N^4-octadecyl-1-β-D-arabinofuranosyl cytosine

(NOAC, Alkasar-18)

Investigator's brochure

Prepared by
Prof. Dr. R.A. Schwendener

June, 2000

TABLE OF CONTENTS

SUMMARY

N^4-octadecyl-1-β-D-arabinofuranosyl cytosine (N^4-octadecyl-ara-C, NOAC, Alkasar-18) is a cytotoxic derivative of cytarabine (cytosine arabinoside, ara-C). Among a series of N^4-alkyl derivatives with alkyl chain lengths of C_{12} to C_{22} , NOAC was found to have the strongest cytotoxic effects in murine L1210 leukemia and various xenografted human solid tumors after intravenous and intraperitoneal application. The oral antitumor activity of NOAC was demonstrated in the L1210 tumor model as well as in the human tumor xenograft CCRF-CEM and acute lymphatic leukemia (ALL).

Due to the lipophilic alkyl chain which is attached at the N^4-position of the cytosine moiety of cytosine arabinoside, the derivative NOAC is highly resistant to deamination and as a consequence cellular pharmacology, pharmacokinetics and mechanisms of action of NOAC are significantly different from those of ara-C.

The chemical stability of NOAC is excellent. At strong acidic and basic aqueous conditions and high temperatures, NOAC degradation is very slow. At acidic conditions (pH 3.15, 70°C) the main degradation product is arabinosyl uracil (ara-U). For parenteral applications NOAC is incorporated into the lipid membranes of small unilamellar liposomes of approx. 100 nm mean diameter which can be stored as a stable lyophilized dry product.

After intravenous application, NOAC is predominantly bound to plasma proteins (HDL and LDL, albumin) and to erythrocyte membranes. Pharmacokinetics in mice revealed a fast distribution half-time ($t_{1/2(\alpha)}$) of 5-15 min and a long lasting elimination half-time ($t_{1/2(\beta)}$) of ± 20 hours. In mice, NOAC is excreted in the feces in its unaltered form and as hydroxylated and sulfated metabolites, whereas in urine ara-C and ara-U were found.

The dose of acute toxicity determined in healthy mice after a single i.p. application is 524 mg NOAC/kg. The therapeutic index (MTD/minimal therapeutic dose) in mice is approximately 16. The maximal tolerated dose (MTD) in tumor bearing nude mice is 150 mg/kg given every 3 days in 4 injections.

Cellular pharmacology and cytotoxicity data give evidence that NOAC is not exclusively a prodrug of ara-C and that other, presently unknown mechanisms of action are involved in the cytotoxic action of the drug. Experimental data show that NOAC is active in ara-C resistant tumor cells and due to its lipophilic properties the drug was also found to be active in multidrug resistant (e.g. doxorubicin resistant) cells

These unique properties of NOAC make this new compound a very promising candidate of a parenterally as well as orally active drug with significant cytostatic effects in leukemias and in solid tumors.

1 INTRODUCTION

N^4-octadecyl-1-β-D-arabinofuranosyl cytosine (N^4-octadecyl-ara-C, NOAC, Alkasar-18) was selected from a series of N^4-alkyl derivatives of cytarabine (ara-C), due to its strongest cytotoxic effects in murine L1210 leukemia and various xenografted human solid tumors after intravenous and intraperitoneal application (Refs. 1-5)

1.1. Advantages and limitations of cytarabine

Cytarabine (ara-C) is a well-established anticancer agent with high activity in acute myelotic and lymphoblastic leukemia (AML, ALL). It is included in standard combination therapy protocols resulting in cure rates of approximately 5 - 15 % in AML and 30 - 70 % in ALL. Cytarabine is administered at 100 - 200 mg/m^2 per day for 5 - 7 days or in high dose protocols with 1 - 3 g/m^2 per day for 3 days. Cytarabine shows also activity in non-Hodgkin and in Hodgkin lymphomas. In solid tumors the drug is inactive or it has not been adequately tested.

Cytarabine side effects include myelosuppression, gastrointestinal toxicity, alopecia and at high doses CNS dysfunction and severe conjunctivitis. It is being administered intravenously and subcutaneously. It is not active by the oral route.

Cytarabine acts as an analog of the physiologic nucleoside deoxycytidine and has multiple effects on DNA synthesis. It inhibits DNA polymerase-α, is incorporated into DNA, and terminates DNA chain elongation.

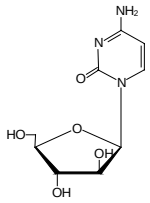
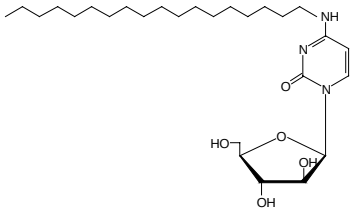
In order to exert its cytotoxic activity, cytarabine has to be activated intracellularly to its triphosphate derivative. Degradation to inactive arabinosyluracil (ara-U) occurs by the action of cytidine deaminase which is present in plasma, liver, intestinal mucosa and in granulocytes. 70 - 80 % of a given dose of cytarabine are excreted as ara-U. Cytarabine has a plasma elimination half-life of $T_{1/2(\alpha)}$ = 7 - 20 min and $T_{1/2(\beta)}$ = 2 h.

1.2. Advantages and potential of the present novel compound, NOAC

New analogs of cytarabine have long been searched for in order to improve the properties of cytarabine or to avoid its drawbacks and limitations in its clinical use. Some of the desirable properties of a new cytarabine analog include: altered metabolism, resulting in a longer plasma elimination half-life and in higher tissue concentrations, cytostatic activity in solid tumors, higher activity and/or a different toxicity spectrum in leukemias, low non-hematologic toxicity and low toxicity to hematopoietic stem cells, new, unknown mechanisms of action, no requirement for intracellular activation and possible circumvention of multidrug resistance. A significant oral availability and a potential for dermal applications would open new and broad perspectives of use.

The present compound NOAC shows many of these desirable properties, as can be seen from the following comparative table (Table 1). NOAC appears as much more than a prodrug or a structural analog ("me-too") of cytarabine. The spectrum of properties and activities render NOAC a promising new cytostatic drug for the treatment of leukemias as well as solid tumors.

1.3 **Table 1.** Comparative properties of cytarabine and NOAC

	Cytarabine	**NOAC**
Molecular structure		
Chemical name	1-β-D-arabinofuranosyl cytosine	N^4-octadecyl-1-β-D-arabino-afuranosyl cytosine
International non-proprietary name (INN)	Cytarabine (rec. INN)	----
CAS Registry Number		
Molecular weight	243.2	495.7
Water solubility at 25°C	0.1 g/mL	<0.05 mg/mL
Presentation for clinical use	Freeze dried 200- or 500 mg ampoule	Lyophilized liposomal formulation (500 or 1000 mg)
Spectrum of activity in experimental models *in vitro*	Human and murine leukemias	Leukemias Solid tumors (prostate, lung, breast)
Spectrum of activity in experimental models *in vivo*	Leukemias (murine, human)	Leukemias Solid tumors (prostate, lung, breast)
Pharmacokinetics in mouse	Terminal elimination half-life (β): $t_{½}$ = 21 min	Terminal elimination half-life (β): $t_{½}$ = 7 h
Therapeutic index in mouse (MTD/Min. effective dose)	?	16 (L1210 leukemia model)
Human pharmacokinetics	Terminal plasma elimination half-life: $t_{½}$ = < 30 min	Terminal plasma elimination half-life: $t_{½}$ = 11-16 h
Clinical efficacy	AML, ALL, CML	? leukemias ? lymphomas ? ? solid tumors ? MDR-tumors ?

