Clinical Study

ONCOLOGY

Oncology 1996;53:476–481

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Experimental and Clinical Efficacy of 2′,2′-Difluorodeoxycytidine (Gemcitabine) against Renal Cell Carcinoma

Abstract

Preclinical and clinical studies have been performed to evaluate the efficacy of gemcitabine (2′,2′-difluorodeoxycytidine; dFdC) in human renal cell carcinoma. Experimental data corroborated dFdC as an effective drug against cell lines from renal cell carcinomas (ACHN, A-498, SN12C) at concentrations much below clinically achievable doses. ACHN-bearing nude mice showed an overall response rate of 27% to dFdC (3 mice with complete response, 1 with partial response, 3 with stable and 8 with progressive disease). Objective response from 37 evaluable patients was 8.1% (1 patient with complete response and 2 patients with partial response). Gemcitabine was well tolerated thus, although gemcitabine at the dosage and schedule chosen had only small activity, the observed toxicity may permit further dose escalation or a more frequent administration of the drug.

Key Words

2′,2′-Difluorodeoxycytidine
Renal cell carcinoma
In vitro studies
Xenografts
Phase II trial

Introduction

Metastatic renal cell carcinoma (RCC) has been treated with monochemotherapy with variable success. Among a panel of more than 30 different drugs, fludarabine, lomustine, vinblastine, ifosfamide or bisantrene did show some activity. Response rates did vary between 3 and 34% [1]. Overall, the results with chemotherapy have been disappointing, with most studies revealing response rates below 10%. Several forms of immunotherapy with interferons and interleukin-2 have been applied, resulting in a limited number of durable responses [2]. Therefore, further studies with new agents are warranted. Gemcitabine (2′,2′-difluorodeoxycytidine; dFdC; LY 188011) is a novel nucleoside analogue that inhibits DNA synthesis and repair, leading to cell death. For activation, the drug requires an intracellular phosphorylation to tri (dFdCTP)-, di (dFdCDP)- or mono (dFdCMP)-5′-phosphorylated metabolites. The modes of action have been described in detail elsewhere [3–5]. Briefly, DNA elongation is inhibited by incorporation of wrong nucleosides [3, 4] or by direct inhibition of DNA polymerases [5]. Interestingly, the cytotoxicity of the drug is enhanced by several self-potentiating mechanisms [4].

In vitro dFdC has already been found to be cytotoxic against human leukemia cells [5–7], ovarian cancer cells [8], colon carcinoma [9] or squamous cell carcinoma cells [10].

Therefore, we performed preclinical studies and a clinical trial to evaluate the efficacy of gemcitabine in human RCC.

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0030–2414/96/0536–0476$10.00/0

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Patients, Materials and Methods

Cell Culture. The cell lines derived from human RCC: SN12C, [11], ACHN (intrinsically vincristine resistant; ATCC CRL 1611, American Type Culture Collection, Rockville, Md., USA) and A-498 [12] were grown under cell culture conditions in (D)MEM standard cell culture medium (GIBCO, Grand Island, N.Y., USA) at 37°C and a humidified atmosphere of 6% CO_2. Media were supplemented with 10% (v/v) heat-inactivated fetal calf serum, penicillin (100 IU/ml) and streptomycin (100 µg/ml).

Colorimetric Cytotoxicity Assay. The cytotoxicity of gemcitabine for ACHN, A-498 and SN12C cells was measured by use of the sulforhodamine B assay [13]. In brief, 25,000 tumor cells were seeded per well of a microtiter plate. After 18 h incubation, cells were exposed to gemcitabine for 48 h (0.1–1,000 ng/ml). Controls received supplemented media. Fixation, staining and washing steps have been described elsewhere [14]. Bound stain was solubilized with 50 µl Tris buffer (pH 10.5). Optical densities were read at a single wavelength of 530 nm on an automated spectrophotometric plate reader (EAR 400 AT, SLT-Labinstruments, Crailsheim, Germany). Results from duplicates per microtiter plate were repeated at least twice. Cytotoxicity was expressed as arbitrary T/C percent values (optical density of treated cells/optical density of control cells × 100).

Animal Experiments. 5×10^6 tumor cells were subcutaneously injected in 3- to 4-week-old male balb/c nu/nu mice. Therapy was initiated at tumor volumes of 100 mm^3. Test group animals received gemcitabine (40 mg/kg body weight intraperitoneally; Lilly Deutschland, Giessen, Germany) compared to controls that received NaCl 0.9% intraperitoneally (n = 11–15).

