Anti-Cancer Drugs, 2, pp. 495–502

Biotransformation and *in vitro* activity of an arabinosylcytosine 5′-chloro-5′-deoxy analog

Ladislav Novotný,[CA] Viera Reichelová, Eliška Balážová, Werner Scheithauer, Mária Benesová and Viliam Ujházy

L Novotný, V Reichelová, E Balážová, M Benesová and V Ujházy are at the Department of Experimental Therapy, Cancer Research Institute, Slovak Academy of Sciences, Špitálska 21, 812 32 Bratislava, Czechoslovakia. Fax: 427 561 40.
W Scheithauer is at the Department of Gastroenterology and Hepatology II, Vienna University School of Medicine, Garnisongasse 13, 1090 Vienna, Austria.

5′-Chloro-5′-deoxyarabinosylcytosine (5′-chloro-araC), a lipophilic and cytidine-deaminase resistant analog of the cytotoxic agent arabinosylcytosine (araC) was evaluated in terms of bioactivation, transformation and its cytotoxic activity *in vitro*. 5′-Chloro-araC interferes with DNA synthesis (IC_{50} = 2.8 μmol/l) and inhibits the growth of L1210 cells in suspension culture (IC_{50} = 1.05 μmol/l) and in the soft agar assay (IC_{50} = 0.65 μmol/l). Being phosphorylated to the triphosphate of araC–araCTP (5′-triphosphate of araC), 5′-chloro-araC has the same mechanism of action as arabinosylcytosine. In alkaline solutions 5′-chloro-araC is transformed to another (cytostatically inactive) araC analog—2′,5′-anhydroarabinosylcytosine—but at physiological pH and temperature conditions, it has sufficient stability to be phosphorylated and thus activated. A lower rate of araCTP formation from 5′-chloro-araC explains the somewhat lower cytotoxic effect of this compound against various established cell lines *in vitro* compared to araC. Lipophilicity that would allow an oral drug formulation and certain other superior physico-chemical and biochemical characteristics of 5′-chloro-araC make this compound an interesting candidate for further investigations.

Key words: 2′,5′-Anhydroarabinosylcytosine, cell growth inhibition, 5′-chloro-5′-deoxyarabinosylcytosine, cytotoxic activity *in vitro*, mechanism of action, phosphorylation, 5′-triphosphate of arabinosylcytosine formation.

Introduction

Arabinosylcytosine (AraC) is a cytotoxic compound successfully used in the treatment of acute myeloid leukemia.[1] Although it is chemically an analog of cytidine, in biological systems it competes with deoxycytidine.[2] AraC is biologically activated by phosphorylation to araC 5′-mono-phosphate and subsequently to 5′-triphosphate. 5′-Triphosphate of araC (araCTP) is the active form of this nucleoside interfering with DNA synthesis while lowered kinases activity is an important mode of resistance to araC.[1] Potential disadvantages of araC are related to its low distribution volume in the human organism, its rapid deamination to arabinosyluracil[3] and elimination from *in vivo* systems.[4]

These disadvantages stimulated the interest of several investigators in new analogs of araC. We have previously shown that the 5′-chloro-5′-deoxy analog of araC[5] is more stable against deamination by cytidine deaminase than araC *in vitro*[6] and *in vivo*.[5] Because the 5′-hydroxyl is substituted in this compound by a chlorine atom, it is more lipophilic than araC.[5] A different behavior in biological models may be expected, thus making this drug interesting for a pharmacological study. Since 5′-hydroxyl was determined as a basic structural condition for phosphorylation of nucleoside analogs,[7] one aim of our study was to find out whether 5′-chloro-5′-deoxyarabinosylcytosine (5′-chloro-araC) can be phosphorylated in leukemia cells. Another aspect of our work was to determine whether 5′-chloro-araC would be stable enough to form araCTP and not be degraded to 2′,5′-anhydroarabinosylcytosine (2′,5′-anhydro-araC).

Apart from defining these basic parameters of 5′-chloro-araC bioactivation and possible biotransformation, we describe the preclinical anticancer activity data obtained against several different established tumor cell lines.

[CA] Corresponding Author

© *1991 Rapid Communications of Oxford Ltd*

Materials and methods

Compounds

5'-Chloro-araC (NSC No. 318 799), 2',5'-anhydro-araC (NSC No. 340 843) and 5'-chloro-5'-deoxycytidine (5'-Cl-cytidine) were prepared as described elsewhere.[8,9] AraC was synthesized according to Brokeš and Beránek.[10] Chemical structures of these compounds are shown in Figure 1. Potassium dihydrogenphosphate crystalline extra pure was purchased from Merck (Darmstadt, Germany), high performance liquid chromatography (HPLC)-grade methanol was a product of Fluka (Buch, Switzerland). The water used for the preparation of all buffers, solutions and for HPLC was purified by passing deionized water through Elgast Spectrum SC 20 (Elga Ltd., Lane End, Wycombe, Bucks, UK). AraC and araCTP were obtained from Sigma Chemical Co. (St. Louis, MO, USA), EDTA was purchased from Fischer Scientific Co. (Fair Lawn, USA).