1. 4 Advantages of NOAC over other lipophilic Derivatives of Ara-C

NOAC has several advantages compared to cytarabine ocfosfate (ara-CMP-stearyl ester, YNK 01; Nippon Kayaku and ASTA Medica) which is an orally active prodrug of ara-C and the N^4-acylated prodrug behenoyl-ara-C (BH-AC, Asahi). NOAC is both orally and parenterally active, whereas cytarabine ocfosfate cannot be applied parenterally due to its strong hematolytic toxicity. Furthermore, NOAC is active against solid and drug resistant tumors, whereas cytarabine ocfosfate and BH-AC are classical prodrugs of ara-C with an activity spectrum not significantly different from the parent drug ara-C.

1.5 Patent and Licenses

Patent Priority:	US Patent 5'641'758 (24.06.1997); PCT-EP 92-00706
Licensee Company:	open
Consultants:	open
WHO ATC:	L01B (Antineoplastic)
EphMRA ATC:	L1B (Antimetabolites)

2 CHEMICAL, PHYSICAL AND ANALYTICAL DATA

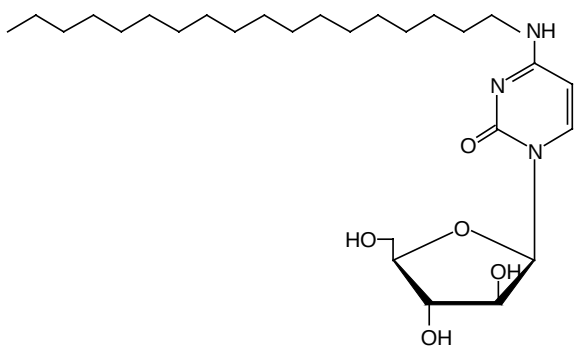
2.1 Chemical and physical properties (Refs.1-5)

2.1.1 Chemical name
1-(β-D-arabinofuranosyl)-4-octadecylamino-2(1H)-pyrimidinone

2.1.2 International Non-proprietary Name
Not yet available

2.1.3 Abbreviations and other names
N^4-octadecyl-ara-C, NOAC, Alkasar 18

2.1.4 Structural formula



2.1.5 Molecular formula

$C_{27}H_{49}N_3O_5$

2.1.6 Molecular weight

495.7

2.1.7 Physical appearance

White crystalline powder

2.1.8 Melting point

160-61° C

2.1.9 Spectroscopic data

NOAC was analyzed by the following spectroscopic methods: H^1 NMR, C^{13} NMR, C^{13} DEPT NMR, IR, MS. Standard operating procedures (SOP) of the analysis of NOAC are available. The spectroscopic data are in agreement with the chemical structure of NOAC.

UV spectroscopic data (methanol):

λ_{max} at 208, 240 and 274 nm

E_{240} = 9'813 $mol^{-1}dm^3cm^{-1}$, E_{274} = 12'848 $mol^{-1}dm^3cm^{-1}$

2.1.10 Chemical synthesis

NOAC is prepared from octadecylamine and 4-(1,2,4-triazol-1-yl)-1-β-D-2',3',5'-tri-O-acetylarabinofuranosyl) pyrimidine-2(1H)-one in dioxane. Yields are > 95% (Refs. 1-3).

Purity by HPLC > 97.5 %. Solvent residues (methanol, chloroform) are < 0.3%.

2.1.11 Solubility

DMSO, > 50 mg/ml	Cremophor EL, < 1 mg/ml
Pyridine, > 50 mg/ml	Cremophor EL/EtOH (1:1,v/v),<3 mg/ml
CH_2Cl_2/MeOH (1:1 v/v), < 20 mg/ml	Soybean oil, < 1 mg/ml

Ethanol, < 3 mg/ml	NaCl 0.9%, < 0.05 mg/ml
Chloroform, < 1 mg/ml	Phosphate buffer < 0.05 mg/ml
Methanol, < 1 mg/ml	

2.1.12 Chemical stability of bulk NOAC

NOAC is not volatile, hygroscopic or light and oxidation sensitive. The long term, temperature, humidity and light dependent stability of NOAC in its crystalline form is excellent and in the course of assessment.

3 PHARMACEUTICAL FORMULATION AND STABILITY

3.1 Parenteral application form

Due to its lipophilic properties, NOAC is formulated in liposomes. Depending on batch size, the NOAC-liposome formulations are prepared by high pressure filter extrusion, detergent dialysis, or microfluidization

For preclinical investigations NOAC is formulated as a lyophilized preparation of small unilamellar liposomes of 100 nm average size (Ref. 6).

An example for the formulation of 1.0 ml liposome suspension prepared by filter extrusion, microfluidization or detergent dialysis (4) is given below

Soy phosphatidylcholine (SPC)	5.00	g	(6.5×10^{-3} mol)
Cholesterol	0.50	g	(1.3×10^{-3} mol)
α-Tocopherol	0.03	g	(6.5×10^{-5} mol)
NOAC	0.330	g	(0.66×10^{-3} mol)

in buffered medium containing cryoprotectants (e.g. 180 mM sucrose + 30 mM phosphate buffer, pH 7.4).

The NOAC concentration per milliliter can be varied from 1 mg/ml to about 10 mg/ml, depending on the concentration of phospholipid (SPC) per ml and the method of liposome preparation. Reconstituted liposomes retain their size and homogeneity longer than 72 h after reconstitution. NOAC remains chemically stable during lyophilization and reconstitution. Liposome preparations are ready for parenteral use within less than 30 minutes after reconstitution.

3.2 Chemical stability of liposomal NOAC in aqueous media (Ref. 7)

3.2.1 Effect of acidic pH

Liposomal NOAC decays at 70° C and pH 3.12 with a half-time of approx. 20 h under decomposition to an unstable intermediate product and to arabinosyl uracil (ara-U). In comparison, cytarabine has a decay half-time of 20 min under identical conditions (Ref. 8). Thus, at 26°C and 37°C NOAC is extremely stable against dealkylation and deamination.

3.2.2 Effect of basic pH

NOAC stability in strong basic medium is excellent. At 26° C and 37° C no degradation products were found, whereas ara-U formation at 70° C / pH 13 is less than 2%.

