Surgical Results After Preoperative Chemoradiation Therapy for Patients With Pancreatic Cancer

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Objectives: The results of surgical therapy alone for pancreatic cancer are disappointing. We explored surgical results after neoadjuvant chemoradiation therapy (NACRT) for patients with pancreatic cancer that extended beyond the pancreas.

Methods: Sixty-eight consecutive patients with pancreatic cancer who underwent pancreatic resection were included. Twenty-seven patients underwent surgical resection after NACRT (NACRT group). The other 41 patients were classified as surgery-alone group. Surgical results were compared in patients who underwent curative resection (R0/1) who were followed up for at least 25 months and underwent no adjuvant therapy.

Results: A lower frequency of lymph node metastasis was observed in the NACRT group ($P < 0.05$). The frequency of residual tumor grading in the NACRT group was significantly different from that in surgery-alone (R0/1/2%, 52/15/33 vs 22/51/27; $P = 0.0040$). In R0/1 cases, overall survival and disease-free survival rates in the NACRT group (n = 18) were significantly longer than in surgery-alone (n = 30, $P < 0.05$). The rate of local recurrence in the NACRT group was significantly less than in surgery-alone (11% vs 47%, $P = 0.0024$).

Conclusions: This single-institution experience indicates that NACRT is able to increase the resectability rate with clear margins and to decrease the rate of metastatic lymph nodes, resulting in improved prognosis of curative cases with pancreatic cancer that extended beyond the pancreas.

Key Words: curative resection, retrospective analysis, gemcitabine, 5-FU, CDDP, survival analysis

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The results of surgical therapy alone for pancreatic ductal cancer are still disappointing. Surgical resection for patients with pancreatic cancer at an early stage, which corresponds to cancer growth within pancreatic parenchyma, is the only curative treatment option; however, both distant and local/regional patterns of relapse are common within a year, even after curative resection.[1] In approximately 50% of resected pancreatic tumors, the surgical margins contain tumor cells.[2] The aggressive features of pancreatic cancer can lead to a dismal prognosis, and surgery alone is not optimal for achieving locoregional control of pancreatic cancer.[3,4]

To achieve 5-year survival exceeding 50% in patients with pancreatic cancer, Traverso LW advocated appropriate patient selection for curative resection by accurate staging, balanced resection, centralized treatment in high-volume centers, and the use of an effective adjuvant or neoadjuvant therapy.[4] Neoadjuvant (preoperative) chemoradiation therapy (NACRT) has several possibilities such as improved patient selection after the restaging evaluation, increased resectability rate with clear margins (R0 resection),[5] decreased rate of metastatic lymph nodes, and decreased rate of local relapse.[6] We previously reported that preoperative chemoradiation (5-fluorouracil [5-FU] or gemcitabine + 40 Gy) enabled the selection of 24 of 32 patients for surgery and resulted in acceptable toxicity.[7]

The objectives of this retrospective study were to compare the pathological results, overall survival (OS) and disease-free survival (DFS) rates, and type of recurrence in pancreatic cancer patients who underwent surgical resection after NACRT with those of patients who underwent surgery alone.

MATERIALS AND METHODS

One hundred seventy-five consecutive patients with a clinical diagnosis of pancreatic ductal adenocarcinoma were evaluated for the staging of tumor extension between January 2000 and December 2005 in Kansai Medical University Hospital. Cases involving an endocrine tumor of the pancreas, intraductal papillary mucinous cancer, acinar cell cancer, anaplastic cancer, duodenal cancer, distal common bile duct cancer, or ampullary cancer were excluded. During this period, 68 consecutive patients with pancreatic cancer who underwent pancreatic resection were included in this study. All tissues of the resected patients were pathologically proven ductal adenocarcinoma of the pancreas. Between 2001 and 2004, NACRT was performed in 35 patients who had radiologically diagnosed pancreatic cancer that extended beyond the pancreas (T3/T4 pancreatic cancer by TNM staging), and who were regarded as potentially resectable ([PR] n = 19) and locally advanced ([LA] n = 16), defined by National Comprehensive Cancer Network (NCCN) guideline.[8] Treatment consisted of concurrent radiotherapy (40 Gy within 4 weeks), and chemotherapy with 5-FU and cisplatin (CDDP) ([FP] n = 13) or with gemcitabine ([GEM] n = 22), as described in the previous article.[7] Finally, 27 patients (PR, n = 16; LA, n = 11; FP, n = 8; GEM n = 19) underwent surgical resection (NACRT group). The other 41 patients were classified as the surgery-alone group that consisted of pancreatic cancer patients who had a tumor limited to the pancreas (T1/T2 TNM staging) between 2001 and 2004, and the resected cases from 2000 and from 2005. Forty-eight patients with residual tumor staging of R0/1 were abstracted from 68 resected patients between 2000 and 2005, and the clinical and pathological characteristics, OS rate, DFS rate, and type of relapse were compared (NACRT group, n = 18; surgery-alone group, n = 30). All patients were followed up for at least 25 months and underwent no adjuvant chemotherapy.

As shown in the previous article,[9,10] local tumor unresectability was defined as (1) vascular involvement of a major

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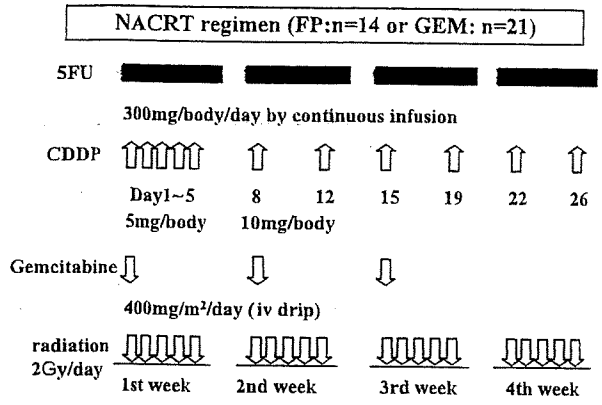

FIGURE 1. The regimen of NACRT.

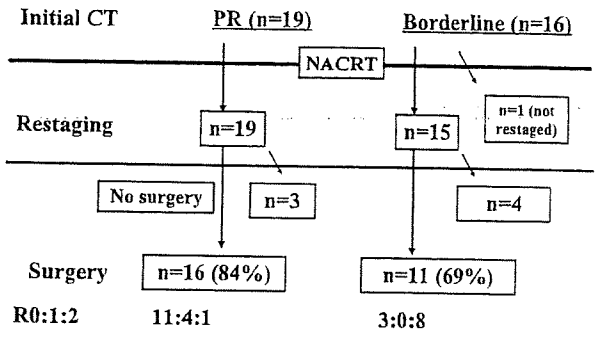

FIGURE 2. Clinical course of the NACRT group. PR indicates potentially resectable; borderline, borderline resectable in the NCCN guidelines.

peripancreatic artery (defined as tumor ingrowth with >50% vessel contiguity in the celiac trunk [CA], common or proper hepatic artery, or superior mesenteric artery); (2) extended obstruction of the portal vein to distal branches of the superior mesenteric vein; or (iii) with cavernous transformation of the porta hepatis. Patients with peritoneal carcinomatosis or distant organ metastasis were also excluded from this study. On the other hand, patients with cancer in the pancreatic body and tail, with CA invasion, and without superior mesenteric artery (SMA) invasion were also classified as candidates for the Appleby operation. All operations were performed by 2 experienced hepatopancreatobiliary surgeons who were in agreement about the extent of surgery to be performed. Preoperative staging was performed using contrast-enhanced computed tomography (CECT), abdominal angiography, CT-assisted hepatic arteriography, and CT during arterial portography before August 2002, and using CE multidetector row CT after September 2002.[9,10]

The detailed eligibility criteria were reported in the previous article.[8] Informed consent was obtained from all patients according to institutional regulations, and this study was approved by the local ethics committee. Patient data were obtained from the prospective database of pancreatic disease at Kansai Medical University Hospital.

Treatment Protocol

Patients received a continuous infusion of 5-FU (200 mg/m^2 5 times per week, 1–4 weeks), accompanied by CDDP (3 mg/m^2 on days 1–5, 6 mg/m^2 on days 9, 12, 14, 19, 23, and 26) in the FP-NACRT arm (Fig. 1). Gemcitabine at a dose of 400 mg/m^{-2} per day was given intravenously over 30 minutes starting 2 hours before radiotherapy 3 times weekly for 4 weeks in the GEM-NACRT arm (Fig. 1). Concomitantly, 10 mg of azasetron was routinely given before chemotherapy administration. Chemoinfusion was started approximately 60 minutes before radiation therapy. The protocol for radiation therapy was as follows. A total of 40 Gy was concurrently delivered in 2-Gy fractions to the tumor bed Monday through Friday for 4 weeks by a linear accelerator using megavoltage photon beams (6 MV). The clinical target volume was delineated slice by slice on the planning CT scan using CT simulation software. It encompassed the gross tumor volume as defined by the preoperative CT scan, plus a margin of 0.8 cm. Also included were retroperitoneal paraaortic lymphatic vessels between the CA and the upper mesenteric artery to the anterior level of the vertebral bodies. The gross tumor volume was defined as the gross tumor mass detected by CT scans. The planning target volume included the clinical target volume, with a 1-cm margin. Usually, a 4-field approach was chosen using anteroposterior and left and right lateral beams.

Surgical resection was performed 3 to 4 weeks after NACRT completion if none of the following were found: disease progression to an unresectable status (as previously mentioned) as determined by repeated abdominal CECT, a prohibitive decline in performance status, or other evidence of metastatic disease. Pancreatectomy was performed with portal vein resection, if portal vein resection was predicted to provide a surgical- or pathological-free margin. For resected patients, curative surgery was performed with extended lymph node dissection including paraaortic lymph nodes. Median time from the last day of NACRT to surgical resection was 28 days (range, 15–60 days). If tumor progression was evident, additional treatment with chemotherapeutic regimens was determined on an individual basis.

TABLE 1. Patient and Operative Factors of the NACRT and Control Groups

	NACRT	Control	P
Total no. patients	27	41	
Age	64 (47–74)	66 (50–83)	n.s.
Gender (male/female)	10:17	23:18	n.s.
CA19-9, U/mL	110 (1–8116)	89.7 (1–9116)	n.s.
Comorbid disease (+/−)	14:13	23:18	n.s.
Site of primary lesion			
Head/body-tail	21:6	28:13	n.s.
Tumor size, mm	30 (16–80)	30 (13–90)	n.s.
CDRS (PR/borderline)	16:11	24:17	n.s.
Type of surgery (PD/TP/DP)	20:1:6	27:1:13	n.s.
PV resection (+/−)	4:23	12:29	n.s.
CA resection (+/−)	2:25	1:40	n.s.
Operative duration, min	560 (325–840)	515 (265–900)	n.s.
Extent of blood loss, mL	1390 (400–6420)	1045 (390–7250)	n.s.

Data are expressed as the median (range).

Borderline indicates borderline resectable; CA19-9, carbohydrate antigen 19-9; CDRS, criteria defining resectability status; DP, distal pancreatectomy; n.s., not significant; PD, pancreaticoduodenectomy; PR, potentially resectable; PV, portal vein; TP, total pancreatectomy.

TABLE 2. Tumor Factors of the NACRT and Control Groups

	NACRT	Control	P
Total no. patients	27	41	
Site of primary lesion			
Head/body-tail	21:6	28:13	n.s.
Tumor size, mm	30 (16–80)	30 (13–90)	n.s.
Pathological differentiation (well/mod/por/other)	6:18:1:2	10:22:5:4	n.s.
Stage I–III : IVa/IVb	10:17	13:28	n.s.
LN mets positive/negative	11:16	28:13	0.0440
INF β/γ	19:8	33:8	n.s.
Ly 0/1:2/3	16:11	13:28	0.0440
V 0/1:2/3	22:5	20:21	0.0102
Ne 0/1:2/3	7:20	6:35	n.s.
Ch positive/negative	12:15	22:19	n.s.
Du positive/negative	14:13	20:21	n.s.
S positive/negative	12:15	16:25	n.s.
Rp positive/negative	16:11	28:13	n.s.
PV positive/negative	8:19	15:26	n.s.
A positive/negative	6:21	7:34	n.s.
PL positive/negative	9:18	19:2	n.s.
R grading 0:1:2 (n)	14:4:9	9:21:11	0.0040
R grading 0:1:2, %	52:15:33	22:51:27	
Radiological response*			
Grade Ia:Ib:II	12:6:9	N/E	

Data are expressed as the median (range).

Mod indicates moderately; por, poorly; other, papillary/adenosquamous cell carcinoma; R0, negative margin; R1, positive microscopic margin; R2, positive gross margin; LN met, lymph node metastasis; INF, mode of histological infiltration; Ly, grade of infiltration of the lymphatic vessels; V, grade of venous infiltration; Ne, grade of perineural invasion; Ch, grade of invasion to intrapancreatic common bile duct; Du, grade of invasion to the duodenum; S, grade of invasion to the anterior capsule; Rp, grade of invasion of the retroperitoneal tissue; Pv, grade of invasion of the portal vein; A, grade of invasion of the large artery; Pl, invasion of the extrapancreatic nerve plexus.

*Radiological response was defined as the amount of degenerated cancer cells.

Ia indicates less than 33% population of degenerated cancer cells; Ib, between 34% and 66%; II, more than 67%.