Determination of the constant of dissociation (p*K*)

The values of p*K* of 5'-chloro-araC and both araC and cytidine as standards were determined by titration of 0.1 mol/l solution of the nucleoside with 0.1 mol/l solution of hydrochloric acid. Consumption of the hydrochloric acid solution for neutralization of the nucleoside was measured by a laboratory digital pH meter OP-211/1 (Laboratorní přístroje, Prague, Czechoslovakia). The value of p*K* was determined as the value of pH when half of the nucleoside was neutralized.

Determination of the rate constants of the transformation of 5'-chloro-araC to 2',5'-anhydro-araC

Solutions (1%) of 5'-chloro-araC in buffer were prepared. The transformation of 5'-chloro-araC to 2',5'-anhydro-araC in solutions with different pH was studied at room temperature and at 37°C using HPLC. For the HPLC analysis the Separon SGX C-18 column (7 μm) obtained from Tessek Ltd. (Prague, Czechoslovakia) was used. The mobile phase consisted of 0.01 mol/l KH_2PO_4 and 10% of methanol, pH 5.81. Flow used was 0.5 ml/min and pressure was 60 bar. The analysis was performed at 275 nm.

DNA synthesis

Inhibition of DNA synthesis was determined using 6-[^{3}H]thymidine (sp. act. 980 GBq/mmol) purchased from the Institute for Research, Development and Production of Radionuclides (Prague, Czechoslovakia). The incorporation into trichloro acetic acid (TCA)-insoluble fraction was measured. Cells were incubated in culture medium as described below at 37°C using labeled precursor for 10 min (185 kBq/ml, and 5×10^6 cells/ml of suspension). All experiments were carried out in triplicate.

L1210 in suspension culture

Murine leukemia L1210 cells were cultured in RPMI-1640 medium (Flow Laboratories, Irvine, UK), supplemented with 5% fetal bovine serum and 15% heat-inactivated bovine serum, antibiotics

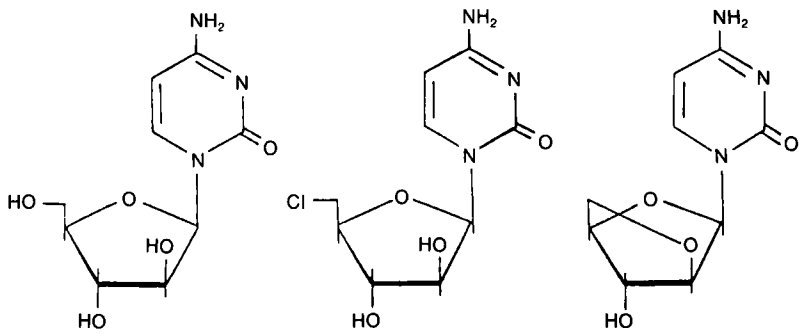

Figure 1. Chemical structures of araC, 5'-chloro-araC and 2',5'-anhydro-araC.

(penicillin and streptomycin) and L-glutamine. Petri dishes containing the suspension cultures were maintained in a humidified atmosphere of 7% CO_2 at 37°C. In their logarithmic phase of growth cells were transferred to Petri dishes, each containing 10^3 cells in 5 ml of suspension. Freshly prepared drug solutions were added immediately after plating. Percentage of inhibition was determined from the growth inhibition curve.

L1210 cells in the soft agar assay

L1210 cells were suspended in 0.9% agar (Difco, Detroit, USA), in RPMI 1640 medium, supplemented with 10% fetal bovine serum, penicillin, streptomycin and L-glutamine. Five ml of the cell suspension, containing 10^3 cells per ml were pipetted into 60 mm Petri dishes. Cell suspensions containing the tested drug were incubated for 10 days at 37°C. Colonies containing ≥ 30 viable cells were then counted using an inverted microscope. Percentage of inhibition was determined and ID_{50} extrapolated from the growth inhibition curve at various drug concentrations. Petri dishes for ID_{50} calculations in continuous treatment cultures were routinely set up in triplicates. The number of colonies in controls was approximately 700–1000 per dish.

HPLC instrumentation

The HPLC equipment consisted of a Pye Unicam PU 4003 gradient elution high performance liquid chromatograph, a Rheodyne model 7125 injector and PU 4030 controller, PU 4031 oven and PU 4020 UV detector with variable wavelength (Philips Scientific, Cambridge, UK). A dual-channel SP 4200 computing integrator (Spectra Physics, Darmstadt, Germany) was used to record detector signals.

