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Antitumor effect induced by dendritic cell (DC)-based immunotherapy against peritoneal dissemination of the hamster pancreatic cancer

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Abstract

Establishing a method to control peritoneal dissemination is one of the most pressing issues in the postsurgical treatment of pancreatic cancer. In the present study, we investigated the effect of dendritic cell (DC)-based immunotherapy on peritoneal disseminations of hamster pancreatic cancer cells, PGHAM-1. After the orthotopically inoculation of 2×10^6 PGHAM-1 cells, DC pulsed with PGHAM-1-derived tumor lysates, DC alone or PBS as a vehicle was injected intraperitoneally (i.p.) three times at weekly intervals. The group treated with DC or DC + lysate was found to have smaller disseminated tumors than the vehicle-treated. In addition, mean survival time in the DC + lysate groups was significantly longer than the PBS group. These findings suggested that DC-based immunotherapy might be efficient for the treatment of peritoneal disseminations of the pancreatic cancer.

Keywords: Hamster DC; Pancreatic cancer; Orthotopic model; Peritoneal dissemination; DC migration; Cytotoxic T-lymphocyte

1. Introduction

In almost all cases of pancreatic cancer patients, because of liver metastasis, peritoneal dissemination and local recurrence in the early postoperative stage, even macroscopically radical operations have not been successful in improving the prognosis of

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pancreatic cancer. Peritoneal dissemination is the most important factor in the prognosis of pancreatic cancer patients. Therefore, a therapeutical approach to control peritoneal dissemination is urgently required in clinical oncology.

Recently, using several DC-based immunotherapeutic approaches against intractable cancers, some positive results were reported for melanoma [1], kidney [2] and prostate cancers [3], lymphoma [4] and colorectal cancers [5]. However, few clinical approaches to the treatment of peritoneal dissemination have been successful, because control of it is very difficult in the devastating conditions of terminal cancer and the poor self-immune response of such patients. Previously, our group already demonstrated that hamster bone marrow (BM)-derived DCs loaded with tumor lysate could induce tumor-specific cytotoxic T lymphocyte (CTL) activity and a significant anti-tumor response in subcutaneously pancreatic cancer-bearing hamsters [6].

In the present study, based on the promising therapeutical effect on a subcutaneous tumor, we have focused on peritoneal dissemination, the worst of the local progressions or recurrences after surgery in pancreatic cancer resulting in death, and investigated the therapeutical effect of DC treatment on pancreatic cancer-induced peritoneal disseminations in hamsters in terms of tumor weight and survival time.

2. Materials and methods

2.1. Hamsters and cell lines

Specific pathogen-free 3 to 4-week-old female Syrian hamsters were purchased from Japan SLC, Inc. (Shizuoka, Japan) and housed under pathogen-free conditions. All animal used in this study were cared for and used humanely according to the principles of laboratory animal care (NIH publication No. 85-23, revised 1985) and the Guidelines for animal experiments of the National Cancer Center, Tokyo. The PGHAM-1 hamster pancreatic cancer cell line was kindly provided by Dr Uchida (Nippon Medical School, Tokyo, Japan), and maintained in Dulbecco's modified Eagle's medium (DMEM) (SIGMA, St. Louis, MO) containing 10% fetal bovine serum (Invitrogen, Rockville, MD), 2 mM L-glutamine, 100 U/ml of penicillin and 100 μg/ml of streptomycin. The DDT1MF-2 cell line, Syrian hamster leiomyosarcoma cells and the YAC-1 cell line were purchased from the American Type Culture Collection (Manassas, VA).

2.2. Culture of bone marrow-derived DCs and antigen pulsing

The method of BM-derived DC culture and pulsing with tumor lysates was previously described [6]. Briefly, unfractioned BM cells were cultured with mGM-CSF and mIL-4 at a concentration of 2×10^6 cells per ml in a 24-well culture plate for 7 days. Non-adherent, DC-enriched cell populations were collected by gentle pipetting. Then 1×10^7 DC-enriched cells resuspended in Opti-MEM medium and 2 mg of tumor lysate from PGHAM-1 cells mixed with 50 μg of *N*-[1-(2,3-Dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methylsulfate (DOTAP) were incubated 4 h at 37 °C in a water bath with constant gentle shaking. The DCs were washed three times with PBS (−) and used for intraperitoneal injection.