Follow-Up

After the completion of all treatments, patients were evaluated by physical examination every month, chest radiography, and CECT every 3 months. The development of a new low-density mass in the region of the pancreas bed and root of the mesentery was considered evidence of local recurrence even in the absence of symptoms. Cytological or histological confirmation of recurrent disease was not routinely required. Radiographic evidence of a new low-density region in the liver or lung was considered evidence of distant recurrence; biopsy was rarely performed. Peritoneal recurrence was defined as new ascites on physical examination or on CT and was confirmed by cytological examination of ascites. Sites of recurrent disease were documented at the time of initial recurrence. If the patients had any useful tumor markers at the first admission, these were checked again for confirmation of recurrence. In all patients, the date of first treatment was chosen as the starting point for survival analysis. All patients had a minimum follow-up of 25 months.

End Points and Statistical Analysis

The countable data were expressed as the median and range. The χ test or Fisher exact test was used for comparison of categorical variables when appropriate. The OS and DFS rates were calculated from the start of study treatment until death or the final date of follow-up and determined by the Kaplan-Meier method. Patients alive at the time of the study report were censored. The log-rank test was applied for the comparison of survival rates between different groups. Results were considered significant at $P < 0.05$.

RESULTS

Clinical Course of the NACRT Group

From 2001 to December 2004, 35 patients diagnosed as having pancreatic cancer were treated with NACRT using FP or GEM. Fourteen patients received FP-NACRT, and 21 patients received GEM-NACRT. According to NCCN guidelines, the 35 patients were divided into PR (n = 19) and borderline (n = 16), as shown in Figure 2. After NACRT, 34 patients were restaged using CECT or CE multidetector row CT, and 1 patient refused restaging. Three patients in the PR category (16%) did not undergo surgical resection because of liver metastasis, and 5 patients in the borderline category (31%) who had peritoneal metastasis (n = 2), liver metastasis (n = 1), and progressive disease (n = 1), in addition to 1 patient who refused restaging, did not undergo surgical resection. Finally, 16 patients (84%) in PR and 11 patients (69%) in borderline underwent surgical resection. The frequency of R0/1 in PR was 94%, significantly superior to 27% in borderline ($P < 0.0001$).

When radiological response was defined as the amount of degenerated cancer cells, only 9 patients (33%) in this NACRT regimen had more than 67% population of degenerated cancer cells (Table 1).

TABLE 3. Patient and Operative Factors in R0/1 Cases of the NACRT and Surgery-Alone Groups

	NACRT	Surgery-Alone	P
Total no. patients	18	30	
Age	65 (51–74)	68 (50 – 83)	n.s.
Gender (male/female)	7:11	18:12	n.s.
CA19-9, U/mL	90 (1–8116)	87 (1–9116)	n.s.
Comorbid disease (+/−)	10:8	18:12	n.s.
Site of primary lesion			
Head/body-tail	15:3	18:12	n.s.
Tumor size, mm	29 (16–80)	30 (13–90)	n.s.
PR/borderline	15:3	21:9	n.s.
Type of surgery (PD/TP/DP)	15:0:3	17:1:12	n.s.
PV resection (+/−)	3:15	9:21	n.s.
CA resection (+/−)	1:17	1:29	n.s.
Operative duration, min	557 (330–795)	512 (265–900)	n.s.
Extent of blood loss, mL	1078 (400–6420)	970 (390–5030)	n.s.

Data are expressed as the median (range).

Borderline indicates borderline resectable (NCCN, criteria defining resectability status); DP, distal pancreatectomy; LN, lymph node; PD, pancreaticoduodenectomy; PR potentially resectable; PV, portal vein; TP, total pancreatectomy.

TABLE 4. Tumor Factors in R0/1 Cases of the NACRT and Control Groups

	NACRT	Surgery Alone	P
Total no. patients	18	30	
Pathological differentiation (well:mod:por:other)	5:11:1:1	8:16:2:4	n.s.
Stage I-III/IVa/IVb	10:8	13:17	n.s.
LN met positive:negative	6:12	18:12	n.s.
INF β:γ	12:6	27:3	n.s.
Ly 0/1:2/3	12:6	11:19	n.s.
V 0/1:2/3	15:3	14:16	0.0158
Ne 0/1:2/3	5:13	4:26	n.s.
Ch positive/negative	8:10	13:17	n.s.
Du positive/negative	10:8	12:18	n.s.
S positive/negative	12:15	16:25	n.s.
Rp positive/negative	7:11	18:12	n.s.
PV positive/negative	2:16	8:22	n.s.
A positive/negative	1:17	2:28	n.s.
PL positive/negative	3:15	11:19	n.s.
Radiological response			
Grade Ia:Ib:II	7:4:7	N/E	

Data are expressed as the median (range).

Mod indicates moderately; por, poorly; other, papillary/adenosquamous cell carcinoma; LN met, lymph node metastasis; INF, mode of histological infiltration; Ly, grade of infiltration of the lymphatic vessels; V, grade of venous infiltration; Ne, grade of perineural invasion; Ch, grade of invasion to intrapancreatic common bile duct; Du, grade of invasion to the duodenum; S, grade of invasion to the anterior capsule; Rp, grade of invasion of the retroperitoneal tissue; Pv, grade of invasion of the portal vein; A, grade of invasion of the large artery; Pl, invasion of the extrapancreatic nerve plexus.

Comparisons of Surgical Results Between NACRT and Surgery-Alone Groups

The operative and tumor characteristics of all resected patients are listed in Tables 1 and 2. There were no significant differences in patient and operative characteristics between NACRT and surgery-alone groups. On comparison of tumor characteristics, significantly lower frequencies of lymph node metastasis, infiltration of lymphatic vessels, and venous infiltration in the NACRT group were found relative to those in the surgery-alone group ($P < 0.05$). Moreover, the frequency of pathologically curative resection (R0) in the NACRT group was significantly higher that that in the surgery-alone group (R0/1/2%, 52/15/33 vs 22/51/27; $P = 0.0040$). On abstracting R0/1 cases in NACRT and surgery-alone groups (Tables 3 and 4), although there was a tendency of a lower frequency of lymph node metastasis and infiltration of lymphatic vessels in the NACRT group relative to the surgery-alone group, a significant difference was not achieved. A significantly lower frequency of venous infiltration only was found in the NACRT group relative to the surgery-alone group ($P = 0.0158$). There was no difference in the survival curve of R2 cases between them.

Comparisons of OS and DFS Rates

All patients were followed up for at least 25 months without adjuvant chemotherapy. The median follow-up time after NACRT was 20.5 months (range, 3–84 months) for all patients and 56 months (range, 34–84 months) for censored patients. No

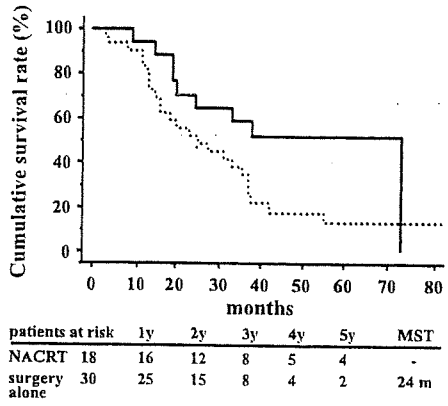

FIGURE 3. Overall survival rates in the NACRT and surgery-alone groups. Solid line indicates NACRT group; broken line, surgery-alone group. MST indicates median survival time.

treatment death occurred. Although the 1-, 3-, and 5-year OS rates in the NACRT group were 85%, 39%, and 34%, superior to 68%, 30%, and 9% in the surgery-alone group ($P = 0.0792$), there was no significant difference. The median survival time in the NACRT and surgery-alone groups was 24.5 and 18.5 months, respectively.

When patients who underwent curative resection (R0/1) were abstracted from all patients, there was a significant difference in the OS curve between the NACRT and surgery-alone groups (OS rates at 1 year, 3 years, and 5 years: 94%, 59%, and 52% in the NACRT group versus 83%, 34%, and 13% in the surgery-alone group; $P = 0.0425$; Fig. 3). The median survival time in the NACRT group was not reached, and in the surgery-alone group was 24 months. At a minimum of 36 months' follow-up, 8 patients in the NACRT group (44%) and 5 patients in the surgery-alone group (17%) were alive. Disease-free survival rates at 1 year, 3 years, and 5 years were 59%, 47%, and 47% in the NACRT group, significantly better than 53%, 12%, and 8% in the surgery-alone group (Fig. 4, $P = 0.0359$). Although the DFS rate at 1 year was similar, the difference in the DFS curve dramatically extended over 1 year after surgical resection. At the minimum follow-up of 25 months, 8 patients (44%) in the NACRT group and only 2 patients (7%) in the surgery-alone group were disease-free, and a significant difference was found between them ($P = 0.0024$). In the

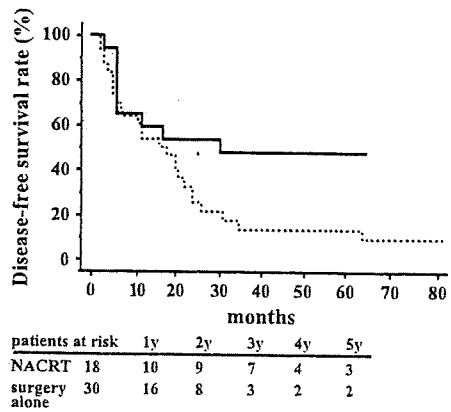

FIGURE 4. Disease-free survival rates in the NACRT and surgery-alone groups. Solid line indicates NACRT group; broken line, surgery-alone group.

NACRT group, all patients disease-free for more than 1 year have survived between 36 and 65 months.

There were no significant differences in OS and DFS rates between the use of GEM- and 5-FU–based chemoradiation. Moreover, there was no significant difference in survival curves between patients with R0 and R1 resection.

Type of Recurrence in Patients Who Underwent Curative Resection (R0/1)

The major pattern of recurrence was distant metastasis such as the liver and peritoneum (39%) in the NACRT and local recurrence (47%) as well as distant metastasis (43%) in the surgery-alone group. The frequency of local recurrence in the NACRT group was 11%, significantly lower than 47% in the surgery-alone group ($P = 0.0024$).

DISCUSSION

Even after curative resection, patients with pancreatic cancer face a 50% to 80% local recurrence rate and a 25% to 50% chance of developing distant metastases at the peritoneum and liver. The dreadful prognosis associated with this disease has mandated studies of combined multimodality therapies with both radiation and chemotherapy.[11,12] Crane et al[13] mentioned that NACRT had its own intrinsic advantages in that it theoretically increased the vulnerability of cancer cells because of intact vasculature, better tumor cell oxygenation, and the probability of sterilizing cells at the resection margin. Neoadjuvant CRT can clinically provide improved patient selection because patients with rapidly progressive systemic disease are identified as part of the restaging evaluation performed after NACRT before the planned surgery. Another advantage is better tolerability, which consecutively allows multimodal treatment in a higher number of patients, and the avoidance of late radiation-related toxicity. Furthermore, NACRT is able to facilitate resectability with free margins and a low frequency of lymph node metastasis. The Duke University group[14] reported that NACRT was associated with a marked reduction in the incidence of pancreatic leak, as well as leak-associated morbidity and mortality. On the other hand, Tse et al[15] referred to the theoretical disadvantages of potential overtreatment for a subset of patients with early-stage disease or with benign disease and of the potential risk of biliary stent–related morbidity.

Our previous study demonstrated that 5-FU/CDDP- or GEM-based CRT could reduce pain at a high rate without affecting Karnofsky performance status and body weight, resulting in acceptable toxicity.[7] Subsequently, we attempted to compare surgical results after NACRT in patients with pancreatic cancer that extended beyond the pancreas with patients who underwent surgery alone in this study. As a result, a lower frequency of lymph node metastasis and a higher frequency of pathologically curative resection were observed in the NACRT group. In patients who underwent curative resection, OS and DFS rates in the NACRT group were significantly longer than in the surgery-alone group. At a minimum follow-up of 25 months, the actual DFS rate in the NACRT group was 44%, significantly better than 7% in the surgery-alone group. Moreover, the frequency of local recurrence in the NACRT group was significantly less than in the surgery-alone group.

Neoadjuvant CRT ultimately leads to patient selection, as patients who show tumor progression during chemoradiation do not undergo surgery. As many as approximately 20% to 40% of patients initially presenting with resectable pancreatic tumors, but which had become unresectable at restaging evaluation, avoided unnecessary laparotomy.[7,16–22] In this article, 16% of PR and 31% of borderline resectable patients in the NACRT group were excluded from the subsequent surgical resection.