One course of therapy (4 weeks) included intraperitoneal injections once a week for 3 weeks followed by 1 week rest. A complete therapy schedule consisted of four courses. Tumor size was measured with calipers; tumor volume was calculated as tumor length × $(width)^2/2$. Tumor volumes after 16 weeks of therapy were compared using the Wilcoxon test. A p value <0.05 was designated statistically significant.

Clinical Trial. A multicenter study group conducted a phase II nonrandomized clinical trial to treat patients with advanced RCC with gemcitabine monotherapy. Details have been described elsewhere [15]. Briefly, patients (18–75 years) with a histologically or cytologically confirmed metastatic or inoperable advanced RCC were eligible, if they had no previous chemotherapy, a WHO performance status of 0–2 and a life expectancy of at least 3 months. Previous therapy with biological response modifiers was allowed (>4 weeks before therapy), as well as palliative radiotherapy if the measurable lesion remained nonirradiated. Exclusion criteria were: bilateral RCC, bony lesions only, second malignancies, CNS envolvement or any serious systemic disorder.

Treatment was performed in accordance with the declaration of Helsinki, and informed consent of the patients was obtained. 800 mg/m^2 body surface gemcitabine was administered once weekly for 3 weeks followed by 1 week rest. Patients who completed a cycle of therapy could have a dose escalation up to 20% in each subsequent cycle (maximum: 1,200 mg/m^2). A 50% dose reduction was administered if white blood cell counts >2 but <3 giga/l leukocytes and 50–99 giga/l platelets or if nonhematologic toxicity WHO grade III occurred. Any disease progression led to withdrawal from the treatment with gemcitabine.

Efficacy was examined in each patient before each therapy cycle and additionally in 8-week intervals (chest X-ray, CT scan if appropriate).

Efficacy analysis included tumor response rate and 95% confidence intervals. Each investigator-determined responder was reevaluated by independent experts. The duration of a partial response (PR) was measured from the time of the first administration of the drug until progressive disease was documented. The duration of a complete response (CR) was measured from the time of a documented CR until the first observation of disease progression. Survival was measured from the time the first dose gemcitabine was administered until death or the patient was last known to be alive.

Results

In vitro Studies

The growth of all three tumor cell lines was inhibited by treatment with gemcitabine (fig. 1). ACHN cells were inhibited more effectively than A-498 cells. SN12C cells were nearly 25 times less sensitive than ACHN cells. The drug concentrations that led to a 50% reduction in cell proliferation compared to controls were approximately 2 ng/ml (0.0076 µmol/l) for ACHN cells, 10 ng/ml (0.038 µmol/l) for A-498 and 45 ng/ml (0.1711 µmol/l) gemcitabine for SN12C cells (fig. 1).

Xenografts

Xenografts of SN12C cells did not respond to treatment with gemcitabine ($p < 0.1$). In contrast, the growth of tumors induced by ACHN cells was statistically significantly inhibited by gemcitabine ($p < 0.0014$; fig. 2). Interestingly, late responses have been observed even weeks after the end of therapy. Table 1 summarizes the best responses. The overall response rate was 4/15 (27%) for ACHN xenografts (3 CR; 1 PR and 3 whith stable disease).

Clinical Data

39 patients with measurable metastatic RCC were enrolled. Their characteristics are listed in table 2. 34 have been subjected to previous surgery, 5 to previous radiotherapy and 20 have had prior treatment with interferon-α and/or interferon-γ.

37 patients were eligible for evaluation of efficacy (29 males, 8 females). The age range was 38–74 years (median 56.6). The WHO performance statuses were 0 in 15 patients, 1 in 19 patients and 2 in 2 patients. There was 1 patient with a WHO performance status of 3 who, although this was a protocol violation, qualified for efficacy analysis. Metastatic sites were mainly located in the lung (81.1%) and lymph nodes (40.5%).