2.3. Tracking of GFP (green fluorescence protein)-labeled BM-derived DCs in vivo

The method of construction of the recombinant adenoviral vector containing the GFP gene and generation of adenovirus was described before [7]. After a 7-day-culture, DCs were harvested and resuspended at 5×10^6 cells per ml. Supernatant of adenovirus containing GFP cDNA (AdGFP) or mock virus was added to DCs at multiplicity of infection (MOI) of 100 and incubated for 2 h. After two washes, the cells were incubated for another 24 h with GM-CSF and IL-4. 1×10^7 Hamster DCs transduced with GFP cDNA (DC-GFP) or mock DCs were resuspended in 200 ul of PBS (−) and injected intraperitoneally (i.p.) into the hamsters. After 24 h, omentum, mesenteric and paraaortic lymphnodes were resected and single cell suspensions were made. Fluorescence-positive cells were measured using a FACSCalibur (Beckton-Dickinson, SanDiego, CA).

2.4. Adenoviral-retroviral sequential infection

PGHAM1 cells with 50% confluency on a 6 cm culture plate were infected with the adenovirus containing a human amphotropic retrovirus receptor cDNA (1×10^8 PFU) for an hour [7]. After a change of medium to DMEM supplemented with 10% FBS, they were incubated at 37 °C overnight. Then they were infected with the retrovirus containing cDNAs of EGFP (Clontech) and Ampho [8] by adding viral stock with polybrene (8 μg/ml) (adenoviral-retroviral sequential infection method; manuscript in preparation). At 2 h after the infection, the medium was replaced with DMEM supplemented with 10% FBS and incubated overnight. Blasticidin (108 μg/ml) was added to the medium and maintained until colonies were formed. GFP-expressing PGHAM-1 cells were used for the DC-based treatment model in animal experiments.

2.5. Animal experiments

The cohorts consisted of hamsters injected with PBS (−), DCs or DCs + lysate. On day 0, all hamsters were inoculated into the pancreas (orthotopically) with 2×10^6 non-transduced or GFP gene-transduced PGHAM-1 cells. On day 3, 9 hamsters per group were injected i.p. with PBS (−), 1×10^7 DCs, or 1×10^7 tumor lysate-pulsed DCs per an animal. The injections of vehicle or DCs were repeated 2 more times at weekly intervals. On day 24, one week after the final injection, three hamsters from each group were sacrificed for the measurement of resected tumor weight and metastatic nodule numbers and the investigation of CTL activity using spleen cells. A small amount of resected tumor from each group was also used for histological examinations. All 6 remaining hamsters were monitored until death, and the therapeutical effect on survival time was analyzed. The therapeutical effect on GFP-expressing tumor-inoculated hamsters was investigated using a digital CCD camera (Hamamatsu Photonics K.K., Hamamatsu, Japan).

2.6. CTL assays

Spleens were harvested from three hamsters per group on day 24, 7 days after the last DC injection, and used for the assay of CTL activity. The method of CTL assay was described before [6]. Briefly, spleen cells (5×10^6/ml) were co-cultured with irradiated (18,000 rads) PGHAM-1 cells (5×10^5/ml) in 6-well plates for 5 days. Restimulated spleen cells were used as effectors for the ^{51}Cr release assay. PGHAM-1 cells, DDT1MF-2 cells as an irrelevant tumor or YAC-1 cells as a target of NK cells were labeled with $Na_2\,^{51}CrO_4$. The labeled cells were mixed with effector cells at various *E/T* ratios in 96-well flat bottomed culture plates for 4 h. The method of measuring the radioactivity in supernatant was described previously [6].

2.7. Statistical analysis

Statistical differences were analyzed using the two-tailed Student's *t* test. Survival in vivo was analyzed by comparing differences in mean survival times using the Kaplan–Meier method. A comparative analysis of survival times between groups was then made using the Logrank test. Values of $P<0.05$ were considered statistically significant.

3. Results

3.1. Tracking of GFP-labeled BM-derived DCs in vivo

Almost all (more than 85%) GFP cDNA-transduced hamster DCs were GFP-positive and the MFI was very high, 5147 (data not shown). In vivo, the frequency of fluorescence-positive cells in the lymph node cell suspension was highest in omental lymph node tissue (6.8%, Fig. 1). No significant number of positive cells was seen in other lymph node tissues.