In general, the favorable prognostic factors for survival and recurrence in patients with pancreatic cancer have been reported as curative resection and negative lymph node metastasis.[4,5] The quality of surgery and examination of the pathological specimens can vary. Raut et al[23] proposed the term *SMA margin*, which indicates perivascular soft tissue, primarily perineural and mesenteric tissue, adjacent to (and posterior to) the right lateral border of the proximal SMA. In pancreatic cancer, the retroperitoneal margin is very close and often positive. It seems reasonable to conclude that locoregional therapy in pancreatic cancer can be optimized with complete gross tumor resection and treatment of microscopic disease at the SMA margin with chemoradiation. Factors that define resectability include the surgeon's opinion on the necessity of venous or arterial resection and whether high-risk margins for tumor resection are acceptable. In this study, our surgical indication included not only "potentially resectable" but also "borderline resectable," defined by NCCN,[9] and subsequently, we allowed R2 resection in this study. We performed aggressive pancreatectomy with portal vein or CA resection in some cases in which resection had been predicted to generate surgical-free margins. During this study, surgical indication was fixed, and 2 experienced surgeons performed all resections. Two pathologists closely examined pathological specimens of the dependently removed surgical stump of perineural and retroperitoneal fat tissues between the pancreatic parenchyma and the SMA or CA under surgical exposition of the right-sided adventitia of the proximal SMA and CA. Some authors reported that the frequency of pathologically curative resection (R0) after NACRT was 60% to 90%,[7,16,17,19–23] which was similar to our results of 52% (69% in PR and 27% in borderline resectable cases). It has been reported that the frequency of negative lymph node metastasis after NACRT and surgical resection was 40% to 80%, lower than after surgery alone.[7,16,17,19,21,22] In this experience, 59% negative lymph node metastasis in the NACRT group was significantly higher than 32% in the surgery-alone group. Better patient selection and the direct effect of chemoradiation in the NACRT group are able to facilitate resectability with free margins and a low frequency of lymph node metastasis. On the other hand, 20 (29%) of 68 resected patients had R2 residual tumor staging. There were no differences in survival analysis between R2 surgery in the NACRT and surgery-alone groups. Although it is difficult to interpret the results in a small population, surgical results in R2 cases after NACRT were disappointing.

Previous studies have shown that surgery alone yielded local recurrence rates of 50% to 80%, whereas preoperative chemoradiation reduced local failure rates to 5% to 13%.[7,17,20–22,24] The low local recurrence rate (11%) in the NACRT group at a minimum follow-up of 25 months was encouraging and was similar to previous reports.[7,17,21,22] Interestingly, there was a similar DFS rate within 1 year in the NACRT and surgery-alone groups with the absence of adjuvant chemotherapy, but a significant difference of the DFS curve over 1 year was observed among those who underwent curative resection. When all observed patients were followed up for 2 years, 44% of patients in the NACRT group were disease-free, significantly better than 7% in the surgery-alone group. All surviving patients in the NACRT group have been disease-free with a range of follow-up of 36 and 65 months, and the median survival time in the NACRT group was not reached. Over time, the difference in the DFS curve was clearly extended. It is important to note that all patients did not undergo adjuvant

chemotherapy, but patients with recurrent disease underwent weekly GEM administration on recurrence.

The median survival time in 5-FU–based neoadjuvant trials ranged from 15.7 to 45 months, which compares favorably with the survival rate of patients in the observation arms of previous randomized adjuvant trials (range, 11–19 months),[25,26] and is similar to that of the treatment arms of randomized adjuvant trials (range, 20–44 months).[25,27,28] The M.D. Anderson Cancer Center group[29] reported favorable results that the median survival time in GEM-based chemoradiation was 33 months, and most patients were noted to have greater than 50% nonviable tumor cells in the specimen, and 2 pathological complete responses were noted. Moreover, a phase II trial of neoadjuvant GEM (400 mg/m^2) with concurrent radiation of 30 Gy showed that 61 (73%) of 71 patients underwent surgical resection, and the median survival time was 36 months at 2 years' follow-up.[30] A phase II multi-institutional trial of NACRT using full-dose GEM conducted at the University of Michigan[16] demonstrated that 17 of 20 patients underwent surgical resection with 94% R0 grading, and the median survival time and 2-year OS rate were 26 months and 61%, respectively, after a median follow-up of 18 months. Thus, some studies of NACRT have demonstrated favorable outcomes compared with similar series of patients treated with surgery alone; however, the efficacy results must be interpreted with caution because the reports of NACRT for pancreatic cancer are heterogeneous with regard to patient population, treatment methods, modalities, and limited accrual. The University of Liverpool group[27,31] criticized that some studies using NACRT resulted in a median survival time of 9 to 39 months, and the largest comparative study found that neither the survival nor the pattern of disease recurrence was significantly different between neoadjuvant and adjuvant therapy. There have been no randomized controlled trials of neoadjuvant therapy despite the positive outcomes of single-institutional series of neoadjuvant therapy. New trials are being developed to address the neoadjuvant therapy question.[32,33] Brunner et al[32] initiated a multicenter prospectively randomized phase II study that aimed to answer the question of whether NACRT with GEM and CDDP can prolong the OS of patients with ductal adenocarcinoma of the pancreatic head in comparison with primary resected patients.

Chemoradiation therapy followed by curative resection seems to improve survival in patients with pancreatic cancer that extended beyond the pancreas in this study; however, several questions remain controversial: Should therapy be given preoperatively or postoperatively? Which chemoagent with external radiation has the high efficacy to induce tumor cell necrosis? How long is needed? How much radiation and chemotherapy should be used? More effective and less toxic regimens are necessary for neoadjuvant therapy to realize the ultimate goal of maximizing the number of patients who receive curative resection with less frequent metastatic lymph nodes, resulting in 50% or more 5-year survival rates.

REFERENCES

1. White RR, Shah AS, Tyler DS. Pancreatic cancer since Halsted how far have we come and where are we going? *Ann Surg*. 2003;238:132–144.
2. Willett CG, Lewandrowski K, Warshaw AL, et al. Resection margins in carcinoma of the head of the pancreas. Implications for radiation therapy. *Ann Surg*. 1993;217:144–148.
3. Chandler NM, Canete JJ, Stuart KE, et al. Preoperative chemoradiation in resectable pancreatic cancer. *J Hepatobiliary Pancreat Surg*. 2003;10:61–66.
4. Traverso LW. Pancreatic cancer: surgery alone is not sufficient. *Surg Endosc*. 2006;20:446–449.
5. Pingpank JF, Hoffman JP, Ross EA, et al. Effect of preoperative chemoradiotherapy on surgical margin status of resected adenocarcinoma of the head of the pancreas. *J Gastrointest Surg*. 2001;5:121–130.
6. Breslin TM, Hess KR, Harbison DB, et al. Neoadjuvant chemoradiotherapy for adenocarcinoma of the pancreas: treatment variables and survival duration. *Ann Surg Oncol*. 2001;8:123–132.
7. Takai S, Satoi S, Yanagimoto H, et al. Neoadjuvant chemoradiation in patients with potentially resectable pancreatic cancer. *Pancreas*. 2008;36:e26–e32.
8. National Comprehensive Cancer Network (NCCN) *Clinical Practice Guidelines in Oncology* 1:2005, Vol. 3, No. 5, 598.
9. Satoi S, Yamamoto H, Takai S, et al. Clinical impact of multidetector row computed tomography on patients with pancreatic cancer. *Pancreas*. 2007;34:175–179.
10. Satoi S, Yagagimoto H, Toyokawa H, et al. Preoperative patient selection of pancreatic cancer patients by multi-detector row CT and surgical results. *Hepatogastroenterology*. 2008. In press.
11. Wolff RA, Evans DB, Gravel DM, et al. Phase I trial of gemcitabine combined with radiation for the treatment of locally advanced pancreatic adenocarcinoma. *Clin Cancer Res*. 2001;7:2246–2253.
12. Takai S, Satoi S, Toyokawa H, et al. Clinicopathologic evaluation after resection for ductal adenocarcinoma of the pancreas: a retrospective, single-institution experience. *Pancreas*. 2003;26:243–249.
13. Crane CH, Varadhachary G, Pisters PW, et al. Future chemoradiation strategies in pancreatic cancer. *Semin Oncol*. 2007;34:335–346.
14. Cheng TY, Sheth K, White RR, et al. Effect of neoadjuvant chemoradiation on operative mortality and morbidity for pancreaticoduodenectomy. *Ann Surg Oncol*. 2006;13:7–9.
15. Tse RV, Dawson LA, Wei A, et al. Neoadjuvant treatment for pancreatic cancer—a review. *Crit Rev Oncol Hematol*. 2008;65:263–274.
16. Talamonti MS, Small W Jr, Mulcahy MF, et al. A multi-institutional phase II trial of preoperative full-dose gemcitabine and concurrent radiation for patients with potentially resectable pancreatic carcinoma. *Ann Surg Oncol*. 2006;13:150–158.
17. Mornex F, Girard N, Scoazec JY, et al. Feasibility of preoperative combined radiation therapy and chemotherapy with 5-fluorouracil and cisplatin in potentially resectable pancreatic adenocarcinoma: the French SFRO-FFCD 97-04 Phase II trial. *Int J Radiat Oncol Biol Phys*. 2006;65:1471–1478.
18. Magnin V, Moutardier V, Giovannini MH, et al. Neoadjuvant preoperative chemoradiation in patients with pancreatic cancer. *Int J Radiat Oncol Biol Phys*. 2003;55:1300–1304.
19. White RR, Hurwitz HI, Morse MA, et al. Neoadjuvant chemoradiation for localized adenocarcinoma of the pancreas. *Ann Surg Oncol*. 2001;8:758–765.
20. Hoffman JP, Lipsitz S, Pisansky T, et al. Phase II trial of preoperative radiation therapy and chemotherapy for patients with localized, resectable adenocarcinoma of the pancreas: an Eastern Cooperative Oncology Group Study. *J Clin Oncol*. 1998;16:317–323.
21. Moutardier V, Magnin V, Turrini O, et al. Assessment of pathologic response after preoperative chemoradiotherapy and surgery in pancreatic adenocarcinoma. *Int J Radiat Oncol Biol Phys*. 2004;60:437–443.
22. Pisters PW, Wolff RA, Janjan NA, et al. Preoperative paclitaxel and concurrent rapid-fractionation radiation for resectable pancreatic adenocarcinoma: toxicities, histologic response rates, and event-free outcome. *J Clin Oncol*. 2002;20:2537–2544.
23. Raut CP, Tseng JF, Sun C, et al. Impact of resection status on pattern of failure and survival after pancreaticoduodenectomy for pancreatic adenocarcinoma. *Ann Surg*. 2007;246:52–60.
24. Pisters PW, Abbruzzese JL, Janjan NA, et al. Rapid-fraction

preoperative chemoradiation pancreatoduodenectomy, and intraoperative radiation therapy for resectable pancreatic adenocarcinoma. *J Clin Oncol.* 1998;16:3843–3850.

25. Kalser MH, Ellenberg SS. Pancreatic cancer. Adjuvant combined radiation and chemotherapy following curative resection. *Arch Surg.* 1985;120:899–903.
26. Klinkenbijl JH, Jeekel J, Sahmoud T, et al. Adjuvant radiotherapy and 5-fluorouracil after curative resection of cancer of the pancreas and periampullary region: phase III trial of the EORTC Gastrointestinal Tract Cancer Cooperative Group. *Ann Surg.* 1999;230:776–782.
27. Neoptolemos JP, Stocken DD, Friess H, et al. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. *N Engl J Med.* 2004;350:1200–1210.
28. Picozzi VJ, Traverso LW. The Virginia Mason approach to localized pancreatic cancer. *Surg Oncol Clin N Am.* 2004;13:663–674.
29. Evans DB. Preoperative chemoradiation for pancreatic cancer. *Semin Oncol.* 2005;32:25–29.
30. Wolff RA, Evans DB, Crane CH, et al. Initial results of a preoperative gemcitabine-based chemoradiation for resectable pancreatic adenocarcinoma. *Proc Am Soc Clin Oncol.* 2002;21:130a [abstract 516].
31. Ghaneh P, Smith R, Tudor-Smith C, et al. Neoadjuvant and adjuvant strategies for pancreatic cancer. *Eur J Surg Oncol.* 2007; 11;1–9.
32. Brunner TB, Grabenbauer GG, Meyer T, et al. Primary resection versus neoadjuvant chemoradiation followed by resection for locally resectable or potentially resectable pancreatic carcinoma without distant metastasis. A multi-centre prospectively randomised phase II-study of the Interdisciplinary Working Group Gastrointestinal Tumours (AIO, ARO, and CAO). *BMC Cancer.* 2007;7:41.
33. Crane CH, Varadhachary G, Wolff RA, et al. The argument for pre-operative chemoradiation for localized, radiographically resectable pancreatic cancer. *Best Pract Res Clin Gastroenterol.* 2006;20: 365–382.

Is a Nonstented Duct-to-Mucosa Anastomosis Using the Modified Kakita Method a Safe Procedure?

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Objectives: After standardization of the perioperative management of pancreaticoduodenectomy, we retrospectively compared results in nonstented pancreaticojejunostomy with external-stented pancreaticojejunostomy.

Methods: The study population included 129 consecutive patients who underwent pancreaticoduodenectomy between 2004 and 2008. The postoperative mortality and morbidity were compared between 51 patients with restrictive use of external stenting (group A) and 78 patients without external stenting (group B). The patient with a pancreatic duct of less than 3 mm in diameter was 31% in group A and 46% in group B.

Results: There were no differences in postoperative morbidity and mortality between the 2 groups. Although the frequency of overall postoperative pancreatic fistula development was significantly higher in group B than in group A (44% vs 27%, $P = 0.0004$), there was no difference in grade B/C postoperative pancreatic fistula rate (group A: 5.9% vs group B: 14.1%). The length of in-hospital stay in group B was significantly shorter than group A (13 vs 24 days, $P < 0.0001$). There were no differences in postoperative morbidity and mortality between subgroups that were consisted of patients with small pancreatic duct diameter.

