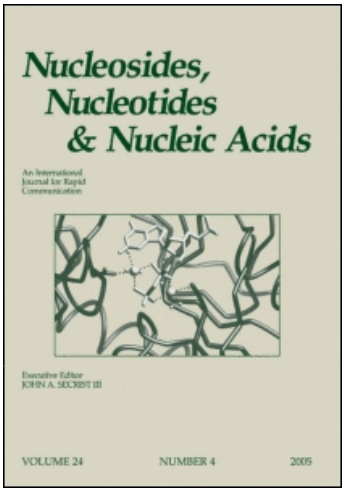

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Antiproliferative Activity and Mechanism of Action of Fatty Acid Derivatives of Gemcitabine in Leukemia and Solid Tumor Cell Lines and in Human Xenografts

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ABSTRACT

Gemcitabine is a deoxycytidine analog, which can be inactivated by deamination catalyzed by deoxycytidine deaminase (dCDA). Altered transport over the cell membrane is a mechanism of resistance to gemcitabine. To facilitate accumulation, the fatty acid derivative CP-4125 was synthesized. Since, the fatty acid is acylated at the site of action of dCDA, a decreased deamination was expected. CP-4125 was equally active as gemcitabine in a panel of rodent and human cell lines and in human melanoma xenografts bearing mice. In contrast to gemcitabine, CP-4125 was not deaminated but inhibited deamination of deoxycytidine and gemcitabine. Pools of the active triphosphate of gemcitabine increased for over 20 hr after CP-4125 exposure,

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while these pools decreased directly after removal of gemcitabine. In conclusion: CP-4125 is an interesting new gemcitabine derivative.

Key Words: Gemcitabine; Fatty acid derivatives; Antiproliferative activity; Leukemia; Solid tumor; Human xenografts.

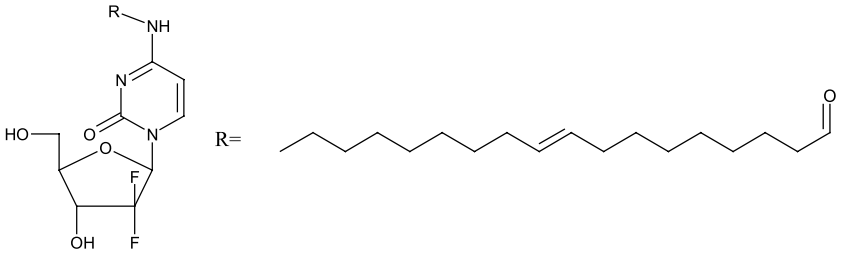
INTRODUCTION

Gemcitabine (2′,2′-difluorodeoxycytidine, dFdC) is a deoxycytidine analog, which is active against several solid tumors, including Non-Small Cell Lung Cancer (NSCLC) and pancreatic carcinoma.[1,2] Gemcitabine requires phosphorylation by deoxycytidine kinase in order to be active, and can be inactivated by deamination by deoxycytidine deaminase (dCDA) to 2′,2′-difluorodeoxyuridine (dFdU).[3] In its active form, 2′,2′-difluorodeoxycytidine triphosphate (dFdCTP), can be incorporated into DNA and is a potent inhibitor of DNA-synthesis by inhibition of DNA polymerase.[4] Like other nucleoside analogs gemcitabine is hydrophilic and requires specialized nucleoside transporters to traverse cell membranes.[5] Altered membrane transport of deoxyribonucleosides over the cell membrane is a mechanism of drug resistance for gemcitabine. In order to facilitate the accumulation and increase retention of gemcitabine, a fatty acid ester derivative of gemcitabine was synthesized (Fig. 1). In CP-4125 an elaidic fatty acid group was acylated on the 4-amino group (CP-4125), which might reduce deamination.

MATERIALS AND METHODS

The compounds were tested in four cell lines; the murine leukemia L5, the rat leukemia BCLO, the human ovarian cancer A2780 and the murine colon C26-A cell line. The leukemia cells were grown in suspension, while the sold tumor cells were grown as monolayers.

Growth inhibition tests: cells were plated in 96-wells plates and exposed for 72 hr to the drugs. Cell growth of the solid tumor cell lines was evaluated by the sulforhodamine B (SRB) assay and growth inhibition was expressed as the 50% growth



CP-4125 Elaidic acid; 18 carbon, 1 double bond

Figure 1. Chemical structure of the fatty acid derivative.

Table 1. IC50 values of gemcitabine and its derivatives.

	Leukemia cells		Solid tumor cells	
Compound	L5	BCLO	C26-A	A2780
Gemcitabine	1.9 ± 8.3	8.3 ± 0.9	16 ± 4	1.5 ± 0.6
CP-4125	5.3 ± 2.1	2.1 ± 1.4	20 ± 4	5 ± 2.7

Values are Means ± SEM.

inhibiting concentration (IC50).[6] For the leukemic lines we used cell counting to evaluate cytotoxicity.