3.3 Oral and dermal application forms

In view of the excellent oral anti-tumor activity of NOAC (see below), the development of an oral application form (e.g. micronized NOAC in a capsule) is being planned. Formulations for cutaneous applications are also envisaged.

4. ANALYTICAL METHODS

4.1 Thin layer chromatography (TLC) (Refs. 2,3)

TLC of alkylated ara-C derivatives is done on silicagel plates:

Mobile phase A: chloroform/methanol/25% ammonia = 8 : 2 : 0 (v/v)
Mobile phase B: chloroform/methanol/25% ammonia = 1 : 1 : 0.3 (v/v)
Mobile phase C: chloroform/methanol/1-chlorobutane = 8 : 3 : 1 (v/v)
Mobile phase D: tetrahydrofurane/ethyl acetate/ water = 9 : 3 : 0.5 (v/v)

Detection: Fluorescence quenching under UV 254 nm, dark spots
Spraying with 2,7-dichlorofluorescein: bright orange spots at 366 nm. (see also Refs. 2,3)

R_f-values: A: R_f = 0.57; B: R_f = 0.88; C: R_f = 0.48; D: R_f = 0.39

4.2 High pressure liquid chromatography (HPLC) (Ref. 9)

4.2.1 For bulk drug and liposomal formulations

a) Under isocratic conditions with a RP-C_{18} column:
25 x 0.4 cm) with methanol/water = 93 : 7 v/v% as mobile phase.
Flow rate 0.6 ml/min. Detection at 275 nm.
Retention times: 8.4 min, N^4-hexadecyl-ara-C (= internal standard)
11.3 min, N^4-octadecyl-ara-C
5.1 min, ara-C

b) Under isocratic conditions with a Zorbax C_8 column (5 µm, 150 x 4.6 mm); mobile phase 80% acetonitrile in 0.5% acetic acid, 10 mM hexane sulfonic acid. Flow rate 1 ml/min. Detection UV at 225 nm.
Retention time: 9.6 min

4.2.2 HPLC analysis for biological samples

Columns: 2 x RP-18 (25 x 0.4 cm), first column at RT, second column at 45° C
Mobile phase: methanol/ ammonium formiate 0.16 M, pH 2.7, 9 : 1 v/v parts.
Detection: UV 275 nm
Retention times: 12-13 min, N^4-hexadecyl-ara-C (= internal standard)
19-24 min, N^4-octadecyl-ara-C

4.2.3 Plasma extraction method

1ml plasma is incubated together with 0.1 ml of 0.05 mM N^4-hexadecyl-arabinoside (= internal standard) and 1 ml of 10M urea during 15-18 hours (over night) on a horizontal shaker at room temperature. Solid phase extraction is done on Bondelut C_{18} columns (3 ml). After sample application, the columns are washed with 1 ml water, 0.5 ml of 33% methanol and 3 ml methanol. The methanol phase is concentrated and the residue dissolved in mobile phase.

5 EXPERIMENTAL PHARMACOLOGY

5.1 Anti-tumor activity

5.1.1 *In vitro* anti-tumor activity

5.1.1.1 National Cancer Institute Developmental Therapeutics Program (DTP) (Ref. 10)

NOAC (NSC 685096) was tested in the NCI in vitro testing program against a standard panel of 56 human tumor cell lines. In Table 2 the growth inhibitory concentrations (GI_{50}) of the tumors with high NOAC activity are summarized. NOAC was also included in the COMPARE analysis (Table 3). The COMPARE algorithm allows the comparison of the cytotoxic activity of NOAC in the standard tumor cell line panel with the data of the entire NCI drug database. The COMPARE analysis with NOAC as “seed” compound can be run online on the internet.

The internet address of the National Cancer Institute DTP is: http://dtp.nci.nih.gov The in vitro test results and the COMPARE analysis of NOAC can be run by entering the NSC number which is 685096.

Table 3 shows the paired correlation coefficients obtained of a COMPARE analysis where NOAC was compared with the standard NCI drug database. Hydroxyurea with a coefficient of 0.825 in the TGI (tumor growth inhibition values) analysis is the only compound to which NOAC has a certain similarity in the growth inhibitory responses to the 56 tumor cell lines included in the test. The low values of ara-C (c= 0.501) and of ara-AC (Fazarabine) (c= 0.541), the chemically most related compounds to NOAC, allow to assume that NOAC does not share the mechanisms of action of ara-C or Fazarabine.

Table 2 In vitro growth inhibitory (GI50) concentrations of NOAC

Cell line	GI_{50} (μM)		Cell line	GI_{50} (μM)	
	NOAC	ara-C		NOAC	ara-C
Leukemias:			CNS†:		
CCRF-CEM	5.4	0.025	SNB-19	25	8
HL-60	2.5	0.94	SNB-75	30	>100
MOLT-4	4.6	0.015	U251	37	6.5
RPMI-8226	34	>100			
SR	23	0.04	Melanoma:		
			LOX IMVI	63	0.21
NSCL*:			M14	55	0.4
NCI-H522	20	42			
A549/ATTC	80	0.16	Ovarian:		
			IGROV1	15	23
Colon:					
Colo 205	38	55	Renal:		
HCC-2998	50	5	RXF 393	7.6	2.4
HT-29	95	29			
KM12	84	>100	Breast:		
			MCF7	57	4.5
Prostate:			HS 578T	29	>100
PC-3	2.45	1.2			

*; NSCL, non-small cell lung cancer

†; CNS, central nervous system cancer

Table 3 COMPARE correlation coefficients with NOAC as seed compound

Drug	**COMPARE GI_{50}**	**Drug**	**COMPARE TGI**
NOAC (NSC 685096)	1.000	**NOAC** (NSC 685096)	1.000
Flourodopan	0.514	Hydroxyurea	0.825
Melphalan	0.464	Ara-AC (Fazarabine[1])	0.541
BCNU	0.455	Ara-C	0.501
Chlorambucil	0.432	Fluorodopan	0.495
Hydroxyurea	0.429	BCNU	0.439
Rifamycin SV	0.421	Melphalan	0.427
Carmethizole	0.414	5-HP[2]	0.410
Ara-C	0.287		
5-Azadeoxycytidine	0.223		
Azacytidine	0.100		
Ara-AC	0.098		
Gemcitabine	0.050		

1: Ara-AC = 1-β-D-arabinofuranosyl-5-azacytosine, Fazarabine

2: 5-HP = 5-hydroxypyridine-2-carboxaldehyde semicarbazone

5.1.1.2 In vitro cytotoxicity

In vitro, NOAC cytotoxicity was tested on a panel of human tumor cell lines using the MTT dye reduction assay. The IC_{50} values for NOAC and those for cytarabine as comparison are summarized in Table 4 :

Table 4. In vitro cytotoxicity of NOAC in comparison to cytarabine (Refs. 4,5,11)

Tumor cells	**IC_{50} NOAC (μM)**	**IC_{50} Cytarabine (μM)**
HL-60	8	> 200
HL-60/ara-C resistant	16.5	> 400
U937	< 100	> 200
K562	< 200	> 400
CCRF-CEM	50	< 1
CCRF-CEM (dCK^-)[1]	< 140	> 550

[1] Deoxycytidine kinase (dCK^-) deficient cell line

5.1.1.3 Inhibitory activity of NOAC against freshly biopsized human tumors in vitro

NOAC was tested in vitro against a panel of fresh human tumor specimens in a colony forming assay using a long- and short- term exposure schedules at the Technical University Munich, Division of Hematology/Oncology, Klinikum Rechts der Isar, (Prof. A.-R. Hanauske, Ref. 12).