3.2. Inhibition of tumor growth and peritoneal dissemination

On day 24, 7 days after the last DC injection, 3 hamsters from each group were harvested and the remaining tumors were investigated. Tumor weight and the number of metastatic nodules were significantly reduced in the groups treated with DC or DC + lysates compared with the PBS group (Table 1). The amount of bloody ascites was also decreased in the groups injected with DC or DC + lysates. In terms of external appearance of hamsters, there was

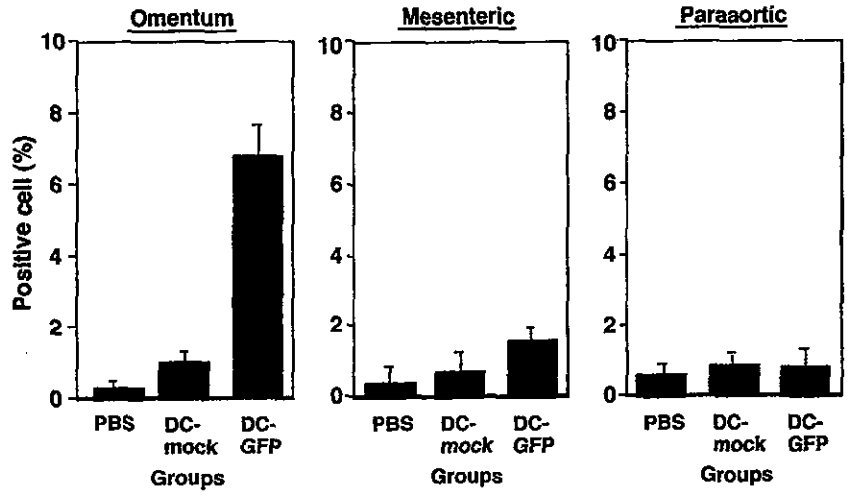

Fig. 1. Tracking of green fluorescence protein (GFP)-labeled BM-derived hamster DCs in vivo. Hamster DCs (1×10^7) transduced with GFP cDNA (DC-GFP) or mock gene (DC-mock) were injected intraperitoneally into hamsters. After 24 h, omentum, mesenteric and paraaortic lymph nodes were harvested and fluorescence-positive cells were measured using a FACSCalibur. Each column represents the mean±SD of lymph node samples from three hamsters.

a marked difference between the PBS group and the treated groups; remarkable abdominal distention was noted in the PBS group, whereas in the treated groups, the abdomen looked flat (data not shown). There was also a significant difference in the number of metastatic nodules and ascites between the DC group and DC+lysate group (Table 1). Additionally, in the GFP-expressing PGHAM-1 tumor treatment model, DC+lysate group showed no significant GFP spot compared with the PBS group, which showed multiple GFP spots in accordance with metastatic lesions (Fig. 2).

3.3. Cytotoxic T cell activity against PGHAM-1 tumors after intraperitoneal injection of BM-derived DCs

The patterns of CTL activity induced by i.p. injected BM-derived DCs and DC pulsed with tumor lysate were consistent with the anti-tumor activity of these groups shown in PGHAM-1 tumor-bearing hamsters. CTL assays revealed that the DC and DC+lysate groups showed more potent CTL activity against PGHAM-1 tumor cells than the PBS control group ($P<0.01$, Fig. 3) at *E/T* ratios greater than 11. In addition, the DC+lysate group exhibited greater CTL activity than the DC group at *E/T* ratios greater than 33 ($P<0.05$). Antibody-based blocking test for CTL activity also demonstrated that anti-CD8 MoAb inhibited moderately but significantly DC-stimulated CTL activity by 32 and 38% in DC alone and DC+lysate group, respectively (data not shown). No significant differences in NK activity were detected. Most importantly, CTL activity against DDT1MF-2 sarcoma cells, another irrelevant tumor syngeneic to Syrian hamsters, was not induced at any treated group. These results suggested that tumor-specific CTL activity may be an important key factor to

Table 1
Inhibition of pancreatic cancer growth and peritoneal dissemination by DC injections

Groups	Tumor weight (g)	No. of tumor nodules	Ascites (ml)
PBS	2.22±0.75	29.0±9.27	16.0±4.48
DC	1.01±0.85 ($P=0.0482$)	10.6±4.72 ($P=0.0058$)	4.55±3.10 ($P=0.0103$)
DC+lysate	0.24±0.39 ($P=0.0061$)	1.60±2.61 ($P=0.0014$)	0 ($P=0.0014$)

The data show the mean±SD from three hamsters. Values in parentheses indicate *P* values for the DC or DC+lysate group compared with the PBS group.