Conclusion: This retrospective single-center study showed that nonstented duct-to-mucosa anastomosis was a safe procedure and was associated with a shortened in-hospital stay.

Key Words: grade B/C, pancreatic duct diameter less than 3 mm, early drain removal, in-hospital stay, morbidity, mortality

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In recent years, pancreaticoduodenectomy (PD), including pylorus-preserving PD (PpPD), has been increasingly performed to treat a variety of diseases of the pancreas and periampullary region. Advances in surgical techniques and appropriate perioperative management have improved the short-term outcome of PD. In most high-volume centers, the mortality rate has decreased to less than 5%, although postoperative morbidity rates remain at approximately 40%.[1–8] To reduce the frequency of postoperative pancreatic fistula (POPF) development, we introduced the following departmental guidelines[9]: (i) modified Kakita method[10] for performing a pancreaticojejunostomy (PJ), (ii) omental wrapping around the PJ, (iii) early removal of closed-suction drain, and (iv) restrictive use of pancreatic and biliary duct stenting. Following those approaches, postoperative morbidity (39%), frequency of grade B/C pancreatic fistula (6%), and delayed gastric emptying (6%) have all been significantly reduced. According to this policy, external stents were inserted across the anastomosis to drain the pancreatic duct in the limited number of patients who had a pancreatic duct diameter of less than 3 mm and/or with bile duct diameter of less than 10 mm. However, there have been reports of some severe complications associated with the stenting tube.[11–13] These have included acute pancreatitis due to subsequent occlusion or bending of the stenting tube, late anastomotic stenosis after iatrogenic injury sustained when withdrawing the external stenting tube, or hepatic abscess formation caused by internal stent migration.[11–13] After standardization of the anastomotic method and perioperative management of PD,[9] since September 2006, we have performed nonstented PJ even in patients with a pancreatic duct diameter of less than 3 mm and/or with a bile duct diameter of less than 10 mm. The data on postoperative complications were collected from the prospective database in Kansai Medical University. We herein retrospectively compared the results from nonstented PJ with external-stented PJ after pancreaticoduodenectomy.

MATERIALS AND METHODS

From June 2004 to September 2008, 129 consecutive patients with pancreatic and periampullary disease underwent PD including PpPD by 2 pancreatic surgeons at Kansai Medical University Hospital. Cases were excluded from the study if they had undergone: duodenal-preserving pancreatic head resection (n = 1), medial segment–preserving pancreatectomy (n = 1), median pancreatectomy (n = 3), total pancreatectomy (n = 3), PD without PJ (post–median pancreatectomy, n = 1), partial pancreatectomy (n = 5), and emergent PD for patients with the lasting duodenal bleeding from gastrointestinal tumor (n = 1). The PJ was performed using the modified Kakita method as described in a previous paper.[9] Briefly, 8 absorbable interrupted stitches were placed in the pancreatic duct and jejunal mucosa in end-to-side fashion, and an approximation of the jejunal wall and the pancreatic stump was made using 3 or 4 nonabsorbable interrupted penetrating stitches. We routinely used the internal thoracic artery (ITA) holder to obtain good visualization of the anastomosis.[14] Duct-to-mucosa anastomosis can be difficult to perform when the pancreatic duct is not dilated and the lumen of duct is easily flattened in patients with normal pancreatic parenchyma, and the insertion of an ITA holder into the duct lumen in such cases enables excellent visualization without retaining the duct or pancreatic remnant. Before closing the abdomen, the omentum was wrapped around the pancreatic anastomosis. Routinely, 2 closed-suctioned drains were placed in the pancreatic anastomosis area. Our policy was to remove drains early between 3 and 6 days after the operation in patients without infection-induced systemic inflammatory response syndrome (SIRS)[15] when POPF defined by the International Study group of Pancreatic Fistula[16] was absent or grade A, or when fluid drained was less than 200 mL/d. When POPF was clinically diagnosed as grade B, the perianastomotic drain was

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replaced, and in some cases, closed lavage was performed with 500 to 3000 mL of natural saline solution, depending on the amylase level in the drained fluid. On developing POPF, all attempts were made to maintain per os and enteral feeding. All patients with obstructive jaundice first underwent endoscopic nasogastric/retrograde bile drainage or percutaneous transhepatic drainage, and it was performed in 61% of patients. All patients received prophylactic antibiotics intraoperatively and for 1 or 2 days postoperatively. The nasogastric tube was routinely removed on postoperative day (POD) 1 after confirmation that the volume of drainage fluid was less than 200 mL/d. Postoperative enteral nutrition through a jejunostomy tube was initiated at POD 3 in all patients. Oral food intake was initiated at POD 5 or 6. Once the patient was getting 50% of required nutrition from an oral diet, enteral nutrition was stopped, and the jejunostomy tube was finally withdrawn at POD 21 at an outpatient clinic. Peripancreatic drainage fluid was collected, and the amylase level was measured and monitored on PODs 3 and 6 and every 3 days thereafter as needed. The date of discharge from hospital was decided by the senior surgeons and was taken once the patient could take more than 50% of solid food served and was afebrile with falling C-reactive protein levels less than 5 mg/dL. No patients had prophylactic octreotide to prevent POPF development and thromboembolic prophylaxis with low-molecular-weight heparin. All complications were prospectively recorded into a prospective pancreatic database.

Group A

Group A consisted of 51 consecutive patients who underwent PD from June 2004 to August 2006; in these patients, the semiclosed external stenting of pancreatic and biliary ducts (polyethylene tube; Sumitomo Bakelite Co, Tokyo, Japan) was used in cases with a pancreatic duct of less than 3 mm in diameter (16 patients, 31%) and with a bile duct of less than 10 mm in diameter (13 patients, 25%). This group has the same population as presented in the previous article.[9]

Group B

Group B consisted of consecutive 78 patients who underwent PD from September 2006 to September 2008. All patients underwent PJ without external stenting of the pancreatic and biliary duct. In this group, 36 patients (46%) had a pancreatic duct of less than 3 mm in diameter.

Definition of Postoperative Complications

The overall general and surgery-related complications were recorded in this study. These included delayed gastric emptying, POPF, wound dehiscence, intra-abdominal infection, abdominal abscess formation, cardiopulmonary disorder, hemorrhagic complication, peritoneal/pleural effusion, anastomotic leakage, marginal ulcer formation, and diarrhea and other complications. Each day that a patient demonstrated clinical symptoms of SIRS[15] was recorded. Sepsis was considered to be present if a patient had any complication involving clinical symptoms of infection-induced SIRS that continued for more than 2 inpatient days. Clinical symptoms of SIRS within the first 4 PODs were excluded as a systemic response to surgical stress.

The POPF was defined according to the International Study group of Pancreatic Fistula criteria.[16] Abdominal abscess, including liver abscess, was defined as a collection of pus or infected fluid confirmed by ultrasound, computed tomography–guided aspiration and culture, or a second laparotomy. Delayed gastric emptying was defined according to the International Study Group of Pancreatic Surgery.[17] Wound dehiscence was diagnosed as an open wound with or without the clinical presence of pus or microbiological findings of bacteria. Intra-abdominal infection was regarded as radiological findings of fluid collection (pleural effusion and/or ascites) or microbiological findings of bacteria with infection-induced SIRS. Peritoneal/pleural effusion was defined as more than 200 mL/d of drained fluid beyond the 14th POD. Anastomotic leakage was radiologically diagnosed by leakage of contrast agents from the anastomosis. Upper gastrointestinal endoscopy for marginal ulcer formation was performed when patients showed signs of appetite loss, epigastralgia, or bloody discharge from the nasogastric tube or in the stools. Hemorrhagic complication was defined as intra-abdominal or intestinal bleeding requiring blood transfusion, operation, and/or radiological intervention. Aspartate aminotransferase or alanine aminotransferase levels of more than twice the upper limit of normal reference indicated postoperative liver dysfunction, including cholangitis. Death of a patient for any reason was regarded as in-hospital death.

Statistical Analysis

Mortality and morbidity after PD were calculated for each group and were compared between groups A and B. Patient variables included age, sex, clinical diagnosis, comorbid disease, obstructive jaundice, operative variables, preoperative blood tests, and preoperative chemoradiation. Operative variables included the type and duration of operation, estimated blood loss, type of blood transfusion, and extent of operation, including resection of adjacent organs and portal vein. All data were entered into an electronic database on a personal computer, and continuous variables are expressed as median values and ranges. Statistical analyses including Mann-Whitney U test and Fisher exact test were performed using StatView Version 5.0 for Windows (SAS, Inc, Cary, NC). The profound factors identified by the univariate analysis were further examined by multivariate analysis using logistic regression analysis to determine independent significant factors for grade B/C POPF after PD. $P < 0.05$ was considered significant.

RESULTS

Patient Demographic and Baseline Characteristics Including Surgical Details

Baseline and operative characteristics of the 129 patients enrolled in the study are shown in Table 1. Most variables did not differ significantly between the 2 groups. However, compared with group B (n = 78), in group A (n = 51), the operation time was significantly longer ($P = 0.0086$), and external pancreatic stenting and bile duct stenting were more frequently performed ($P < 0.0001$).

Postoperative Complications

The postoperative course was complicated in 47% of the 129 patients. As shown in Table 2, there were no differences in the frequency of overall complications, septic complications, reoperation, and in-hospital death between the 2 groups. Among the listed complications, although the frequency of overall POPF was significantly higher in group B compared with group A patients (group A: 27% vs group B: 44%; $P = 0.0004$), there was no difference in the incidence of grade B/C of POPF (group A; 5.9% vs group B; 14.1%). Most POPF was classified as grade A (group A: 57%, group B: 68%) that was not clinically relevant.

TABLE 1. Patient Demographics and Baseline Characteristics

Parameters	Group A (n = 51)	Group B (n = 78)	P
Pancreatic duct diameter (≥3 mm:<3 mm)	35 (69):16 (31)	42 (54):36 (46)	NS
Pancreatic duct drainage (+:−)	16:35	0:78	<0.0001
Bile duct drainage (+:−)	13:38	0:78	<0.0001
Age, y	68 (51–84)	66 (36–90)	NS
Male-female ratio	33:18	51:27	NS
Disease (P:B:A)	29:9:13	46:13:19	NS
Benign-malignant ratio	4:47	13:65	NS
Total bilirubin, mg/dL	0.7 (0.3–4.7)	0.7 (0.2–3.6)	NS
AST, U/L	24 (12–77)	25 (13–236)	NS
Amylase, U/L	70 (11–473)	78 (17–300)	NS
Albumin, g/dL	3.7 (2.3–4.5)	3.9 (1.9–4.8)	NS
WBC, $\times 10^2$/mL	50 (31–98)	52 (24–124)	NS
Hb, g/dL	11.6 (8.3–14.1)	11.5 (7.7–15.8)	NS
Comorbid disease (−:+)	19:32	24:54	NS
DM (−:+)	32:19	53:25	NS
Jaundice (−:+)	18:33	32:46	NS
CRT (+:−)	7:44	5:73	NS
Type of operation (PD:PpPD)	33:18	42:36	NS
Operation time, min	523 (355–795)	468 (275–714)	0.0086
Blood loss, mL	1140 (212–6420)	952 (272–5238)	NS
Transfusion (allo:auto:none)	16:30:5	22:49:7	NS
Resection of other organs (+:−)	7:44	19:59	NS
Day of drain removal, POD	7 (4–30)	4 (3–50)	<0.0001
In-hospital stay, POD	24 (11–73)	13 (8–101)	<0.0001

Table shows median value (range) or number of patients (%).

NS indicates not statistically significant; P:B:A, pancreatic disease–biliary disease–ampullary disease; AST, aspartate aminotransferase; WBC, white blood cell count; Hb, hemoglobin; DM, diabetes mellitus; CRT, preoperative chemoradiation therapy; allo, allogenic blood transfusion; auto, autologous blood transfusion; none, no transfusion.

The leakage of PJ, as shown radiologically, was found in only 1 patient in group B. As shown in Table 2, there were no differences in the incidences of other complications. One patient (2%) in group A died at POD 31 after laparotomy because of the development of intestinal necrosis due to sudden onset of superior mesenteric arterial thrombosis. Two patients in group B required relaparotomy because of leakage of colonic anastomosis (n = 1) and intra-abdominal abscess after intractable POPF (n = 1). In group B patients, there were 3 in-hospital death caused by pneumonia (n = 2) and liver failure after leakage of colonic anastomosis. No bleeding complications or POPF-related mortality were reported in this study. The median length of postoperative in-hospital stay was significantly shorter in group B compared with group A: 13 days (range, 11–73 days) vs 24 days (range, 8–101 days), respectively ($P < 0.0001$). In more details, the length of in-hospital stay was (group B vs group A) 64% vs14% (in-hospital of <14 days), 19% vs 55% (15–29 days), and 17% vs 31% (>29 days).

Identification of Risk Factors for Grade B/C POPF

Multivariate logistic regression analyses were used to identify the risk factors associated with grade B/C POPF (Table 3). These risk factors were extracted from the results of univariate analysis for grade B/C POPF. A pancreatic duct with a diameter of less than 3 mm was the only independent risk factor for grade B/C POPF in this study.