At 10 and 100 μM NOAC mamma, lung, ovary carcinomas and non Hodgkin lymphomas were highly responding, whereas melanomas and kidney carcinomas were not inhibited.

Importantly, the comparison of the cytotoxic activity of NOAC with commonly used clinical antitumor agents showed that NOAC was active against tumor cells that were resistant to cisplatin, doxorubicin, 5-fluorouracil, gemcitabine, mitomycin C and etoposide.

5.1.2 ***In vivo*** parenteral and oral anti-tumor activity

5.1.2.1 L1210 murine leukemia (Ref. 3)

1. After intravenous (iv) application

Table 5: Intravenous treatment of L1210 leukemia in BDF1 (female) mice after i.v. implantation of L1210 cells. Drug treatment on days 2 and 6 after tumor cell injection[1].

Preparation	Total Dose		Survival Time (days)		T/C[2]	Survivors
	μmol/kg	mg/kg	Range	Mean ± SD	%	60 Days
NOAC	100	50	> 60	> 60	857	6/6
	50	25	13 - > 60	52.1 ± 19.2	744	5/6
Cytarabine, PB[3]	200	49	10 - > 60	19.8 ± 19.7	282	1/6
Controls	--	--	7.0	7.0	100	0/6

[1] On day 0, 10^5 L1210 cells were injected intravenously into BDF1 mice.
[2] Increase of life span T/C% calculated including 60d survivors. SD; standard deviation.
[3] PB; phosphate buffer (67 mM, pH 7.4)

2. After **oral** drug application (Ref. 13)

Table 6: Cytostatic effect of NOAC in liposomes after ORAL therapy on days 1 - 5 after intravenous L1210 inoculation[1].

Preparation	Total Dose		Survival Time (days)		T/C[2]	Survivors
	mmol/kg	mg/kg	Range	Mean ± SD	%	60 Days
NOAC	1	496	23 - > 60	54 ± 15	673	5/6
	2	992	31 - > 60	55 ± 12	689	
Cytarabine, PB[3]	2	486	10 - 16	12 ± 2.1	172	0/6
	4	972	12 - 16	14 ± 1.5	193	0/6
Controls	-	-	7.2 ± 0.4		100	0/6

1: On day 0, 10^5 L1210 cells were injected intravenously into BDF1 mice.
2: Increase of life span T/C% calculated including 60d survivors. SD; standard deviation.

5.1.3. Human tumor xenografts in nude mice (Ref. 5)

5.1.3.1 Intraperitoneal application

NOAC was studied in 2 human leukemias growing as subcutaneous tumors as well as in 6 solid tumor xenografts in part in comparison with cytarabine.
The results are shown in Tables 8 and 9.

5.1.3.2 Toxicity

The maximal tolerated dose (MTD) levels in tumor bearing nude mice are shown in Table 8 The MTD of NOAC was 150 mg/kg/day, which resulted in a lethality of 14 % (7/49). NOAC was slightly more toxic than cytarabine for which the MTD was between 300 - 500 mg/kg/day.

Table 7: Toxicity of NOAC and cytarabine in tumor bearing nude mice

Compound	Daily Dose[1] mg/kg	Schedule days	Lethality/Total[1]	Lethality (%)
NOAC	200	1,4,7,10	4/7	57
	150	1,4,7,10	7/49	14
	100	1,4,7,10	2/30	7
	50	1,4,7,10	2/21	10
Cytarabine	500	1,4,7,10	3/14	21
	300	1,4,7,10	3/25	12
	200	1,4,7,10	2/10	20
	50-100	1,4,7,10	0/10	0

[1] Lethality recorded until 10 days after last therapy

5.1.3.3 Antitumor activity

Table 8: In vivo activity of NOAC in human tumor xenografts growing subcutaneously in the nude mouse1 (Ref. 5)

Tumors		Type	Growth inhib. (%) NOAC[2]	ara-C[3]
Leukemias				
Leukemia	HL-60	Acute promyelocytic	97	48
Leukemia	CCRF-CEM[4]	Acute lymphatic	96	95
Solid tumors				
Mammary carcinoma	MAXF 401	Adenocarcinoma	85	58
Prostate carcinoma	PC3M	Adenocarcinoma	83	nd[5]
Lung carcinoma	LXFS 605	small cell	73	44
Lung carcinoma	LXFL 529	large cell	79	nd
Melanoma	MEXF 276	amelanotic	39	nd

[1]: Therapy: intraperitoneal (ip) on days 1, 4, 7 and 10 after randomization.
[2]: Tumor growth inhibition at maximal tolerable dose (MTD): 150 mg NOAC/kg/day.
[3]: At MTD 300 - 500 mg/kg/day (1,4,7,10) or 200 mg/kg/day (days 1-3 and 15-17)
[4]: Treatment on days 1 - 4, ip.; [5]: nd, not determined.

Acute leukemias: NOAC was tested in the human promyelocytic leukemia HL-60. NOAC was tested at 200, 100 and 50 mg/kg i.p. given on days 1,4,7,10. The dose of 200 mg/kg/day was toxic, 100 mg/kg/day resulted in a marked tumor inhibition, the tumor volume being less than 10% of the initial volume and 3% of the control after 17 days. The activity was confirmed in other experiments with a T/C of 4% (= 96 % growth inhibition). Cytarabine was less active, the tumors grew progressively. For cytarabine, the T/C was 52% at the highest dose of 300 mg/kg/day.
In the acute T-lymphocytic leukemia CCRF-CEM, a daily schedule for 4 days was studied. The 2 highest dosages (200 and 150 mg NOAC/kg/day) were toxic. CCRF-CEM is known to be sensitive against alkylating agents and vinca alkaloids.

NOAC was also active in a xenotransplanted ALL-SCID-3 leukemia after intraperitoneal and oral treatment (Ref. 11)

Solid tumor xenografts: NOAC was tested in 3 models known to be sensitive to standard agents (mammary cancer MAXF 401, small cell lung cancer LXFS 605 and large cell lung cancer LXFL 529) and 2 others known to be resistant (prostate cancer PC3M and melanoma MEXF 276). In MAXF 401 NOAC effected on days 7-14 a regression to 62-65% of the initial tumor volume. The control tumor volumes were 139-366 % of the initial volume. MAXF 401 is known to respond to alkylating agents only as standard agents.