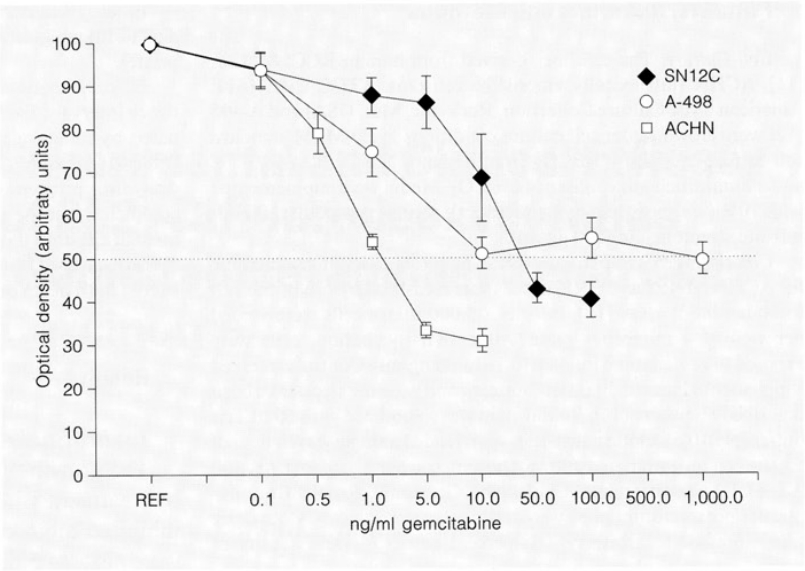

Fig. 1. Growth inhibition of three different human RCC cell lines (SN12C, A-498, ACHN) by gemcitabine compared to unexposed tumor cells (REF).

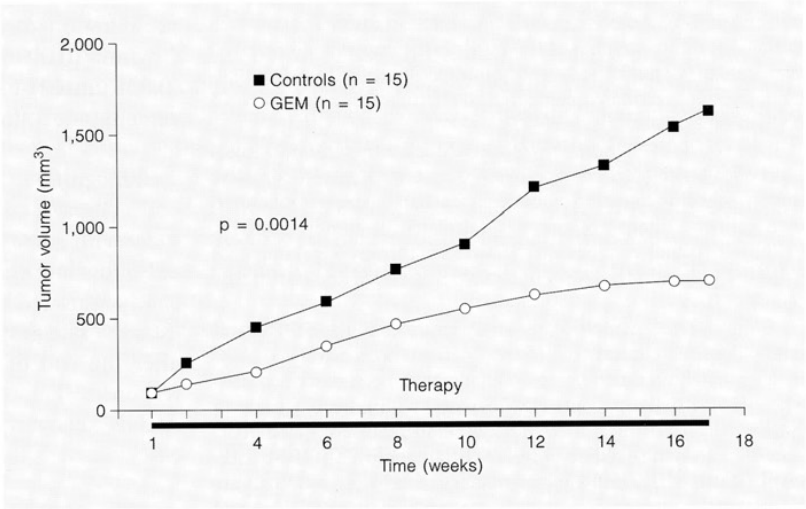

Fig. 2. Growth inhibition of xenografts derived from ACHN cells by treatment with gemcitabine (GEM) compared to untreated animals (controls).

39 patients received at least one dose of gemcitabine. The mean number of cycles was 3.7 (0–16). 1.6% of all injections were omitted, only 12.8% reduced in dose. Most dose reductions (70%) occurred in the first three cycles, usually due to leukopenia. 4.6% of injections could be subsequently escalated.

At present, 37 of 39 patients are evaluable, with 3 patients being independently confirmed as responders (1 CR; 2 PR) giving a response rate of 8.1% (95% confidence interval: 1.7–21.9%). Responses lasted 32, 12 and 19 months, respectively. Table 3 gives a summary of best tumor responses. The median time to disease progression was 3.7 months (0.7–33.9). The median survival was 12.3 months (0.7–33.9). In 13 patients disease progressed. The major sites of metastases were the lung in 23 patients, followed by the lymph nodes in 4 patients and the brain in 3 patients.

Overall, the drug was well tolerated. A toxicity of WHO grade III due to leukopenia was seen in 5.3% of infusions as well as <8% grade III toxicity in liver function tests. No grade IV changes in laboratory parameters occurred.

Table 1. Response of xenografts derived from SN12C or ACHN cells during therapy (0–16 weeks) and during follow-up (17–32 weeks)

Cells	0–16 weeks		17–32 weeks	
	PD	SD	PD	R + SD
SN12C				
CO (n = 15)	15	–	15	–
GEM (n = 11)	11	–	11	–
ACHN				
CO (n = 15)	15	–	15	–
GEM (n = 15)	13	2	8	7 (3 × CR, 1 × PR, 3 × SD)

CO = Untreated animals; GEM = gemcitabine-treated animals; PD = progressive disease; SD = stable disease; R = objective response.