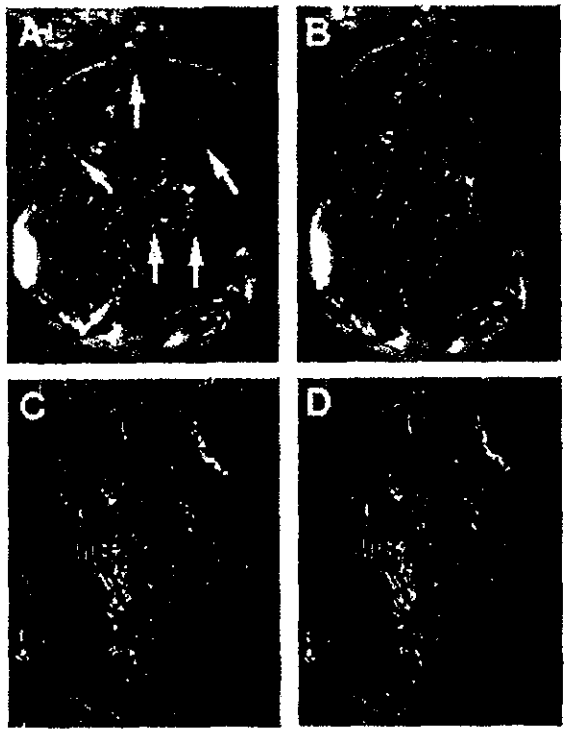

Fig. 2. Remarkable growth suppression of orthotopically inoculated PGHAM-1 tumor after repeated DC vaccinations. (A) and (B) PGHAM-1 cell-inoculated hamster treated with PBS. Multiple tumor nodule disseminations were seen in the peritoneal cavity (arrows). (C) and (D) GFP-positive PGHAM-1 cell-inoculated hamsters treated with DCs+tumor lysates. (A) and (C) regular image, (B) and (D) GFP image overlayed.

the anti-tumor effects of tumor lysate-pulsed DC against PGHAM-1 tumors.

3.4. Histological observations in PGHAM-1 tumors from hamsters treated with tumor lysate-pulsed DCs

PGHAM-1 tumors from hamsters injected with tumor lysate-pulsed DCs showed a remarkable less of viable tumor cells and massive necrosis with heavy infiltration of mononuclear and polymorphonuclear leukocytes (data not shown).

3.5. Survival analysis

All hamsters in the PBS group died within 4 weeks of the inoculation of PGHAM-1 cells. In contrast, almost all hamsters from the treated groups were still alive after 4 weeks (Fig. 4). The mean survival time was significantly longer for the DC and DC+lysate groups than PBS group (DC+lysate 48.8 days, DC 35.0 days versus PBS 25.0 days; $P<0.01$ by Logrank test). In addition, survival in the DC+lysate group was demonstrated to be significantly prolonged compared with the DC group ($P<0.05$).

4. Discussion

To date, several therapeutic attempts to control peritoneal disseminations of pancreatic cancers have