In the small cell lung cancer model LXFS 605 NOAC was more active than cytarabine. However, this model is very sensitive against different classes of standard compounds. In the large cell lung cancer LXFL 529 NOAC produced a 21 % tumor inhibition, which is less than for other agents. In melanoma NOAC was ineffective (Table 8).

An unexpected activity of NOAC was found in the prostate model PC3M. In this tumor model, standard agents do not produce tumor regression. Treatment with NOAC resulted in a marked tumor inhibition with a T/C of 17% (= 83 % growth inhibition, Table 9). Among the standard agents, vindesin was the most active with a T/C of 23% followed by alkylating agents and adriamycin (Table 9). The activity of NOAC in this prostate model is promising since chemotherapy of prostate cancers is relatively ineffective.

5.1.3.4 Antitumor effects after oral drug application

In the CCRF-CEM leukemia model NOAC was administered also orally. The dose of 800 mg/kg/day given on days 1 - 4 was almost equally active as 100 mg/kg/day given intraperitoneally. The corresponding T/C values were 4.1 % for i.p. and 7.3 % for the oral application of NOAC. Body weight loss and lethality were slightly higher in the i.p. group. Overall, in the L1210 model (see Table 7) and in the CCRF-CEM leukemia comparable activities were found for oral and i.p. or i.v. application of NOAC.

Antitumor activity was also found after oral treatment in the ALL-SCID-3 model (see Ref. 11).

Table 9: Cytostatic activity of NOAC compared to standard antitumor agents against prostate cancer PC3M in nude mice

Compound	Daily Dose[1] mg/kg	Schedule days	Application	% Inhibition of Tumor Growth[2]
NOAC	150	1,4,7,10	i.p.	83
Vindesine	1.5	1,8,15	i.v.	77
Cyclophosphamide	200	1,15	i.p.	73
Ifosfamide	130	1-3, 15-17	i.p.	63
Cisplatin	6.4	1,15	s.c.	59
Adriamycin	8	1,15	i.v.	43
DTIC[3]	300	1,15	i.p.	29
HECNU[3]	10	1	i.v.	26
Orchidectomy	-	-	-	9

[1] at maximally tolerated dose (MTD); [2] Inhibition of tumor growth compared to untreated controls

[3] DTIC, dacarbazine; HECNU, nitrosourea derivative

5.1.3.5. Antitumor activity in cytarabine-resistant cells

NOAC is cytotoxic in cytarabine resistant HL-60 cells as well as in deoxycytidine kinase deficient cells (Table 4 and Ref. 11).

5.2. Cellular pharmacology (Refs. 4,11)

In a number of classical experimental systems, NOAC was shown to behave differently, as compared with cytarabine:

a) Uptake by tumor cells

Total uptake after incubation of NOAC with tumor cells is generally 5 - 10-fold higher than that of cytarabine. Furthermore, the uptake mechanism of NOAC was shown to be independent of the nucleoside transport mechanism involved for cytarabine and known to be sensitive to dipyridamole and deoxycytidine. NOAC uptake/cytotoxicity were not decreased in the presence of dipyridamole. Similarly, deoxycytidine concentrations known to inhibit cytarabine activity did not interfere with NOAC cytotoxicity.

b) Intracellular distribution

After fractionation of tumor cells (HL-60, K562, U937, L1210) into nuclei/membranes and cytoplasma, NOAC is distributed by more than 90% to the membrane fraction. In contrast, cytarabine is distributed by more than 95% to the cytoplasmic fraction.

c) Intracellular fate and metabolism

The intracellular half-life of NOAC is more than five times longer than that of cytarabine. In HL-60 cells, NOAC half-life was found to be >7 h, compared to 1.5 h for cytarabine.

In vitro formation of intracellular phosphorylated metabolites (e.g. cytarabine-triphosphate) of NOAC is significantly lower than from cytarabine. Correspondingly, DNA incorporation of NOAC is also reduced by factors of 50 – 120.

d) Activity on the cellular reproduction cycle

NOAC is less S-phase specific than cytarabine as determined by cytometric methods with HL-60 cells (Refs. 11).

e) Resistance to deamination

In vitro formation of ara-U by deamination in human plasma was shown to be more than 40 times lower than with cytarabine after 4 hours incubation of NOAC at 37° C. Cytarabine is deaminated by more than 80%, whereas only 2% of NOAC is found as ara-U. In mouse liver microsomes, deamination of NOAC is more than fivefold lower than that of cytarabine.

f) Other properties of possible relevance to the antitumor effect of NOAC

In HL-60 promyelocytic leukemia, NOAC induced apoptotic cell death at concentrations of 10-50 μM.

NOAC is not a substrate for deoxycytidine kinase (dCK), the key enzyme of ara-C phosphorylation, pointing to other mechanisms of action (Ref. 11).

5.3 Distribution to blood cells and plasma proteins (Ref. 14, 15)

In vivo, after intravenous injection of liposomal NOAC the drug is transferred within short time to erythrocyte membranes (30%), plasma proteins (<67%) and leukocytes (<2%). NOAC has no amphiphilic properties and has no hemolytic toxicity. *In vitro*, NOAC was also found to strongly bind to albumin, HDL and LDL.

In vitro, low density lipoprotein (LDL) mediated uptake and cytotoxic effects of NOAC were studied in Daudi lymphoma cells. NOAC was either incorporated into LDL or liposomes. Specific binding of NOAC-LDL to Daudi cells was 5 times higher than to human lymphocytes. LDL receptor binding could be blocked and up- or downregulated. In an in vitro cytotoxicity test the IC_{50} of NOAC-LDL was about 160 μM. Blocking the LDL receptors with empty LDL protected 50% of the cells from NOAC cytotoxicity. The natural affinity of NOAC for LDL provides an interesting rationale for the specific delivery of the drug to tumours that express elevated numbers of LDL receptors.

5.4. Mechanism of action

NOAC is a cytarabine derivative that is substantially protected from deamination and is taken up by tumor cells through a nucleoside transporter independent system. Due to its lipophilic properties, NOAC may be taken up by passive membrane diffusion and the drug is distributed at high concentrations in cell membranes. In the cell, phosphorylation of NOAC is very low, as is the DNA incorporation of NOAC metabolites. The fact that NOAC is not a substrate for deoxycytidine kinase, is highly cytotoxic in kinase-deficient and cytarabine-resistant cells, suggests that other, presently unknown, mechanisms of action are responsible for its excellent cytotoxic activity.

It is conceivable that due to its strong affinity to cell membranes NOAC could influence and/or perturb various signal transduction pathways, alter cell surface receptor confirmations, or influence the lateral diffusion of proteins in cell membranes.

5.5. Potential therapeutic profile

The present pharmacological data suggest that NOAC has a very high potential to retain its strong cytotoxic activity in tumor cells with low numbers of nucleoside transporting molecules (e.g. CML, CLL, lymphomas), with low kinase activities (cytarabine resistant leukemia) and possibly, due to its different cellular uptake and its lipophilic properties, also in multidrug resistant (MDR) tumor cells. Its antitumor activity was determined in a number of solid tumor lines, including tumor cells known to be resistant to most standard chemotherapeutic agents,

renders NOAC an interesting candidate for clinical use in such solid tumors (prostate cancer, breast and non-small cell lung cancer, melanoma and possibly CNS tumors (gliomas, etc.).