Clinical toxicity in all courses was mild. As expected nausea and vomiting were the most common adverse events, leaving only 38.5% of patients unaffected. Other frequently reported adverse events included fever (35.9%), flu-like syndrome (17.9%) and skin rash (17.9%). Grade III toxicity was observed in 2 patients, 1 with dyspnea and 1 with myocardial infarction.

Discussion

Due to encouraging preclinical data indicating that gemcitabine is a potent cytostatic drug in vitro as well as in vivo [6, 16–18], clinical studies in patients with advanced colon cancer [19], leukemic diseases [20], squamous cell carcinoma of the head and neck [21], small cell [22] and non-small cell lung cancer [23, 24], breast carcinoma [25], ovarian cancer [26], pancreatic cancer [27], bladder cancer [28], gastric cancer or in patients with advanced malignant melanoma [29, 30] have already been initiated with different success.

The present study supplies comprehensive in vitro, in vivo and clinical data on human RCC. The data obtained in vitro present gemcitabine as a highly effective drug against RCC cell lines at concentrations that were much lower than peak concentrations (dFdC: 50–150 μM = 12.5–37.5 µg/ml; data from Lilly) or even steady state dFdC levels (15–43.8 μM) that could be achieved in human plasma [20, 31]. The relative resistance of SN12C

Table 2. Characteristics of eligible patients (n = 37)

Characteristics	Patients	
	n	%
Males	29	
Females	8	
Performance status		
0	15	40.5
1	19	51.4
2	2	5.4
3	1	2.7
Metastatic sites		
Lung	30	81.1
Lymph node	15	40.5
Bone	7	18.9
Liver	4	10.8
Kidney	5	13.5
Other	15	40.5
Number of sites (n = 39)		
1 ×	20	51.2
2 ×	10	25.6
3 ×	7	17.9
>3 ×	2	5.1
Prior therapy		
Surgery	34	91.9
Radiotherapy	5	13.5
Immunotherapy	20	54.1

The mean age of the patients was 56.62 ± 9.31 years (range 38–74).

Table 3. Best tumor responses of eligible patients treated with gemcitabine

Tumor response	Eligible patients (n = 37)	
	n	%
CR	1	2.7
PR	2	5.4
Stable disease	18	48.6
Progressive disease	13	35.1
Not applicable	3	8.1
Objective response (CR + PR)	3	8.1

cells to gemcitabine in vitro compared to ACHN cells was confirmed in vivo by the fact that SN12C xenografts did not respond to a treatment with 40 mg/kg i.p. gemcitabine. Nevertheless, other groups have shown that nude mice tolerate much higher dosages [17], thus the dose of gemcitabine used in our study might have been to low for SN12C xenografts.

There have been two previous reports of the clinical applications of gemcitabine in RCC before. In a phase I study, the only patient with RCC attained a PR after administration of low-dose gemcitabine (65 mg/m^2) twice a week for 3 weeks [32]. In addition, 18 patients with histologically proven metastatic or locally recurrent RCC were treated in a phase II trial with gemcitabine (800 mg/m^2) on days 1, 8 and 15 of a 28-day cycle. One PR was observed, resulting in an overall response rate of 6% [33]. The overall response rate of 8.1% found in our study for gemcitabine monotherapy indicates that this regimen cannot be considered more active than other chemotherapy approaches in the treatment of metastatic RCC. Although it is not clear whether a dose-response relationship exists for gemcitabine in any tumor entity, it is too early to consider gemcitabine as clinically ineffective, since a 50% higher dose can be tolerated [28].

In conclusion, the present preclinical data confirm gemcitabine as an active chemotherapeutic drug against human RCC cells in vitro and in vivo. Moreover, dFdC does show some minor activity for patients with advanced or metastatic RCC. The hypothesis that higher doses of gemcitabine are more likely to induce an increased antitumor response needs to be clarified. Therefore, future clinical trials are still required. Moreover, besides an estimated benefit by increased dosages, a schedule containing cytokines may lead to an improved antineoplastic response by activated immunologic cascades for cytokines. This prompted further gemcitabine experiments which indicated an additive (or synergistic) effect of sequentially applied interferon-α [34].

Acknowledgements

Experiments were supported by Lilly Deutschland.

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