TABLE 2. Comparison of Postoperative Complications

Parameters	Group A (n = 51)	Group B (n = 78)	P
Overall complications	20/51 (39%)	40/78 (51%)	NS
Septic complications	10/51 (20%)	9/78 (12%)	NS
Reoperation	1/51 (2.0%)	2/78 (2.5%)	NS
In-hospital death	1/51 (2.0%)	3/78 (3.8%)	NS
Pancreatic fistula	7/51 (14%)	34/78 (44%)	0.0004
Grade B/C	3/51 (5.9%)	11/78 (14%)	NS
DGE	3/51 (6%)	6/78 (8%)	NS
Grade A/B/C	0/1/2	3/1/2	NS
Drain infection	3/51 (5.8%)	6/78 (8%)	NS
Abdominal abscess	2/51 (3.9%)	6/78 (8%)	NS
Hemorrhage	0/51 (0%)	0/78 (0%)	NS
Wound dehiscence	10/51 (20%)	12/78 (15%)	NS
Pneumonia	1/51 (2.0%)	3/78 (3.8%)	NS
Bile leakage	1/51 (2.0%)	0/78 (0.0%)	NS
Marginal ulcer	1/51 (2.0%)	0/78 (0.0%)	NS
Peritoneal/pleural effusion	6/51 (12%)	2/78 (3%)	NS
Liver dysfunction	4/51 (7.8%)	6/78 (7.7%)	NS

Figure represents number of patients (%).

NS indicates not statistically significant; DGE, delayed gastric emptying; fluid collection, pleural effusion and/or ascites.

TABLE 3. Logistic Regression Analysis Using Perioperative Parameters for POPF (Grade B/C)

Category	Risk Factors	P	Relative Risk	95% CI
POPF (grade B/C)	P-duct >3 mm	—	1	
	P-duct <3 mm	0.0098	6.391	1.564–26.121

This table shows the most relevant factors in each category by multivariate analysis. The risk factor used for multivariate analysis was abstracted from the results of univariate analyses.

CI indicates confidence interval; P-duct, diameter of pancreatic duct.

Comparison of Postoperative Complications Between the 2 Subgroups in Patients With a Pancreatic Duct With a Diameter of Less Than 3 mm

In groups A and B, 31% (16/51) and 46% (36/78) of patients, respectively, had a pancreatic duct of less than 3 mm in diameter. Regarding the patients' background, there was no difference between the 2 groups (data were not published). Although there was a tendency for a higher frequency of overall POPF in group B versus group A, this did not reach statistical significance. As shown in Table 4, there were no differences between the 2 subgroups in the incidences of grade B/C POPF, delayed gastric emptying, intra-abdominal abscess formation, and other postoperative complications. In comparison of grade B/C POPF rate of 2 groups in patients with a pancreatic duct with a diameter of more than 3 mm, no significant difference was found (data not shown).

DISCUSSION

After the introduction of PD by Kausch[18] and Whipple et al,[19] the mortality rate after PD was approximately 20% in the 1970s. However, in more recent decades, morbidity and mortality rates have decreased because of improvements in perioperative management and preoperative patient selection. The development of POPF often results in severe complications, such as sepsis, intra-abdominal abscess and bleeding, and delayed gastric emptying. The safe reconstruction of pancreaticoenteric continuity after PD continues to be a challenge for the pancreatic surgeon. Although the mortality rates in high-volume centers have fallen in the past 10 years to less than 5%, morbidity still remains at 30% to 50% after PD.[1–7] To date, many efforts have been made to reduce the occurrence of POPF and mortality after PD, including the use of octreotide,[20] methods of pancreaticoenterostomy,[21–23] pancreatic duct stenting,[24,25] drain management,[26] and use of surgical microscopy.[27]

Previously, we reported rates of 5.5% for in-hospital mortality, 56% for postoperative complications, and 31% for septic complications in 198 patients who underwent PD.[8] Consequently, departmental guidelines to reduce postoperative morbidity after PD were developed and could lead to the standardization of perioperative management of PD and to a lower incidence of grade B/C POPF, delayed gastric emptying, and overall complications.[9] In that study, external stenting of the pancreatic duct was used in the limited number of patients with a pancreatic duct of less than 3-mm diameter and with a biliary duct of less than 10-mm diameter. Theoretically, external or internal stenting may help divert the pancreatic secretion away from the anastomosis.[24,25] However, severe complications associated with the stenting tube have been reported[11,12] and include acute pancreatitis due to subsequent occlusion or bending of the stenting tube or late anastomotic stenosis after iatrogenic injury sustained when withdrawing the external stenting tube.[11,12] Recently, Rezvani et al[13] reported that internal stent migration caused liver abscess formation, which was treated by percutaneous transhepatic interventional radiological approach. We have also experienced a very rare case in the early 1990s, when a patient died due to intractable pancreatojejunal anastomotic leakage after withdrawal of the external stenting tube. Other concerns were the occurrence of a catheter-related infection. Based on these experiences, we changed our procedures from duct-to-mucosa anastomosis using external stenting, to nonstented anastomosis.

In this study, there were no differences in grade B/C POPF and other major complications between the 2 groups. The overall incidence of POPF was significantly higher in group B than in group A, and there was a trend for a higher frequency of overall POPF in patients with small-diameter pancreatic ducts in group B, relative to group A. However, nonstented PJ did not increase the frequency of grade B/C POPF cases that were considered to be clinically important, even in patients with small-diameter pancreatic ducts. In this study, the methods used for PJ and perioperative management have been standardized. The population analyzed was relatively homogeneous as to clinical background, underlying diseases, clinical diagnosis, and type of operation; however, there was a tendency for more patients in group B to have small-diameter pancreatic ducts than in group A (46% vs 31%, respectively). Although the significantly higher rate of overall POPF in group B might be associated with relatively higher frequency of patients with small-diameter pancreatic ducts in this group, nonstented PJ might also, in part, have led to the increased rate of grade A POPF. Logistic regression analysis showed that the only risk factor for grade B/C POPF was small-diameter pancreatic ducts, as shown in Table 3. Subgroup analysis of patients with small-diameter pancreatic ducts showed that there was no difference between the 2 subgroups in the overall incidence of POPF, grade B/C POPF, or other complications.

External stenting across PJ anastomosis is widely used by surgeons[9,24,25,28] and may have the potential to drain pancreatic enzymes away from the PJ anastomosis. However, it was unclear whether nonstented PJ was a safe procedure. Roder et al[28] reported in a prospective study that the rate of pancreatic fistula in the external stenting group was decreased, relative to the nonstenting group. Nevertheless, as a proportion of the patients

TABLE 4. Comparison of Postoperative Complications in Subgroup of Patients in Groups A and B With a Pancreatic Duct With a Diameter of Less Than 3 mm

Parameters	Group A (n = 16)	Group B (n = 36)	P
Overall complications	8/16 (50%)	23/36 (64%)	NS
In-hospital death	0/16 (0%)	1/36 (2.8%)	NS
Pancreatic fistula	5/16 (31%)	21/36 (58%)	NS
Grade B/C	3/16 (19%)	8/36 (22%)	NS
DGE	1/16 (6.2%)	3/36 (8.3%)	NS
Grade A/B/C	0/1/0	2/0/1	NS

Figure represents number of patients (%).

NS indicates not statistically significant; DGE, delayed gastric emptying.

underwent PJ using the invagination technique, the findings were based on a heterogeneous population regarding the anastomotic technique used. In contrast, Imaizumi et al[12] retrospectively proposed that a stenting tube was unnecessary even in the normal pancreas if the duct-to-mucosa anastomosis performed was satisfactory. Recently, Poon et al[24] reported results from a prospective randomized trial in 120 patients over a 6-year period, which showed that external drainage of the pancreatic duct with a external stent reduced the leakage rate of PJ after PD. The differences between this study relative to ours were the longer study period, the longer period that the drains were left inserted, and the higher frequency of hemorrhagic complications, and in-hospital deaths due to POPF. In our study, the median POD that the drain was removed was 7 in group A and 4 in group B, which were earlier than POD 10 or more reported by Poon et al.[24] Moreover, there were no hemorrhagic complications or POPF-related mortality in this present study, which was also of a shorter duration than that reported by Poon et al[24] (4 years 4 months vs 6 years 4 months, respectively). The main limitation to our study was the small sample size and the retrospective nature of the analysis, which could have biased the outcome, although the study was conducted throughout by the same staff who all followed standard perioperative management procedures for the relative short study period. It is difficult to elucidate the reason for the difference in results, although the one thing they had in common was that they were single-center studies. To explore further the reasons for the different findings, it will be necessary to run a multicenter trial with the standardization of the anastomotic method and perioperative management across sites.

In this study, we would like to emphasize the shorter postoperative in-hospital stay in group B, relative to group A. In Japan, most patients do not leave the hospital until they have completely recovered because they do not have to pay for the total cost of hospital stay. In the patients who required removal of an external stenting tube, there was a tendency to later discharge from hospital. Therefore, it might be reasonable to assume that the abolishment of external stenting would be associated with shorter in-hospital stay.

Poon et al[24] proposed that stenting of the pancreatic duct allowed more precise placement of sutures, thus protecting the pancreatic duct from suture injury and reducing the risk of iatrogenic pancreatic duct occlusion, pancreatitis, and fistula formation. Indeed, duct-to-mucosa anastomosis with nonstenting can be difficult to perform when the pancreatic duct is not dilated and the lumen of duct is easily flattened in patients with normal pancreatic parenchyma. However, insertion of an ITA holder into the duct lumen enables excellent visualization without retaining the duct or pancreatic remnant.[14] In some cases, a stay-suture may be placed to open the lumen of the pancreatic duct; however, when the suture is pulled, there is a risk in that the suture material can cut easily into the pancreatic duct or parenchyma. Using an ITA holder can minimize incidental laceration of the pancreatic duct or parenchyma by replacing the need for a stay-suture; it can also minimize trauma by protecting the pancreatic duct from crush injuries caused by forceps. Thus, we firmly believe that duct-to-mucosa anastomosis without pancreatic duct stenting, and using the ITA holder, is a safe procedure.

In summary, this retrospective analysis showed that there were no differences in postoperative mortality and morbidity including grade B/C POPF development, between patients who underwent stented and nonstented PJ after PD. The multivariate analysis showed that the only risk factor for grade B/C POPF was a pancreatic duct diameter of less than 3 mm. In conclusion, the nonstented PJ using the modified Kakita method can be safely performed and is associated with a reduced length of in-hospital stay.

REFERENCES

1. Neoptolemos JP, Russel RC, Bramhall S, et al. Low mortality following resection for pancreatic and peri-ampullary tumours in 1026 patients: UK survey of specialist pancreatic units. UK Pancreatic Cancer Group. *Br J Surg*. 1997;84:1370–1376.
2. Yeo CJ, Cameron JL, Sohn TA, et al. Six hundred fifty consecutive pancreaticoduodenectomies in the 1990s. *Ann Surg*. 1997;226:248–260.
3. Boettger TC, Junginger T. Factors influencing morbidity and mortality after pancreaticoduodenectomy: critical analysis of 221 resections. *World J Surg*. 1999;23:164–172.
4. Gouma DJ, van Geenen RCI, van Gulik TM, et al. Rates of complications and death after pancreaticoduodenectomy: risk factors ad the impact of hospital volume. *Ann Surg*. 2000;232:786–795.
5. Gouillat C, Gigot JF. Pancreatic surgical complications-the case for prophylaxis. *Gut*. 2001;49:32–39.
6. Adam U, Makowiec F, Riediger H, et al. Risk factors for complications after pancreatic head resection. *Am J Surg*. 2004;187:201–208.
7. Schmidt CM, Powell ES, Yiannoutsos CT, et al. Pancreaticoduodenectomy. *Arch Surg*. 2004;139:718–725.
8. Satoi S, Takai S, Matsui Y, et al. Less morbidity after pancreaticoduodenectomy of patients with pancreatic cancer. *Pancreas*. 2006;33:45–52.
9. Satoi S, Toyokawa H, Yanagimoto H, et al. A new guideline to reduce postoperative morbidity after pancreaticoduodenectomy. *Pancreas*. 2008;37:128–133.
10. Kakita A, Yoshida M, Takahashi T. History of pancreaticojejunostomy in pancreaticoduodenectomy: development of more reliable anastomosis technique. *J Hepatobiliary Pancreat Surg*. 2001;8:230–237.
11. Ohwada S, Tanahashi Y, Ogawa T, et al. In situ vs ex situ pancreatic duct stents of duct-to-mucosa pancreaticojejunostomy after pancreaticoduodenectomy with Billroth I–type reconstruction. *Arch Surg*. 2002;137:1289–1293.
12. Imaizumi T, Hatori T, Tobita K, et al. Pancreaticojejunostomy using duct-to-mucosa anastomosis without a stenting tube. *J Hepatobiliary Pancreat Surg*. 2006;13:194–201.
13. Rezvani M, O'Moore PV, Pezzi CM. Late pancreaticojejunostomy stent migration and hepatic abscess after Whipple procedure. *J Surg Educ*. 2007;64:220–223.
14. Satoi S, Toyokawa H, Yanagimoto H, et al. Using an internal thoracic artery holder in pancreaticojejunostomy. *Surgery*. 2006;140: 836–837.
15. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med*. 1992;20:864–874.
16. Bassi C, Dervenis C, Butturini G, et al. Postoperative pancreatic fistula: an international study group (ISGPF) definition. *Surgery*. 2005; 138:8–13.
17. Wente MN, Bassi C, Dervenis C, et al. Delayed gastric emptying (DGE) after pancreatic surgery: a suggested definition by the International Study Group of Pancreatic Surgery (ISGPS). *Surgery*. 2007;142: 761–768.
18. Kausch W. Das Carcinoma der Papilla duodeni and seine radikale Entfernung. *Beitr Z Klin Chir*. 1912;78:439–486.
19. Whipple AO, Parsons WB, Mullins CR. Treatment of carcinoma of the ampulla of Vater. *Ann Surg*. 1935;102:763–779.
20. Yeo CJ, Cameron JL, Lillemoe KD, et al. Does prophylactic octreotide decrease the rates of pancreatic fistula and other complications after pancreaticoduodenectomy? Results of a prospective randomized placebo-controlled trial. *Ann Surg*. 2000;232:419–429.
21. Wente MN, Shrikhande SV, Mueller MW, et al. Pancreaticojejunostomy versus pancreaticogastrostomy: systematic review and meta-analysis. *Am J Surg*. 2007;193:171–183.