6 ANIMAL PHARMACOKINETICS (Refs. 7,16,17)

From NOAC pharmacokinetics in healthy mice (ICR, females; iv drug application), plasma elimination half-lives of $t_{1/2(\alpha)}$ = <10 min and $t_{1/2(\beta)}$ = 7-10 hours were obtained. 40-60% of the drug was found in the liver, with similar elimination kinetics as in plasma. A high proportion of NOAC bound to erythrocytes.

After oral administration in mice, bioavailability of the intact drug was 15-20%.

7 TOXICOLOGY AND METABOLISM

7.1 Acute toxicity after single intraperitoneal treatment (Ref. 13)

The acute toxicity of NOAC determined according to the OECD guideline 420 in healthy mice (ICR, 20 g, females and males) is 524 mg/kg. At this concentration, 50-60% of the treated animals die within 4 - 5 days. Weight loss 6 days after drug treatment is 15- 20%. The total MTD in tumor bearing nude mice is 600 mg/kg given ip on days 1,4,7 and 10 (see 5.1.3.2).

The therapeutic index (TI = MTD / minimal therapeutic dose) is approximately 16 when the MTD in healthy mice is compared with the minimal therapeutic dose determined in the L1210 model after i.v. therapy with NOAC (see Table 8).

7.2 Hematological toxicity (Ref. 13)

Hematological toxicity was studied in female ICR mice after a single i.p. application of 350 mg NOAC/kg, corresponding to 66% of the approximated LD_{50} dose. The effects on leucocytes (myelosuppression), platelets (thrombocytopenia) and erythrocytes were not pronounced.
NOAC treatment resulted in moderate, but rather long lasting (approx. 2 wk) leukopenia and thrombocytopenia. The erythrocyte count was not changed significantly.

Non-hematological side effects were reversible and were only observed at high doses of NOAC (>450 mg/kg) in the gastrointestinal tract, spleen and to a lesser extent liver. Hair loss was seen in <5% of cases.

7.3 Effect of NOAC on hematopoietic precursor cells (Ref. 12)

The cytotoxic effects of NOAC were tested in vitro on hematopoietic precursor cells after short- and long-time exposure. NOAC is in comparison to ara-C and doxorubicin *significantly less toxic by factors of > 100* to clusters and granulopoietic precursor cells.

7.4 Metabolism and excretion in mice (Ref. 16)

Metabolism and excretion of NOAC was compared to ara-C. Analysis by HPLC revealed that 48 h after i.v. application of the drugs 39% NOAC were excreted in urine and 16% in feces, whereas ara-C only found in urine with 48% of the injected dose. Feces extracts and urine were purified by HPLC and further analyzed by LC/MS. Feces extracts of NOAC treated mice was composed of unmetabolized NOAC, hydroxylated NOAC (NOAC+OH), its sulfated derivative (NOAC+OSO_3H) and unidentified metabolites. In urine the hydrophilic molecules ara-C and ara-U were found. Only 2% of the injected NOAC was eliminated in its unmetabolized form, whereas 25% were identified as main metabolite ara-C. Urine collected during 48 hr of ara-C treated mice contained 33% of the injected dose as unmetabolized drug and 13% as the main metabolite ara-U.
Thus, NOAC is metabolized by two major pathways, one leading to the hydrophilic metabolites ara-C and ara-U and the other to hydroxylated and sulfated NOAC.

8 POTENTIAL APPLICATIONS

NOAC is a novel, patented derivative of cytarabine which shows significantly different features of antitumor activity, metabolic inactivation, pharmacokinetic properties, cellular uptake and intracellular phosphorylation, as compared to the parent drug cytarabine. Its mechanisms of action appear to be different from those of cytarabine and are presently not fully known.

NOAC may prove a useful new active cytotoxic agent in acute and chronic leukemias, including cytarabine-resistant cases. In addition, it may prove active in lymphomas, as well as in a number of solid tumors (for which cytarabine has no effect), e.g. prostate, breast and non small cell lung cancer. Its clinical activity in glioblastoma, melanoma and tumors with multiple-drug resistance may also prove valuable.

Its oral bioavailability, which may still be enhanced by appropriate formulations, opens novel avenues of long-term application in leukemias and other tumors. Topical formulations for the treatment of regional malignant lesions (breast cancer, melanomas, Kaposi sarcomas) might further prove the versatility of this new drug.

The development of formulations of NOAC according to GLP and GMP standards would open the possibility to conduct clinical phase I/II trials with this new drug.

9 PRELIMINARY CLINICAL DATA

9.1 Exploratory Phase I trial

A phase-I trial using escalating doses of NOAC given as an 1 h i.v. infusion has been initiated in 1997 at the University Hospital of Zurich, based on the local regulations. It was regularly announced to the Swiss Health Authorities (IKS Notification). The study is performed with a preliminary pharmaceutical liposomal formulation, which is prepared extemporaneously. Pharmacokinetic data are collected during drug administration.
Up-to-now, 11 patients have received the drug (3 patients at 150 mg/m^2, 3 at 300 mg/m^2, 3 at 600 mg/m^2, 2 at 1200 mg/m^2. To date, no relevant hematological and non-hematological toxicities were reported. The pharmacokinetic parameters of NOAC determined by HPLC are as follows: $t_{1/2(\alpha)}$ = 30 min, $t_{1/2(\beta)}$ = 11 – 16 hours and the plasma drug concentrations during the elimination phase range from 1 to 20 mg/liter, depending on the initial dose. (ongoing phase I/II study, unpublished results).