Efficacy of the drugs in vivo was tested in female BALB/c nude (nu/nu) mice bearing EKVX a NSCLC and THX a malignant melanoma human xenografts. Gemcitabine and CP-4125 were administered intra peritoneal every third day, for five times, at their Maximal Tolerated Dose (CP-4125 10 mg/kg, Gemcitabine 120 mg/kg) and tumor size was measured twice weekly.[7]

By incubation of 100 µl of a purified dCDA solution with 500 µM dCyd or gemcitabine as a substrate and 500 µM of gemcitabine or CP-4125, inhibition of deamination by the latter agents was tested.[8]

Accumulation and retention of dFdCTP pools was tested after 4 hr exposure to gemcitabine. dFdCTP pools were measured by HPLC.[8]

RESULTS

CP-4125 was less effective than gemcitabine in L5 cells, but more effective in BCLO cells (Table 1). No significant difference in efficacy between gemcitabine and its derivative was found in the solid tumor cells.

The higher T/C and lower GDF indicate a lower efficacy of CP-4125 than gemcitabine in EKVX bearing mice (Table 2). In MHMX efficacy of CP-4125 and gemcitabine was comparable.

Table 2. Efficacy of gemcitabine and its derivatives in nude mice bearing human xenografts.

		Anti-tumor effect	
Tumor	Compound	T/C	GDF
EKVX	Gemcitabine	3.4	3.4
	CP-4125	26	1.6
MHMX	Gemcitabine	2	> 7.2
	CP-4125	3.3	> 6.9

T/C: volume treated tumor/volume control tumor, GDF: Growth Delay Factor: (Tumor Doubling time $(TD)_{treated} - TD_{control})/TD_{control}$.

Table 3. Effect of gemcitabine and its derivatives on deamination.

Substrate (500 μM)	Test compound (500 μM)	Average product (pmol/hr/μl enzyme)	%	p
dCyd	–	312 ± 5	100.0	–
	Gemcitabine	n.d.	< 1	< 0.001
	CP-4125	167 ± 5	53.4	< 0.01
Gemcitabine	–	924 ± 56	100.0	–
	CP-4125	422 ± 11	45.7	< 0.01

Product is deoxyuridine for dCyd and dFdU for gemcitabine. Values are Means ± SEM.

Gemcitabine inhibited deamination of dCyd completely, but the deaminated product of gemcitabine 2′,2′-difluorodeoxyuridine (dFdU) was found (Table 3). CP-4125 inhibited deamination of dCyd and gemcitabine equally effective.

dFdCTP pools accumulated after gemcitabine exposure, decreased slowly in time. There was a clear dose relationship (Fig. 2). However, a less clear dose relationship was found after CP-4125 exposure, but dFdCTP pools increased even 20 hr after removal of the drug.

DISCUSSION

In this paper we describe the cytotoxic activity and mechanism of action of the gemcitabine derivative CP-4125. Since, the 4-amino position is the side of action of dCDA it was expected that CP-4125 was more toxic compound. However, no difference in cytotoxicity between CP-4125 and gemcitabine was found both in vitro and in vivo. Since gemcitabine is a better substrate for dCDA it inhibited deamination of dCyd completely and dFdU was formed, indicating competition for the enzyme.

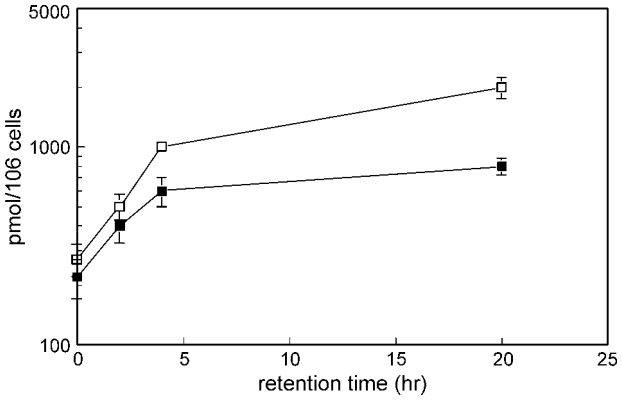


Figure 2. Retention of dFdCTP pools in C26-A cells after 4 hr exposure to 10 μM (■) and 100 μM (□) CP-4125. The values represent means ± SEM.

However, no dFdU was formed with CP-4125, which might be related to reduced deamination by the position of the fatty acid. Although theoretically inhibition of deamination of gemcitabine might result in higher drug levels and higher cytotoxicity, the role of dCDA in the development of resistance is not clear.[9] Accumulation of dFdCTP pools after CP-4125 exposure increased for at least 20 hr, which can most likely be attributed to the fatty acid derivative and might be an advantage in efficacy of the drug. In conclusion, CP-4125 is an interesting gemcitabine derivative by inhibition of degradation of gemcitabine and long retention of DNA damage.

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