22. Bassi C, Falconi M, Molinari E, et al. Duct-to-mucosa versus end-to-side pancreaticojejunostomy reconstruction after pancreaticoduodenectomy: results of a prospective randomized trial. *Surgery*. 2003;134:766–771.
23. Tani M, Terasawa H, Kawai M, et al. Improvement of delayed gastric emptying in pylorus-preserving pancreaticoduodenectomy: results of a prospective, randomized, controlled trial. *Ann Surg*. 2006;243: 316–320.
24. Poon RT, Fan ST, Lo CM, et al. External drainage of pancreatic duct with a stent to reduce leakage rate of pancreaticojejunostomy after pancreaticoduodenectomy: a prospective randomized trial. *Ann Surg*. 2007;246:425–433.
25. Winter JM, Cameron JL, Campbell KA, et al. Does pancreatic duct stenting decrease the rate of pancreatic fistula following pancreaticoduodenectomy? Results of a prospective randomized trial. *J Gastrointest Surg*. 2006;10:1280–1290.
26. Kawai M, Tani M, Terasawa H, et al. Early removal of prophylactic drains reduces the risk of intra-abdominal infections in patients with pancreatic head resection: prospective study for 104 consecutive patients. *Ann Surg*. 2006;244:1–7.
27. Wada K, Traverso LW. Pancreatic anastomotic leak after the Whipple procedure is reduced using the surgical microscope. *Surgery*. 2006; 139(6):735–742.
28. Roder JD, Stein HJ, Bottcher KA, et al. Stented versus nonstented pancreaticojejunostomy after pancreatoduodenectomy: a prospective study. *Ann Surg*. 1999;229:41–48.

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Reproducibility and usability of chronic virus infection model using agent-based simulation; comparing with a mathematical model

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ABSTRACT

We created agent-based models that visually simulate conditions of chronic viral infections using two software. The results from two models were consistent, when they have same parameters during the actual simulation. The simulation results comprise a transient phase and an equilibrium phase, and unlike the mathematical model, virus count transit smoothly to the equilibrium phase without overshooting which correlates with actual biology in vivo of certain viruses. We investigated the effects caused by varying all the parameters included in concept; increasing virus lifespan, uninfected cell lifespan, uninfected cell regeneration rate, virus production count from infected cells, and infection rate had positive effects to the virus count during the equilibrium period, whereas increasing the latent period, the lifespan-shortening ratio for infected cells, and the cell cycle speed had negative effects. Virus count at the start did not influence the equilibrium conditions, but it influenced the infection development rate. The space size had no intrinsic effect on the equilibrium period, but virus count maximized when the virus moving speed was twice the space size. These agent-based simulation models reproducibly provide a visual representation of the disease, and enable a simulation that encompasses parameters those are difficult to account for in a mathematical model.

1. Introduction

All viruses need hosts as a basis for their life. When a virus enters the host body, it invades cells and uses both its own enzymes and those of the host cells to replicate. Host cells infected by viruses launch a self-defense system known as the innate immune system (See and Wark, 2008; Naniche, 2009), which inhibits viral replication and uses the human leukocyte antigen system and cytokines to elicit an immune response. Immune cells that have received signals from host cells activate other immune cells, neutralize viruses in the serum by means of antibodies, and prevent the virus from replicating and proliferating by destroying or curing host cells. Viral infection is a disorder based on the interactions between viruses and cells.

The power relationship between these agents changes along with the progression of the disease. In the very early stages of infection, as the host defense mechanisms are immature, the virus has the ability to overwhelm the host cells, actively replicate, and proliferate. Subsequently, as the capacity of the immune system improves, the speed of viral proliferation drops and the virus count reaches a peak. Infected host cells begin to be disrupted by the immune system or virus particles, and symptoms appear as a result. If the immune system is stronger than the virus, then the viral counts decline, and, in transient viral disorders, the virus is finally eliminated and the host recovers. In chronic viral disorders, however, the power relationship between the virus and host cells reaches equilibrium, and a long-term power balance is maintained with the virus count reaching a plateau.

Mathematical models have been proposed to study the dynamics of such viral disorders, and are regarded as being of value in understanding this phenomenon (Ho et al., 1995; Nowak et al., 1996; Neumann et al., 1998). However, these models are difficult to understand for clinicians, and their applicability is somewhat limited in everyday practice. In clinical research, measurements of viral dynamics in patients for short duration have been made for human

Abbreviations: HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus.

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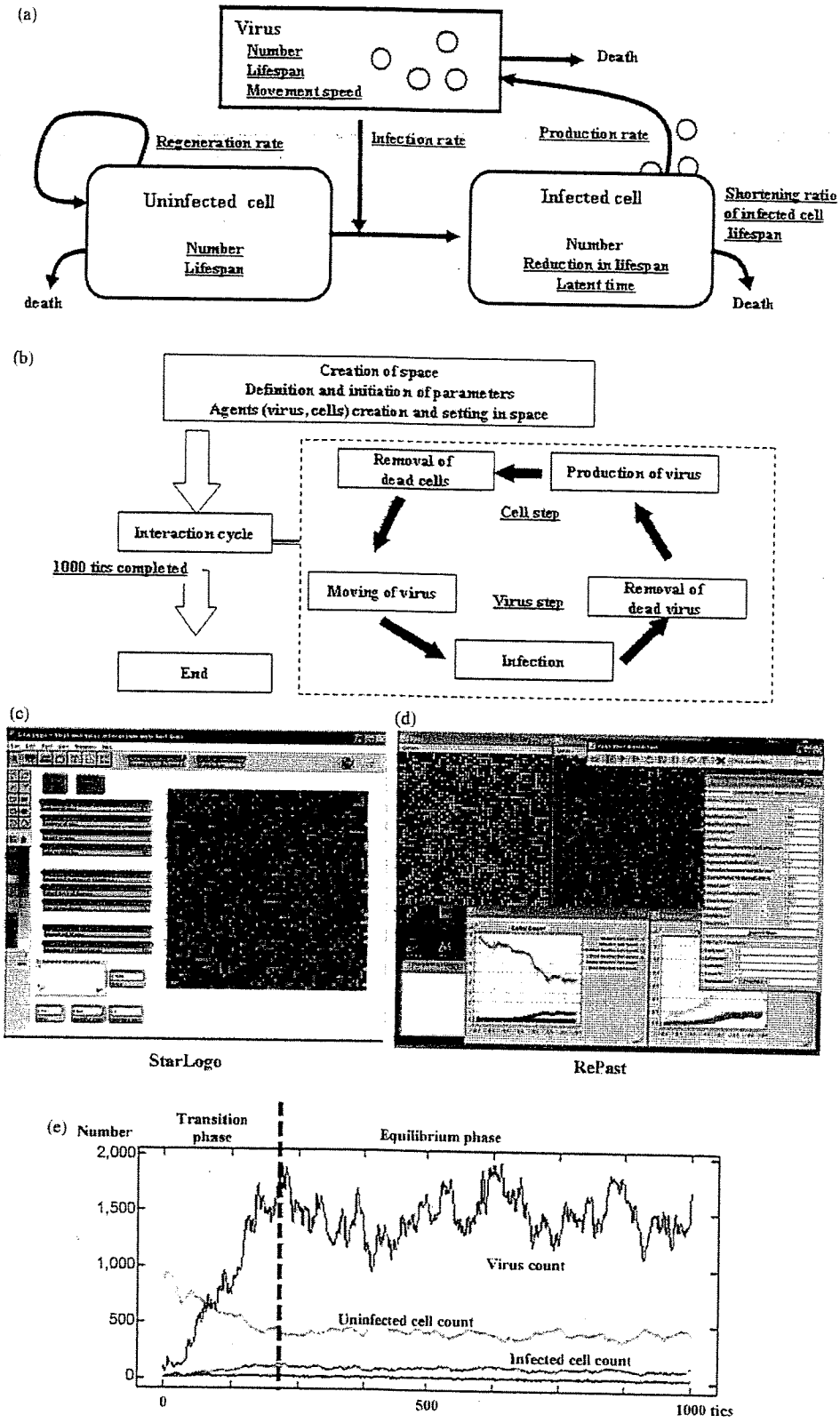

Fig. 1. Simulation design and an example of simulation results. (a) Model concept. Viruses, uninfected cells, and infected cells were treated as agents, and parameters were set for each of these and for interactions between agents (underlined). (b) Flowchart of the program. After preparing the simulation, we entered the interaction cycle, in which virus steps (such as movement) and cell steps were repeated. One cycle was counted as 1 tic, and the simulation concluded after 1000 tics. (c and d) Simulation screen using (c) StarLogo and (d) RePast. Yellow circles are viruses, green squares are uninfected cells, and orange and red indicate infected cells, with orange indicating the latent period. In StarLogo, all the agents are shown on the same screen, but in RePast, viruses and cells are shown in separate windows. (e) Example of a simulation chart in StarLogo. After the start of simulation the virus count and infected cell count increase while the uninfected cell count decreases, with equilibrium state reached after a certain number of tics.

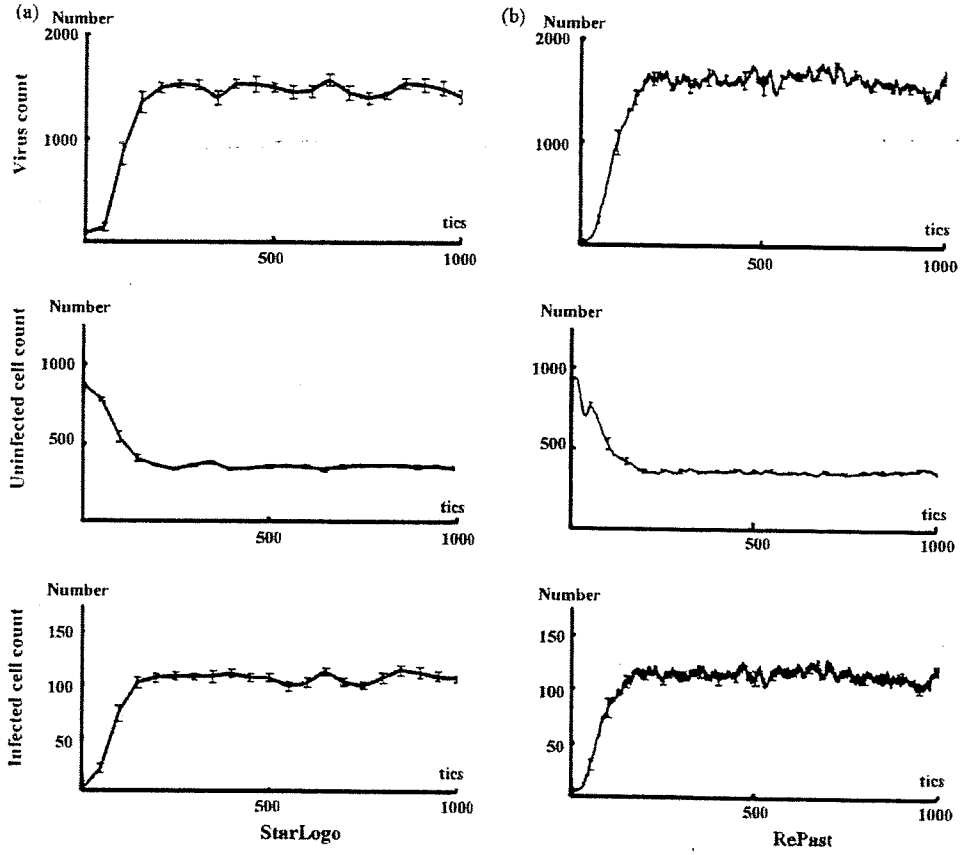

Fig. 2. Comparison of simulation results in (a) StarLogo and (b) RePast. The results were consistent when the parameters were made consistent. (Virus count [average ± SD]: StarLogo 1458.03 ± 173.1, RePast 1462.71 ± 178.8, $p = 0.94$. Uninfected cell count: 364.24 ± 30.4, 368.11 ± 33.4, $p = 0.83$. Infected cell count: 105.73 ± 13.0, 107.74 ± 13.0, $p = 0.24$. Unpaired Student's t-test.) Parameter values were set as follows: initial virus count, 100; uninfected cell count, 880; infected cell count, 0; virus speed of movement, 5 grids/tic; infection rate, 10%; uninfected cell regeneration rate, 1%; latent period, 3 tics; and virus reproduction rate, 5/cells/tic. The following parameter settings were taken from actual measurements: virus lifespan, 4.5 tics; uninfected cell lifespan, 49.8 tics; and infected cell lifespan, 6.7 tics.

immunodeficiency virus (HIV) (Ho et al., 1995), hepatitis B virus (HBV) (Nowak et al., 1996) and hepatitis C virus (HCV) (Neumann et al., 1998), and research is also underway on a range of models based on animal experiments and cell culture systems. As chronic viral disorders persist over long periods of time complete follow-up of viral dynamics is difficult. Furthermore, limitations of items that can be measured, such as the difficulty of measuring whole numbers of host cells, make it extremely difficult to investigate their consistency in mathematical models.