10 REFERENCES

10.1 References quoted

1. US Patent 5'641'758 (1997); Patent PCT-EP 92-00706.
2. Schott, H., Häussler, M.P., and Schwendener, R.A. (1994). Synthese von 4-Alkyl-cytosinnucleosiden und deren zytostatische Wirkung im L1210 Leukämiemodell der Maus. ***Liebigs Annalen der Chemie****,* 465-470.
3. Schwendener, R.A., Schott, H. (1992). Treatment of L1210 murine Leukemia with Liposome - incorporated N^4-hexadecyl-1-β-D-arabinofuranosylcytosine***. Int. J. Cancer*** **51:** 466-469.
4. Schwendener, R.A., Horber, D.H., Ottiger, C. and Schott, H. (1995). Preclinical properties of N^4-hexadecyl- and N^4-octadecyl-1-β-D-arabinofuranosylcytosine in liposomal preparations. ***J. Liposome Res*****. 5:** 27-47.
5. Schwendener, R.A., Horber, D.H., Ottiger, C., Rentsch, K.M., Fiebig, H.H. and Schott, H. (1995). Monograph: Alkasar-18, 1-(β-D-arabinofuranosyl)-4-octadecylamino-2(1H)-pyrimidin-one, N^4-octadecyl-ara-C, NOAC. ***Drugs of the Future*** **20:** 11-15.
6. Schwendener, R. The preparation of large volumes of sterile liposomes for clinical applications. In: G.Gregoriadis, ed. ***Liposome Technology***, 2. edition, 1993, CRC Press, Boca Raton. Vol. I, pp. 487-500.
7. Rentsch, K.M., (1996). Monitoring of cytostatic drugs: Preclinical and clinical applications of Mitoxantrone and N^4-octadecyl-1-β-D-arabino-furanosylcytosine (NOAC). ***Dissertation ETH Nr. 11734*** .
8. Notari, R.E., (1972).Arabinosylcytosine stability in aqueous solutions: pH profile and shelf life predictions. ***J. Pharm. Sci.*** **61:** 1189-96.
9. Rentsch, K.M., Schwendener, R.A., Schott, H., Hänseler, E. (1995). Sensitive high-performance liquid chromatographic method for the determination of N^4-hexadecyl- and N^4-octadecyl-1-β-D-arabino-furanosylcytosine in plasma and erythrocytes. ***J. Chromatogr. B*****, 673:** 259-266.
10. Paull, K.D., Hamel, E., Malspeis, L. Prediction of biochemical mechanism of action from the in vitro antitumor screen of the National Cancer Institute. In: ***Cancer Chemotherapeutic Agents*** (Ed. W.O. Foye) p. 9-45 American Chemical Society, Washington, 1995.
11. Horber, D.H., Cattaneo-Pangrazzi, R.M.C., von Ballmoos, P., Schott, H., Ludwig, P.S., Eriksson, S., Fichtner, I., Schwendener, R.A. (2000). Cytotoxicity, cell cycle perturbations and apoptosis in human tumor cells by lipophilic N^4-alkyl-1-β-D-arabinofuranosylcytosine derivatives and the new heteronucleoside phosphate dimer arabinocytidylyl-(5'-5')-N^4-octadecyl-1-β-D-ara-C. **J. Cancer Res. Clin Oncol**, 126: 311-319.
12. Schwendener, R.A., Friedl, K., Depenbrock, H., Schott, H., Hanauske, A.-R. (2000) In vitro activity of liposomal N^4-octadecyl-1-β-D-arabinofuranosylcytosine (NOAC), a new lipophilic derivative of on biopsized clonogenic human tumor cells and haematopoietic precursor cells. Invest. New Drugs, accepted for publication.
13. Schwendener, R.A., Horber, D.H., Odermatt, B., Schott, H. (1996). Oral antitumor activity and pharmacological properties of lipophilic N^4-alkyl derivatives of 1- β-D-arabino-furanosylcytosine (ara-C*).* ***J. Cancer Res. Clin. Onco****l.* **122:** 102-108.

14. Koller-Lucae, S.K.M, Schott, H., Schwendener, R.A. (1997). Pharmacokinetic properties in mice and interactions with human blood in vitro of liposomal N^4-octadecyl-1-β-D-arabinofuranosylcytosine (NOAC), a new anticancer drug. ***J. Pharmacol. Exptl. Therap.,*****282:** 1572-1580.

15. Koller-Lucae, S.M., Schott, H., Schwendener, R.A. (1999) Low density lipoprotein and liposome mediated uptake and cytotoxic effect of N^4-octadecyl-1-β-D-arabino-furanosylcytosine (NOAC) in mice. ***Brit. J. Cancer 80:*** 1542-1549.

16. Rentsch, K.M., Schwendener, R.A., Schott, H., Hänseler, E. (1997). Pharmacokinetics of N^4-octadecyl-1-β-D-arabinofuranosylcytosine (NOAC) in plasma and whole blood after intravenous and oral application in mice. ***J. Pharm. Pharmacol.,*** **49:** 1076-1081.

17. Koller-Lucae, S.M., Suter, M. J.-F., Rentsch, K.M., Schott, H., Schwendener, R.A. Metabolism of the new liposomal anticancer drug N^4-octadecyl-1-β-D-arabino-furanosylcytosine (NOAC) in mice. ***Drug Metabolism and Disposition*****, 27:** 342-350, 1999.

10.2 Comprehensive list of references on N^4-alkyl-ara-C analogs and liposomes (authors: Schwendener & Schott)

1. Schwendener, R.A., Schott, H. (1992). Treatment of L1210 murine Leukemia with Liposome - incorporated N^4-hexadecyl-1-β-D-arabinofuranosylcytosine***. Int. J. Cancer*** **51:** 466-469.

2. Schwendener, R.A., Horber, D.H., Rentsch, K., Hänseler, E., Pestalozzi, B., and Sauter, Chr. (1994). Preclinical and clinical Experience with Liposome-encapsulated Mitoxantrone. ***J. Liposome Res****.* **4:** 605-639.

3. Schott, H., Häussler, M.P., and Schwendener, R.A. (1994). Synthese von 4-Alkyl-cytosinnucleosiden und deren zytostatische Wirkung im L1210 Leukämiemodell der Maus. ***Liebigs Annalen der Chemie****,* 465-470.

4. Horber, D.H.,. Ottiger, C.,. Schott, H. and Schwendener, R.A. (1995). Pharmacokinetic properties and interactions with blood components of N^4-hexadecyl-1-β-D-arabino-furanosylcytosine (NHAC) incorporated into liposomes***. J.Pharm. Pharmacol****.* **47:** 282-288.

5. Horber, D.H., Schott, H. and Schwendener, R.A. (1995). Cellular pharmacology of a liposomal preparation of N^4-hexadecyl-1-β-D-arabinofuranosylcytosine, a lipophilic derivative of 1-β-D-arabinofuranosylcytosine. ***Br. J. Cancer*** **71:** 957-962.

6. Schwendener, R.A., Horber, D.H., Ottiger, C., Rentsch, K.M., Fiebig, H.H. and Schott, H. (1995). Monograph: Alkasar-18, 1-(β-D-arabinofuranosyl)-4-octa-decylamino-2(1H)-pyrimidin-one, N^4-octa-decyl-ara-C, NOAC. ***Drugs of the Future*** **20:** 11-15.

7. Schwendener, R.A., Horber, D.H., Ottiger, C. and Schott, H. (1995). Preclinical properties of N^4-hexadecyl- and N^4-octadecyl-1-β-D-arabinofuranosylcytosine in liposomal preparations. ***J. Liposome Res*****. 5:** 27-47.

8. Schwendener, R. (1993). The preparation of large volumes of sterile liposomes for clinical applications. In: ***Liposome Technology***, 2.Edition, G.Gregoriadis ed., pp. 487-500, CRC Press: Boca Raton.

9. Rentsch, K.M., Schwendener, R.A., Schott, H., Hänseler, E. (1995). Sensitive high-performance liquid chromatographic method for the determination of N^4-hexadecyl- and N^4-octadecyl-1-β-D-arabinofuranosylcytosine in plasma and erythrocytes. ***J. Chromatogr. B*****, 673:** 259-266.