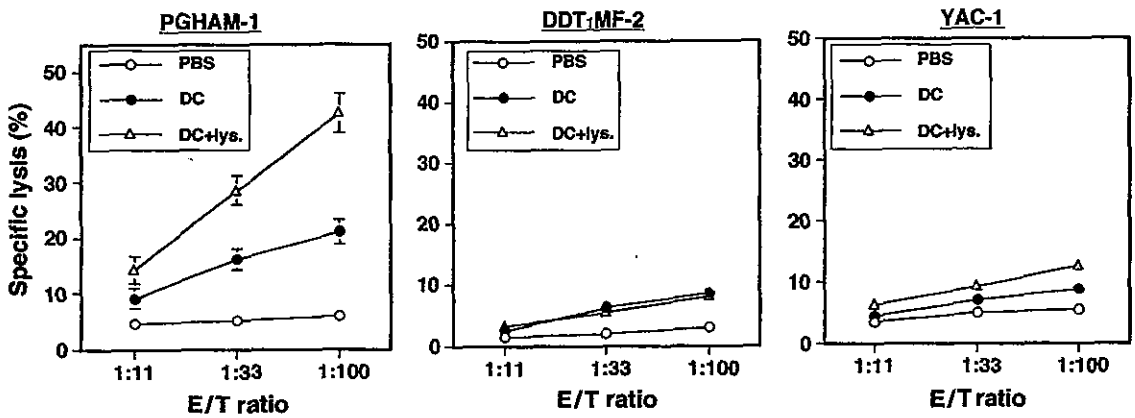

Fig. 3. CTL activity induced by tumor lysate-pulsed DCs in hamsters orthotopically inoculated with PGHAM-1 cells. On day 24, spleen cells were harvested from 3 hamsters per group and pooled. The spleen cell suspension (2.5×10^6 per ml) was co-cultured with 2.5×10^5 per ml of irradiated (180 Gy) PGHAM-1 cells. Restimulated spleen cells were used as effectors in a ^{51}Cr release assay. Killing activities of effector cells at various *E/T* ratios against (A) PGHAM-1 cells, (B) DDT1MF-2 cells and (C) YAC-1 cells are shown. Data show the mean±SD of triplicate samples.

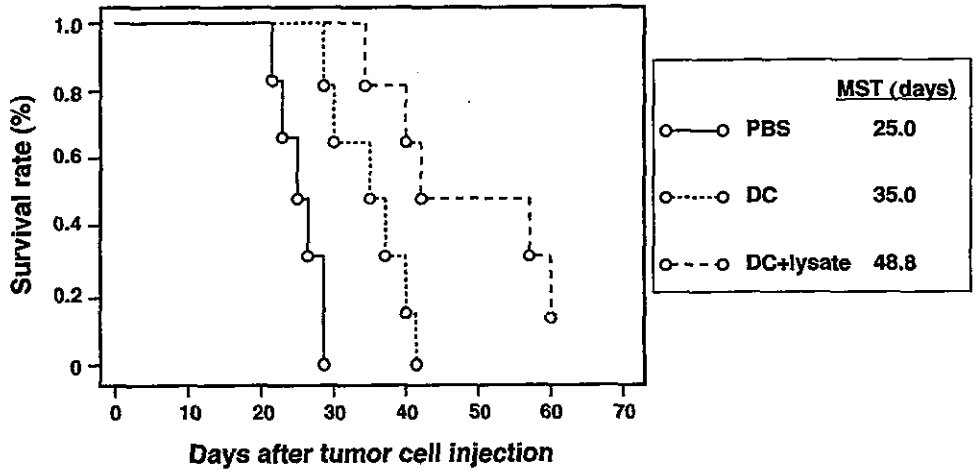

Fig. 4. Prolonged survival of tumor-bearing hamsters treated with DCs pulsed with tumor lysate. All hamsters in the PBS group died within 4 weeks of the inoculation of PGHAM-1 cells. Meanwhile, almost all hamsters from the treated groups survived. Mean survival time was significantly longer for the DC and DC+lysate groups than PBS group (DC+lysate 48.8 days, DC 35.0 days versus PBS 25.0 days; $P<0.01$ by Logrank test).

been reported [9–14]. One group of therapeutic modalities is based on gene therapy. Aoki et al. [9] demonstrated that the herpes simplex virus thymidine kinase (HSV-TK) gene could be transduced intraperitoneally into tumor cells by way of an injection of DNA-lipopolyamine complex, but the feasibility of the gene-delivery system is not clear. Another group uses tumor-antagonizing agents like NK4, a four-kringle fragment of hepatocyte growth factor (HGF). Tomioka et al. [13] reported that NK4 inhibited tumor growth, peritoneal dissemination and ascites accumulation in orthotopic nude mice inoculated with human pancreatic cancer cells into the pancreas, and that the simultaneous targeting at both tumor angiogenesis and the HGF-mediated metastasis might contribute to the anti-tumor effect.

However, few studies of immune cell-based therapeutic approaches based on intensification of the host-defense against pancreatic cancer have been reported. Previously, we demonstrated that hamster BM-derived DCs treated with tumor lysates exhibited obvious anti-tumor effects and caused a tumor-specific CTL induction in hamsters inoculated subcutaneously with syngeneic pancreatic cancer cells [6].

