and retention of the fat property [10].

This enables us to adopt this regeneration method for patients with severe comorbidity such as elderly systemic disease and physical wasting state (data not shown). The