10. Horber, D.H., Schott, H. and Schwendener, R.A. (1995). Cellular pharmacology of N^4-hexadecyl-1-β-D-arabinofuranosylcytosine (NHAC) in the human leukemic cell lines K-562 and U-937. ***Cancer Chemother. Pharmacol.,*** **36:** 483-492.

11. Schwendener, R.A., Horber, D.H., Odermatt, B., Schott, H. (1996). Oral antitumor activity and pharmacological properties of lipophilic N^4-alkyl derivatives of 1-β-D-arabino-furanosylcytosine (ara-C*). **J. Cancer Res. Clin. Oncol.* **122:** 102 108.

12. Rentsch, K.M., (1996). Monitoring of cytostatic drugs: Preclinical and clinical applications of Mitoxantrone and N^4-octadecyl-1-β-D-arabino-furanosylcytosine (NOAC). ***Dissertation, ETH Nr. 11734*** .

13. Schwendener, R.A. and Schott, H. (1996). Lipophilic 1-β-D-arabinofuranosylcytosine derivatives in liposomal formulations for oral and parenteral antitleukemic therapy in the murine L1210 leukemia model*. **J. Cancer Res. Clin. Oncol.,* **122:** 723-726.

14. Rentsch, K.M., Schwendener, R.A., Schott, H., Hänseler, E. (1997). Pharmacokinetics of N^4-octadecyl-1-β-D-arabinofuranosylcytosine (NOAC) in plasma and whole blood after intravenous and oral application in mice. ***J. Pharm. Pharmacol.,*** **49:** 1076-1081.

15. Koller-Lucae, S.K.M, Schott, H., Schwendener, R.A. (1997). Pharmacokinetic properties in mice and interactions with human blood in vitro of liposomal N^4-octadecyl-1-β-D-arabino-furanosylcytosine (NOAC), a new anticancer drug. ***J. Pharmacol. Exptl. Therap.,*****282:** 1572-1580.

16. Koller-Lucae, S.M., Suter, M. J.-F., Rentsch, K.M., Schott, H., Schwendener, R.A. (1999). Metabolism of the new liposomal anticancer drug N^4-octadecyl-1-β-D-arabino-furanosylcytosine (NOAC) in mice. ***Drug Metabolism and Disposition*****, 27:** 342-350.

17. Koller-Lucae, S.K.M, Schott, H., Schwendener, R.A. (1999). Low density lipoprotein mediated uptake and cytotoxic effect of N^4-octadecyl-1-β-D-arabinofuranosylcytosine in Daudi lymphoma cells. ***Brit. J. Cancer 80:*** 1542-1549.

18. Schwendener, R.A., Friedl, K., Depenbrock, H., Schott, H., Hanauske, A.-R. (2000) In vitro activity of liposomal, a new lipophilic derivative of on biopsized clonogenic human tumor cells and haematopoietic precursor cells. ***Invest. New Drugs***, accepted for publication.

19. Horber, D.H., Cattaneo-Pangrazzi, R.M.C., von Ballmoos, P., Schott, H., Ludwig, P.S., Eriksson, S., Fichtner, I., Schwendener, R.A. (2000). Cytotoxicity, cell cycle perturbations and apoptosis in human tumor cells by lipophilic N^4-alkyl-1-β-D-arabinofuranosylcytosine derivatives and the new heteronucleoside phosphate dimer arabinocytidylyl-(5'-5')-N^4-octadecyl-1-β-D-ara-C. ***J. Cancer Res. Clin Oncol, 126:*** 311-319.

10.3 Abstracts

1. Schwendener, R.A., Horber, D.H., and Schott, H. Cellular Pharmacology of N^4-hexadecyl-cytosinearabinoside (NHAC), a new potent Prodrug of Cytosine Arabinoside. 85th Annual Meeting of the American Association for Cancer Research, April 10-13, 1994, San Francisco, CA.

2. Rentsch, K.M., Hänseler, E., Schwendener, R.A., Horber, D.H., Schott, H. N^4-hexadecyl-1-β-D-arabinofuranosyl Cytosine: A new Cytosine-arabinoside Derivative with improved antileukemic Activity - Preclinical Studies in Mice. 8th NCI-EORTC Symposium on new Drugs in Cancer Therapy. Ann. Oncol. 5 (S5), 96, 1994.

3. Horber, D.H., Rentsch, K. M., Hänseler, E., Schott, H., and Schwendener, R.A. N^4-hexadecyl-1-β-D-arabinofuranosyl Cytosine (NHAC), a new Prodrug of Cytosine Arabinoside: Cellular Pharmacology. 8th NCI-EORTC Symposium on new Drugs in Cancer Therapy. Ann. Oncol. 5 (S5), 90, 1994.

4. Horber, D.H., von Ballmoos, P., Schott, H. and Schwendener, R.A. Cell cycle dependent cytotoxicity and induction of apoptosis by N^4hexadecyl-cytosine arabinoside (NHAC), a new lipophilic derivative of cytosine arabinoside. 8th AEK symposium of the division of experimental cancer research. Cancer Res.Clin. Oncol. 121, Suppl. 2, A2 1995.

5. Fiebig, H.H., Dengler, W.A., Drees, M., Schwendener, R.A., Schott, H. In vivo activity of N^4-octadecyl-ara-C in human solid tumors and leukemias. Proc. AACR 36, 408 (1995).

6. Rentsch, K.M., Schwendener, R.A., Schott, H., Hänseler, E. N^4-octadecyl-1-β-D-cytosine arabinoside: A new cytosine-arabinoside derivative with improved antileukemic activity - preclinical studies in mice. 4th Int. Congr. on Therap. Drug Monitoring and Clinical Toxicol.., Vienna, Sept. 4-8, 1995.

7. Koller-Lucae, S.K.M.,Rentsch, K.M.,Schott, H. and Schwendener, R.A. Interactions with human blood in vitro and pharmacokinetic properties in mice of liposomal N^4-octadecyl-1-β-D-arabinofuranosylcytosine (NOAC), a new anticancer drug. *J. Cancer Res. Clin. Oncol.* 123, S1: S36, 1997.

11 Adresses

Prof. H. Schott
Institute of Organic Chemisty
University of Tübingen
Auf der Morgenstelle 18
D-72076 Tübingen

phone +49-7071-297-6220
fax +49-7071-657-82
e-mail: herbert.schott@uni-tuebingen.de

Prof. R.A. Schwendener
Paul Scherrer Institute
Div. of Med. Radiobiology, OFLD

CH-5232 Villigen-PSI
Switzerland

phone: +41-56-310-4728
fax: +41-561-310-4417
e-mail: onkschwn@usz.unizh.ch or onkschwn@bigfoot.com or reto.schwendener@psi.ch
Homepage: http://www.unizh.ch/onkwww/index.html

RS/8 .2000