In the present study, as a next step, anti-tumor effect of lysate-treated DCs against peritoneal dissemination of hamster pancreatic cancer was investigated in vivo. To sum up the results of this study, in the group treated with DC+tumor lysates, the growth of orthotopically inoculated tumor cells, PGHAM-1 cells, and ascites production were significantly inhibited. In addition, even in the survival analysis, the injections of DC+lysates had a greater beneficial effect on the survival of tumor-bearing hamsters than PBS or DC injections alone. For another interesting thing, there was an obvious difference of the strength of cellular immune response including CTL or NK activity against tumor cells between subcutaneously (previously described) and the present intraperitoneally tumor-bearing hamsters. Specifically, CTL killing activity against pancreatic cancer cells at the E/T ratio of 100, was much higher in subcutaneous model (more than 60%) than intraperitoneal model (around 40%, Fig. 3). The one of reasons may be the site of tumor or DC vaccination, which is an immunologically important issue. Subcutaneous injection of DCs is an easy way to migrate to regional lymph nodes, trigger the activation of naive T cell, and expand tumor-specific CTLs. On the other hand, intraperitoneal way of DC injection might has some problems that tumor cells in the site or peritoneal macrophages could hinder inducing potent anti-tumor CTLs by means of Th2 cytokines or cell to cell conatct. Second, the difference of target pancreatic cancer cells may be another reason. PGHAM-1 cell

is rapid-growing pancreatic cancer cells with high malignancy compared with HPD1NR cell used in subcutaneous model. Probably, it is easily considered that the induction of positive cellular immune response in the abdominal cavity of PGHAM-1-inoculated hamsters is a difficult protocol without a strong adjuvant effect like DCs.

It can be speculated that the anti-tumor effects on peritoneal dissemination by PGHAM-1 cells were mediated by well-known biological mechanisms; cell migration and Th1 cytokine production. In the first instance, injected DCs transduced with the GFP gene were demonstrated to migrate to the omentum-associated lymphoid tissues, which are anatomically equivalent to milky spots. It is generally considered that omental milky spots act as the first line of host defense in the peritoneal cavity because macrophages, the major cellular component of the spots are dedicated to facilitating phagocytosis and the processing of circulation antigens and foreign bodies generated from the peritoneal cavity [15–17]. In addition, the administration of a biological response modifier (BRM) is reported to activate macrophages in the milky spots and promote their migration into the peritoneal cavity [18]. On the other hand, given that intraperitoneally injected tumor cells tend to invade the milky spots and form tumor nodules, omental milky spots are also closely involved in the dissemination of cancer cells [19]. Taken these findings into consideration, the migration of injected DCs treated with tumor lysates to the milky spots seems to be an immunologically important event which probably triggers the stimulation and expansion of tumor-specific CTL closely linked to a significant anti-tumor effect in vivo.

In the second instance, the PCR analysis revealed that hamster DCs stimulated with tumor lysates upregulated IL-12 gene transcription, that is to say, IL-12 protein production in our study (data not shown). IL-12, a Th1 cytokine generated by antigen-presenting cells like DCs, is demonstrated to have various effects on effector cells and tumor cells; tumor-specific CTL production or NK cell activation is easily promoted and additionally anti-angiogenic effects and FAS upregulation on tumor cells. Also our previous report demonstrating that intratumoral injection of genetically modified mouse DCs producing IL-12 p70 showed a remarkable anti-tumor effect on B16-bearing mice supports the efficacy of IL-12 [20]. In the present study, there might be other mechanisms besides DC migration and IL-12 production mediating the final tumor-specific CTL production and the inhibition of tumor growth.

Even though tumor lysate-pulsed DC therapy is not a novel modality, from a clinical point of view, it is worth applying DC-based immunotherapy to the treatment of intractable pancreatic cancers, especially peritoneal disseminations. It is recommended that this therapeutical approach should be tested in a phase I clinical trial because there is still no effective treatment for advanced pancreatic cancers.

Acknowledgements

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Systemic Chemotherapy for Pancreatic Cancer

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Abstract: Surgical resection offers the only curative strategy for pancreatic cancer. Yet, because early detection of pancreatic cancer is so difficult and diagnosis is delayed, pancreatic cancer in most patients is surgically unresectable. Even in patients with resectable disease, the long-term outcome remains unsatisfactory due to early recurrence after resection. Early appearance of distant metastasis suggests that systemic treatment, such as chemotherapy, should play a major role in improving patient survival. Although the recently developed gemcitabine has renewed interest in clinical research for pancreatic cancer, other currently available chemotherapeutic agents have little impact on survival. Studies to identify more effective agents or treatment regimens must have the highest priority. The expanding understanding of molecular and genetic biology should facilitate research to develop novel molecule-targeted agents and to establish individualized therapy regimens for this disease.