The recent ascend of dynamic-models owes much to advances in computers. Computer performance has improved markedly in recent years, not only in terms of their calculating capacity but also with regard to image displays, and models that offer a visual representation of viral disorders are now being reported (Gilbert and Bankes, 2002; Duca et al., 2007; Shapiro et al., 2008; Castiglione et al., 2007). One advantage of such visual models is that by providing a visual representation, they make understanding the disease status easy. Another benefit is that they enable parameters to be identified that are hidden as background noise in mathematical models. However, these models have some problems; it is difficult to prove the reproducibility of the simulation results derived from different languages or libraries, difficult to prove the validity of the model's concepts, and difficult to prove that the simulation results accurately reflect the reality. In this study, we created agent-based computer models that visually simulate the conditions of chronic viral infections using two software. The reproducibility of two agent-based computer models and the differences between agent-based models and the mathematical model were analyzed. This agent-based model enabled us to investigate how each parameter included in the concept affects the conditions of chronic viral infections.

2. Methods

2.1. Selection of Software

In this study, we used two different types of softwares: StarLogo version 2.0 (http://education.mit.edu/starlogo/) supplied by MIT Media Laboratory and Recursive Porous Agent Simulation Toolkit (RePast-3.0, http://repast.sourceforge.net/) supplied by the Argonne National Laboratory. StarLogo uses Logo, one of the simplest programming languages, and has a fixed graphical user interface. RePast is a library that uses Java, another programming language, which also has a fixed graphical user interface.

Logo is an assembly language, and StarLogo carries out processing sequentially. Java is an object-oriented language, and RePast has a faster processing speed than StarLogo. In addition, StarLogo has a number of stipulations to simplify simulations, such as parameters can only be set up to five decimal places and the simulation space is also fixed as 51 × 51 square grids. RePast, on the other hand, has fewer such restrictions. Thus, it offers a higher degree of freedom in program settings than StarLogo. Taking simulation space as an example, in spite of the restrictions imposed by the underlying operating system's image display system, any number of grids can be set and a hexagonal grid could also be chosen rather than a square one. However, users must stipulate and set all parameters themselves. This means that they must first declare the shape of the grid and the number of grids they will use to fill the simulation space. Java is also more difficult to learn than Logo, and debugging and correcting the program is also more difficult. Thus, it is difficult to judge whether or not the results agree with the planned simulation.

In effect, these two different types of softwares are polar opposites. It is simple to start a simulation in StarLogo, but producing results takes time and it is difficult to carry out more complex simulations. In RePast it is difficult to compose the program and judge whether or not the planned study has actually been achieved, but the

simulation itself takes only a short time to complete and there are lesser restrictions in the construction of a simulation model.

2.2. Concept for Modeling

We applied the basic virus–host interaction mathematical model to the agent-based simulation system with slight modifications. The mathematical model was used to describe the dynamics of HIV (Ho et al., 1995), HBV (Nowak et al., 1996), and HCV (Neumann et al., 1998) and the only agents involved were host cells and viruses, without the inclusion of immune cells. The effects of the immune system are expressed by varying parameters such as lifespan of host cells and viruses.

Fig. 1a illustrates the study concept. Viruses have the ability to infect healthy host cells (uninfected cells) and the infected cells produce new viruses. Both cells and viruses have definite lifespans, and the lifespan of infected cells is usually shorter than that of uninfected cells. Uninfected cells automatically regenerate within the space, whereas infected cells only arise due to infection of uninfected cells. Viruses also lack the ability to regenerate themselves and are only produced from infected cells.

2.3. Parameter Settings

In the present study, as the StarLogo settings are circumscribed, we limited the simulation space to 51 × 51 square grids. However, we made an exception here while investigating the effects of size of space on the simulation results. The numbers of viruses, uninfected cells, and infected cells could only be set before the start of the simulation. As described in the later, our simulation ran in cycles, with 1 cycle defined as 1 tic.

In mathematical simulation models, the death rate is required as a parameter. However, in our program we set lifespans for viruses and uninfected cells. These lifespans were not uniform, but were set to have a deviation of about 10%. The lifespan of cells was shortened by infection with ratio decided beforehand.

The infection ratio was meaningful only when an infected cell and a virus coincidentally occupied the same grid, and this was used to calculate the probability of the infection occurring in that situation. The virus production rate was set as the number of viruses produced by an infected cell during 1 tic. Infected cells could be set as a parameter indicating the latent period between the time of virus infection and the time of virus replication.

In order to emulate the tissue repair capacity, we set uninfected cell regeneration rate such that grids without any cells had a specified probability of producing uninfected cells on top of themselves. As a result, the more the cell count declined within a space the more regenerated uninfected cells were produced, whereas the number of regenerated cells declined as cell count increased.

The number of grids through which a virus could move in 1 tic was set as the speed of movement, and the direction of movement was set within a range of 90° toward the top of the simulation space. The program used a circulatory method of movement; when a virus arrived at the top of the space, it was translocated to the bottom, and moved again toward the top. Cells were fixed on the grid.

2.4. Simulation Flowchart

Fig. 1b shows a flowchart of the program. First, the simulation space was produced, after which each parameter was defined and the initial settings were made. Next the agents – viruses and uninfected and infected cells – were produced. The simulation cycle was as follows. Viruses moved to a new grid, and if an uninfected cell was present, this was infected with a probability based on the infection rate. The lifespan of the virus decreased, and viruses that had completed their lifespan and those that had caused an infection were removed from the space. Infected cells produced new viruses, the lifespans of both uninfected and infected cells decreased. Then, cells that had completed their lifespan were eliminated and a new cycle began. The program was set such that the simulation ended after this cycle had repeated 1000 times. This meant that one simulation was complete after 1000 tics.

2.5. Data Collection

The RePast model was programmed such that data for each tic was saved automatically as a text file at the end of the simulation. This text file could be opened by a database software. The StarLogo model was programmed to stop the simulation and collect data after every 50 tics.

2.6. Mathematical Model

In order to compare the results of this agent-based simulation, we used a viral infection mathematical model, which we improved as follows.

$$\frac{dT}{dt} = s[2601 - (T+I)] - dT - bVT \quad (1)$$

$$\frac{dI}{dt} = bVT - dI \quad (2)$$

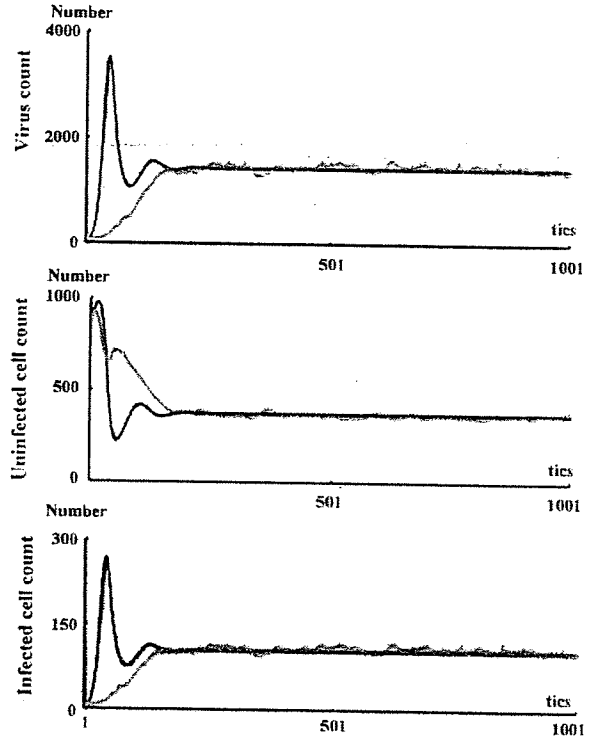

Fig. 3. Comparison of results of agent-based simulation and mathematical simulation. Both sets of results were consistent for the equilibrium phase, but differed in the shift in transition phase. Black line: mathematical model; grey line: results of simulation in RePast. Parameter values were set as follows: initial virus count, 100; uninfected cell count, 880; infected cell count, 0; virus speed of movement, 5 grids/tic; infection rate, 10%; uninfected cell regeneration rate, 1%; latent period, 3 tics; virus reproduction rate, 5/cells/tic; virus lifespan, 10 tics; uninfected cell lifespan, 50 tics; and cell lifespan-shortening ratio as a result of infection, 69%.

$$\frac{dV}{dt} = pI - cV \quad (3)$$

where, T is the uninfected cell count, I is the infected cell count, and V is the virus count. Uninfected cells are supplied to the space with a probability $s[2601 - (T+I)]$, as the number of grids in this agent-based simulation model was 2601 (51 × 51). The death rate of uninfected cells is d, the death rate of infected cells is δ, and the death rate of viruses is c. The infection rate is indicated by β. Viruses are released from infected cells at a probability p.

2.7. Statistical Analysis

Statistical analyses were performed by statistical tests using the program StatView 5.0 (SAS Institute Inc.). All tests of significance were two-tailed, with p values of <0.05 considered to be significant.

3. Results

3.1. Reproducibility of Chronic Viral Infection Disease Models Using Agent-based Simulation Methods

We constructed the chronic viral infection model with StarLogo library. Fig. 1c shows the simulation screen, and Fig. 1e shows one sample result. Immediately after the start of the simulation, the virus count temporarily dropped in accordance with the onset of an infection. Subsequently, the virus count started to increase with an increase in the infected cells and a decrease in the uninfected cells. After a certain number of tics (around 300 in this example), although the virus count, infected cell count, and uninfected cell count had some fluctuation, an equilibrium state was reached. We use the following descriptive terms in this paper: the transient phase is the period during which virus growth peaks, and the equilibrium phase is the period during which an equilibrium state is

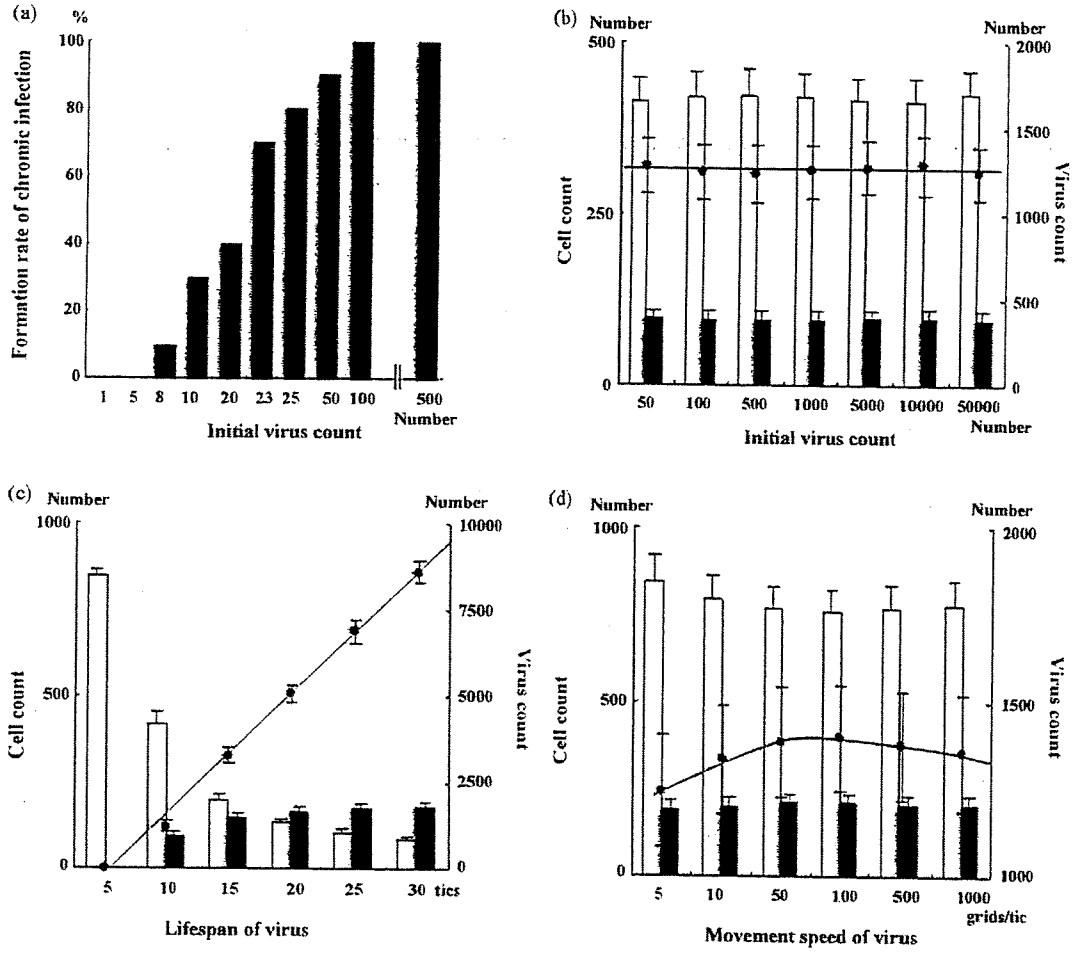

Fig. 4. Effects of changes in viral parameters. (a) The higher the initial virus count, the greater is the increase in the rate of formation of chronic infection, but (b) there was no effect on the conditions in the equilibrium phase. (c) Extending the virus lifespan increased the virus count. (d) Increasing the speed of virus movement to 100 grids/tic increased the virus count, but increasing it to 500 grids/tic had the opposite effect, with a slight declining trend. (a) Black bars: number of infections produced; (b–d) black circles: virus count; line: virus count approximation curve; white bars: uninfected cell count; black bars: infected cell count.

established. When the simulation was performed multiple times, the features described above were maintained, and the average values for virus, infected cell, and uninfected cell counts during the equilibrium state were all consistent.

Fig. 1d shows the simulation screen of the RePast. When we attempted setting all the initial parameters to the same values as those in the StarLogo, the results were not consistent. When we recalculated the parameters from the simulation results, in RePast, the parameters were largely maintained at the levels of the settings, but in StarLogo, the lifespans of both cell types became shorter than the settings while the simulation was in progress. We made the results of both simulations consistent by using the same parameters during the actual simulation (Fig. 2a and b).

3.2. Comparison Between Agent-based Simulation Models and Mathematical Simulation Model

We investigated whether the results of a chronic viral infection disease model produced by RePast would be consistent with the results of a mathematical model. For the mathematical model, we carried out an approximate integration using a four-dimensional Runge–Kutta method to ensure that the uninfected cell count and infected cell count would be in the same class. Parameters were always fixed as constant between simulations. The simulation results were consistent for the equilibrium phase, but transitions in virus count during the transient phase varied, with a shift to equilibrium state following two overshoots in the mathematical model, but a monotonic increase following a logistic curve in the agent-based model (Fig. 3). In the mathematical model, when the equilibrium condition was calculated with $dT/dt = dI/dt = dV/dt = 0$, the equilibrium-phase virus count, uninfected cell count, and infected cell count were very similar to those of the agent-based model (virus count: mathematical model 371.8/space, agent-based model 371.1 ± 32.4/space [average ± SD]; uninfected cell count: mathematical model 1605/space, agent-based model 1454 ± 194/space; infected cell count: mathematical model 115.9/space, agent-based model 108.3 ± 14.2/space).

3.3. Usability of the Models; Effect of Changing Parameters

We investigated the changes in the equilibrium phase brought about by changing each parameter. All the investigations below were carried out by using RePast, and we used the average values from ten simulations.

3.4. Viral Parameters

The lower the virus counts at the beginning of the simulation, the lower the probability of a chronic infection (Fig. 4a). However, the initial virus count did not have any effect on the equilibrium

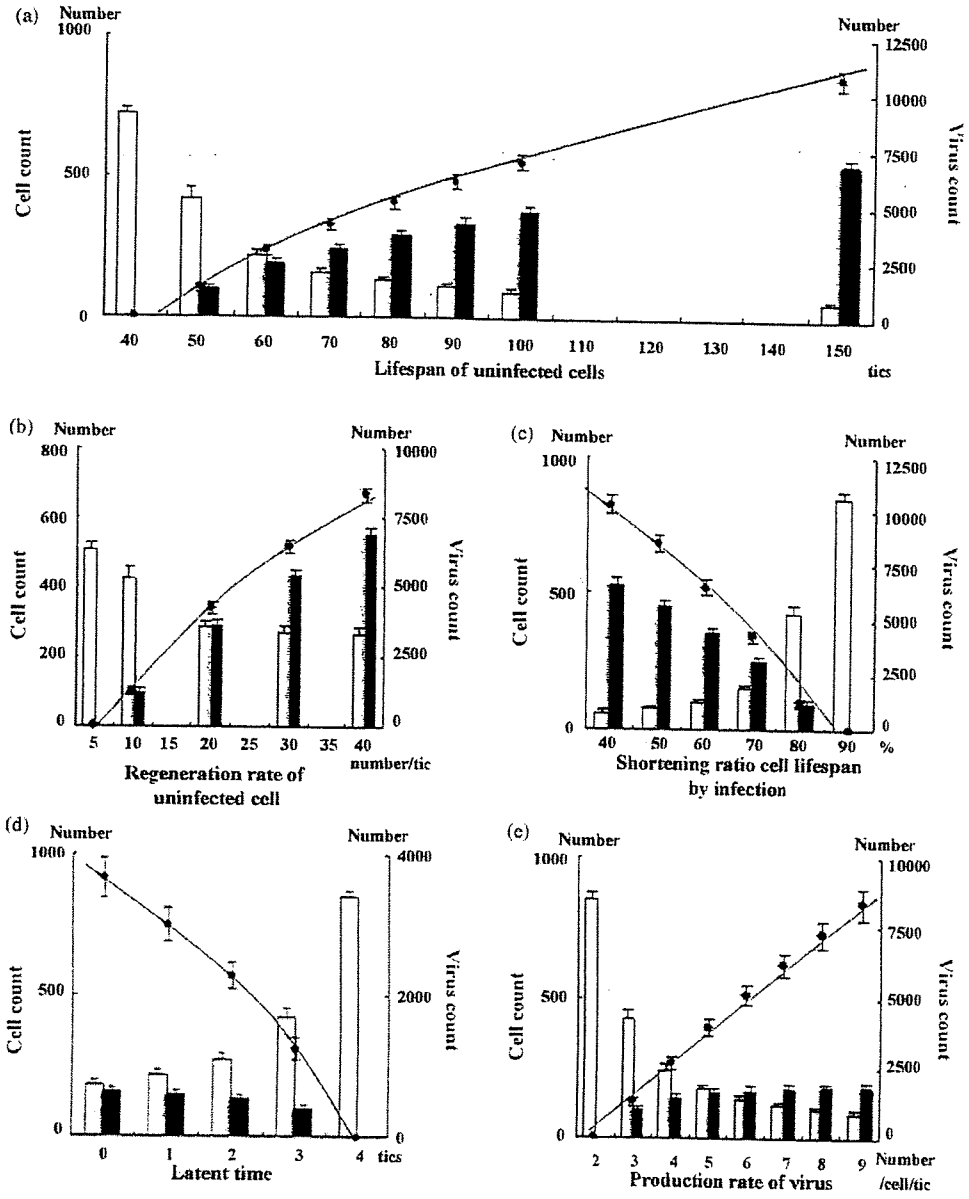

Fig. 5. Effects of changes in cell parameters. (a) Extending the uninfected cell lifespan and (b) increasing the uninfected cell regeneration rate increased the virus count. (c) Raising the lifespan-shortening ratio as a result of infection shortened the lifespan of infected cells, thereby decreasing the virus count. (d) Extending the latent period shortened the period of virus production from infected cells, thereby decreasing the virus count. (e) Increasing the virus production count resulted in a linear increase in equilibrium-phase virus count. Black circles: virus count; line: virus count approximation curve; white bars: uninfected cell count; black bars: infected cell count.

phase itself (Fig. 4b). Extending the lifespan of viruses resulted in a linear increase in equilibrium-phase virus count (Fig. 4c). Although the infected cell count increased, the rate of increase gradually declined. Changing the speed of viral movement resulted in the equilibrium-phase virus count to eventually decline after 100 grids/tic was reached, allowing movement over an area twice the size of the simulation space (Fig. 4d).

3.5. Uninfected Cell Parameters

Extending the lifespan of uninfected cells led to an increased virus count during the equilibrium phase (Fig. 5a). Increasing the uninfected cell regeneration rate also contributed to increased equilibrium-phase virus count (Fig. 5b). In both the cases, the increases in virus count and infected cell count were not linear, but showed a tendency for the rate of increase to decline gradually.

3.6. Infected Cell Parameters

We carried out an investigation of the effects of variation in the lifespan-shortening ratio on the virus count on the assumption that cell lifespan is shortened by infection. When this ratio was increased, the virus count decreased (Fig. 5c). An extended latent period was also related to a decreased virus count (Fig. 5d). However, the virus production from infected cells led to a linear increase in the virus count (Fig. 5e).

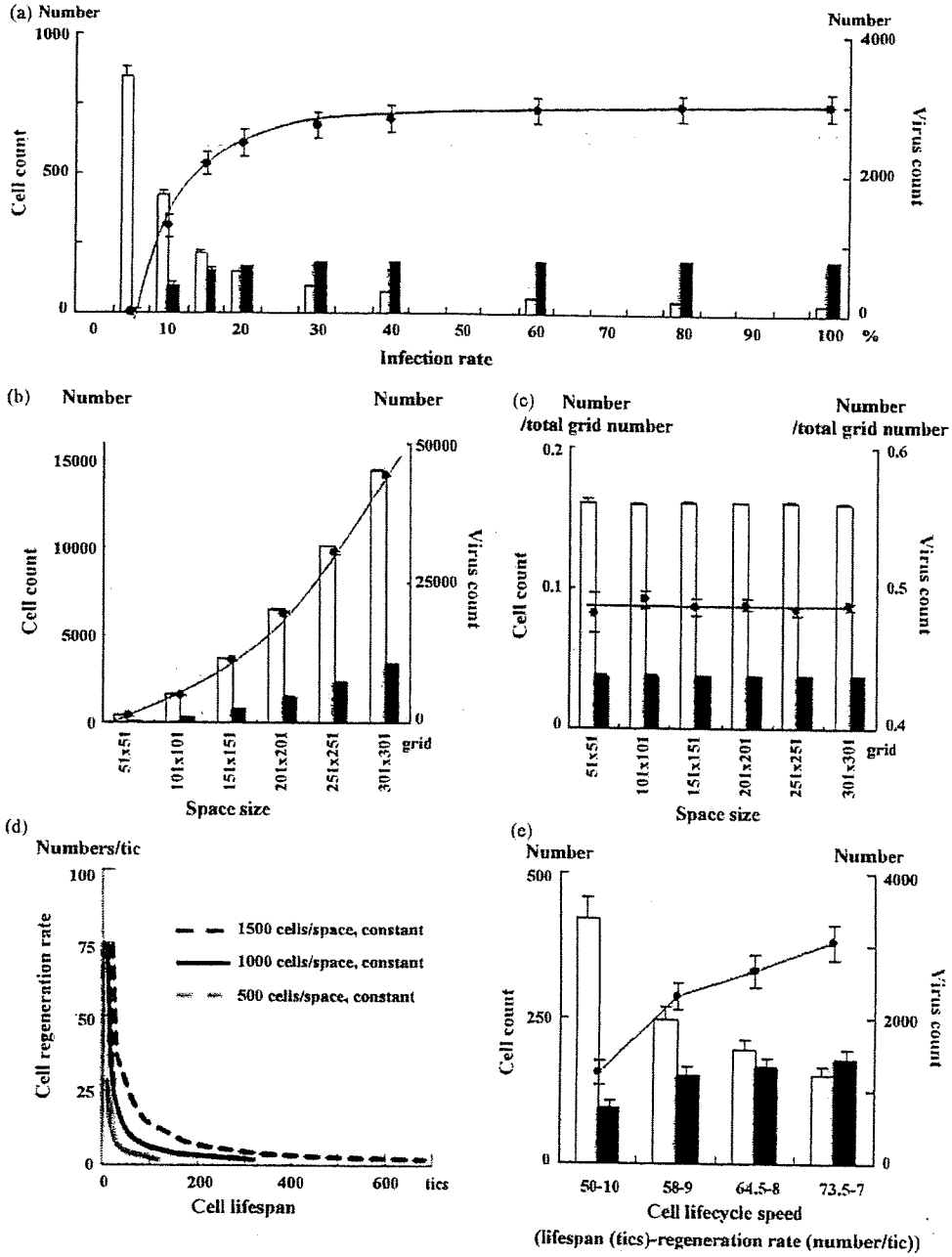

Fig. 6. (a) Increasing the infection rate increased the virus count in equilibrium periods, but the virus count did not change at infection rates of 30% or more. (b) The size of the simulation space increased not only virus count but also the cell count; however, (c) when virus and cell counts were divided by the total number of grids in the space, they were constant for all space sizes. (d) Changing the lifespan and regeneration rate of uninfected cells in opposite directions at the same time makes it possible to change only the cell cycle speed without altering the uninfected cell count. (e) When the cell cycle speed was reduced, the virus count increased toward the right of the graph. This may be because the effect of extending the lifespan of cells exceeds that of reducing their regeneration rate. (a–c and e) Black circles: virus count; line: virus count approximation curve; white bars: uninfected cell count; black bars: infected cell count.

3.7. Infection Rate and Space Size

Increasing the infection rate caused an increase in the virus count, but the change was minimal at an infection rate of 30% or more. The same results were seen for infected cell count, but a decrease in uninfected cell count resulted in a tendency for the infection rate to decrease by up to 60% (Fig. 6a).

The larger the space, higher the increase in both virus and cell counts (Fig. 6b). This increase was proportional to space size, however, when virus and cell counts were divided by the total number of grids in the space they were all constant (Fig. 6c).

3.8. Cell Cycle Speeds

Running a simulation with the initial virus count set to zero enables only the equilibrium condition for uninfected cells to be simulated. Changing the lifespan and regeneration rate of uninfected cells in opposite directions at the same time makes it possible