Key Words: pancreatic cancer, chemotherapy, gemcitabine, S-1, thymidylate synthase inhibitor 5-fluorouracil

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Pancreatic cancer is a virulent disease with an extremely poor prognosis. Of all the treatment modalities for pancreatic cancer, only resection offers the opportunity for cure. However, because of local extension and/or metastatic disease, only a small minority of pancreatic cancer patients are candidates for resection with curative intent. Moreover, even for selected patients, prognosis remains unsatisfactory because of postoperative recurrence, indicating that resection alone has only limited value in treating pancreatic cancer. Accordingly, to improve the overall survival of patients with pancreatic cancer, there is an urgent need to develop effective nonsurgical treatments, including chemotherapy, for this disease. In this article, chemotherapies for pancreatic cancer in Japan are discussed. This review focuses on pancreatic ductal adenocarcinoma because it is the most common form of pancreatic cancer in Japan, accounting for approximately 90% of pancreatic cancer cases.[1]

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RESULTS IN PATIENTS WITH UNRESECTABLE AND RECURRENT DISEASE

Although a variety of chemotherapeutic regimens have been tried and evaluated in advanced pancreatic cancer in Japan, most studies demonstrated little objective evidence of significant activity against this disease. Few agents repeatedly documented high response rates and meaningful impact on patient survival or quality of life. However, the recent development of gemcitabine has renewed an interest in clinical research for pancreatic cancer due to its significant clinical benefit and survival improvement.

Fluoropyrimidine-Based Chemotherapy

Of all chemotherapeutic drugs, the thymidylate synthase inhibitor 5-fluorouracil (5-FU) has been the most extensively evaluated and most widely used agent for pancreatic cancer in Japan. However, despite numerous trials of 5-FU, the optimal dose and administration schedule have yet to be defined. Moreover, results with this agent, regardless of schedule, remain dismal, with reported response rates ranging up to 20%.[2] Based on accumulated clinical evidence suggesting that protracted venous infusion of 5-FU may induce greater antitumor activity than bolus infusion, continuous venous infusion of 5-FU was investigated in a small phase 2 study for advanced pancreatic cancer patients.[3] A dose of 500 mg/m^2 of 5-FU was given for 7 days by continuous venous infusion over a 24-hour period and then followed by a dose of 170 mg/m^2 for more than 28 days. The administration schedule of 5-FU in this study was feasible, but the result of this regimen was disappointing; none of the patients achieved objective response.

There have been various attempts at biochemical modulation of 5-FU through different agents to enhance antitumor activity. We examined sequential administration of methotrexate and 5-FU for metastatic pancreatic cancer patients that showed high response rates in several malignant diseases.[4] Methotrexate (100 mg/m^2) was given, followed by a 600-mg/m^2 infusion of 5-FU. Partial responses were achieved in 4 of the 31 patients (12.9%), with a median survival time of 4.0 months. The antitumor activity of this regimen, therefore, seemed marginal.

UFT, an orally administered drug, is a combination of tegaful, a prodrug of 5-FU, and uracil, a competitive inhibitor of dihydropyrimidine dehydrogenase. A Japanese phase 2 study conducted in the early 1980s of UFT administered at a daily dose of 300–600 mg exhibited a 25% tumor response in 16 evaluable patients with advanced pancreatic cancer.[5] Recently, we conducted a confirmatory phase 2 study of UFT at a dose of 360 mg/m^2/d, but this study failed to confirm the initial response finding; none of 21 patients achieved an objective tumor response.[6]

S-1 is an oral anticancer drug that consists of tegafur (FT) as a prodrug of 5-FU, 5-chloro-2,4-dihydroxypyridine (CDHP), and potassium oxonate (Oxo). The drug was developed in Japan to improve the tumor-selective toxicity of 5-FU by 2 biochemical modulators, CDHP and Oxo. CDHP is a competitive inhibitor of dihydropyrimidine dehydrogenase involved in degrading 5-FU and maintains efficacious 5-FU concentrations in plasma and tumor tissues. Oxo, a competitive inhibitor of orotate phosphoribosyltransferase, inhibits phosphorylation of 5-FU in the gastrointestinal tract and reduces the serious gastrointestinal toxicity of 5-FU. S-1 has already demonstrated a potent antitumor effect on various solid tumors in clinical studies.[7–12] We conducted an early phase 2 study of S-1 in patients with metastatic pancreatic cancer,[13] and our study showed promising results, with a 21% response rate in 19 evaluable patients, and a manageable toxicity profile of this agent. We are conducting a multiinstitutional late-phase 2 study of S-1 for metastatic pancreatic cancer to confirm the results in this study.

There has been hope that improved therapeutic results might be obtained with 5-FU–based multiagent chemotherapy since several agents having at least some activity have been identified. We performed a phase 2 trial of combined chemotherapy using 5-FU and cisplatin, a potential modulator of 5-FU, which itself showed some antitumor activity against pancreatic cancer.[14] 5-FU was administered at 500 mg/m^2/d by continuous intravenous infusion for 5 days and cisplatin was administered at 80 mg/m^2 intravenously on the first day of every 28 days. The therapy on this schedule had limited antitumor activity, with only an 8% response rate in 37 patients. With this treatment, 4 (21%) of the 21 patients obtained remarkable symptom relief.[15] Based on laboratory data suggesting a profound schedule dependency for cytotoxicity of this combination, Tsuji et al[16] conducted a phase 2 trial of continuous-infusion 5-FU and low-dose consecutive cisplatin for 39 patients with advanced pancreatic cancer. 5-FU (160 mg/m^2/d) was continuously infused over 24 hours for 7 consecutive days, and cisplatin (3 mg/m^2/d) was administered over 30 minutes for 5 days, followed by a 2-day rest every 4 weeks. The objective response rate was 28.2%, with a clinical benefit response rate of 48.7% and a median survival time of 6.5 months.

Most studies of 5-FU–based, multiagent chemotherapy have documented little reproducible impact on patient survival, while all of these regimens have exhibited great toxicity. Takada et al[17] failed to demonstrate a survival benefit of the combination chemotherapy consisting of 5-FU, doxorubicin, and mitomycin for Japanese patients with unresectable pancreatic and biliary cancer. Based on the results to date, 5-FU–based chemotherapy cannot be recommended outside clinical trials.

Chemotherapy Using Agents Other Than Fluoropyrimidine

Various agents other than fluoropyrimidine, including drugs developed in Japan, have also been studied in advanced pancreatic cancer patients. CPT-11, a semisynthetic, water-soluble derivative of the plant alkaloid camptothecin, has been tested for this disease. Sakata et al[18] reported a 11.4% response rate in a phase 2 trial employing 100 mg/m^2 given weekly or 150 mg/m^2 given biweekly. However, only 35 of the 57 eligible patients were evaluable for efficacy in this study. A confirmatory phase 2 study is now underway in Japan.

Docetaxel, a semisynthetic taxane, has also been evaluated. In a French study, Rougier et al[19] reported 5 objective responses (29%) in 17 advanced pancreatic cancer patients in the initial report, and 6 responses (15%) in 40 patients in the final report. However, subsequent trials, including a Japanese study, could not confirm the favorable results. None of the 21 patients in the Japanese trial showed a response.[20]

Gemcitabine is a deoxycytidine analog that is capable of inhibiting DNA replication and repair. Gemcitabine has the potential for great activity against various solid tumors, including pancreatic cancer, because of its prolonged inhibition of both cell synthetic function and progression through the cell cycle. In the randomized trial comparing gemcitabine with 5-FU, gemcitabine showed significantly better results in clinical benefit response and survival.[21] Accordingly, gemcitabine has been accepted as first-line chemotherapy for advanced pancreatic cancer. In the phase 1 trial conducted in Japan before this randomized trial, the recommended dose schedule of gemcitabine was 800 mg/m^2 weekly × 3, followed by 1 week of rest, with leukocytopenia as dose-limiting toxicity.[22] However, in most trials of gemcitabine for pancreatic cancer, including the previous randomized study, a dose of 1000 mg/m^2 has been employed and approved in Western countries. Therefore, we conducted a phase 1 trial to confirm the tolerability of weekly scheduled gemcitabine at a dose of 1000 mg/m^2 in Japanese patients with advanced pancreatic cancer.[23] This study showed low incidence of dose-limiting toxicity, suggesting that 1000 mg/m^2 gemcitabine weekly × 7, followed by 1 week rest and again weekly × 3 every 4 weeks may be tolerated in Japanese patients with advanced pancreatic cancer. In this trial, a partial response was obtained in 2 (18%) of the 11 enrolled patients with metastatic pancreatic cancer and a clinical benefit response was achieved in 2 (29%) of the 7 evaluable patients. Based on the consistency in response and toxicity of