K562 cells and assessment of araCTP accumulation

The K562 cell line, derived from a patient with chronic myelogenous leukemia,[11] was obtained from Dr P. Stockbauer (Institute of Hematology and Blood Transfusion, Prague, Czechoslovakia). The cells were maintained in suspension culture at exponential growth in RPMI-1640 medium, supplemented with 10% heat-inactivated fetal calf serum, at 37°C in a humidified atmosphere containing 5% CO_2. Cell number and mean cell volume were determined before and after incubation by a Coulter counter equipped with a model C-1000 particle size analyzer (Coulter Electronics, Hialeah, FL, USA). The cell doubling time was between 24 and 28 h. For measurement of araCTP accumulation exponentially growing K562 cells were incubated with two different concentrations of araC or 5'-chloro-araC for the time indicated. Because 5'-chloro-araC is more lipophilic than araC and its solubility in water is low, both compounds were dissolved primarily in dimethylsulfoxide (DMSO; 2.5% of final volume) and added to the cell culture. Comparison of araCTP accumulation from araC dissolved only in water was made. Cells were washed twice with phosphate-buffered saline (8.1 g NaCl, 0.22 g KCl, 1.14 g Na_2PO_4, and 0.27 g KH_2PO_4 per liter of H_2O, pH 7.4). Nucleotides were extracted using $HClO_4$.[12] AraCTP was separated from other nucleotides by HPLC using a Partisil 10-SAX column (Whatman, Clifton, NJ, USA), and gradient elution with $NH_4H_2PO_4$.[12,13] All results are the average of three experiments.

The identity of araCTP was confirmed by its co-elution with authentic araCTP and by its ratio of absorbance (280 nm/254 nm = 3.11). The quantitation of araCTP was done at 280 nm. The quantitation of nucleosides in $HClO_4$ extracts was determined by electronic integration and reference to preprogrammed response factors. The intracellular concentration of araCTP was calculated by dividing the quantity of araCTP contained in an $HClO_4$ soluble fraction by the number of cells analysed and then multiplying this value by the mean cell volume.

Colorectal cancer cell lines and determination of their drug sensitivity to araC and 5'-chloro-araC with the Bactec system

COLO 320DM and Ht-29 cells were obtained from the American Type Culture Collection (Rockville, MD, USA). The human metastatic colon carcinoma cell line OM-1 was kindly provided by Dr D. Dexter (Biochemical Department, E. I. Du Pont de Nemours and Co., Glenolden, PA, USA). General morphological and biological cell characteristics, as well as culture conditions have been described previously in detail.[14–16]

Growth inhibition was assessed by the Bactec system. Basically, in this short-term metabolic assay,

the amount of $[^{14}C]CO_2$ produced by tumor cells from [^{14}C]glucose incorporated into the culture medium is measured.[17] 1.8 ml of the cytostatically pretreated or untreated (= control) tumor cell suspension, containing 3–6 × 10^4 cells plus 0.2 ml of an aqueous solution of $[^{14}C]CO_2$ (37 kBq/ml) are mixed and injected aseptically into 20 ml glass vials. For initial adjustment of the gaseous atmosphere in the sealed glass vials to 5% CO_2 and 95% air, as well as for subsequent measurements of the amount of [^{14}C]glucose metabolism (representative counts were usually obtained on day 6), the vials are simply placed into the Bactec 460 instrument (Johnston Laboratories, Towson, MD, USA). Automatically the $[^{14}C]CO_2$ containing gaseous atmosphere is flushed into a ionization chamber, replaced, and the disintegrations are directly converted to growth index values. Cytotoxic effects are calculated by comparing the growth index values of drug-treated samples to those of triplicate control vials. The variability of drug-induced changes in the [^{14}C]glucose uptake, estimated by the coefficient of variation, ranged from 2 to 10%.

Results

Determination of the constant of dissociation (p*K*)

The p*K* value of 5′-chloro-araC determined in our experiments was 3.84. The p*K* values of cytidine, araC[18] and 5′-chloro-araC are shown in Table 1, together with the retention time for HPLC measurement at pH 5.5 which is directly dependent on the p*K* value.

Table 1. The p*K* values of some pyrimidine nucleosides and their HPLC retention-times (t_R)

	p*K*	t_R[a] (min)
Cytidine	4.13 (14) 4.22 (17)	3.27
AraC	4.15 (14)	3.68
5′-Chloro-araC	3.84	15.90
2′,5′-Anhydro-araC	–	4.38

[a] The isocratic mobile phase used on reversed phase column consisted of 0.01 mol/l potassium dihydrogen phosphate with 5% methanol, pH 5.5.

Table 2. Inhibition of DNA synthesis and cell growth in the leukemia L1210 cell line in suspension culture and the soft agar assay

Compound	Inhibition of DNA synthesis (IC_{50}, μmol/l)	Inhibition of cell growth in suspension culture (IC_{50}, μmol/l)	Soft agar assay (ID_{50}, μmol/l)
AraC	0.048	0.08	0.23
5′-Chloro-araC	2.8	1.05	0.65
5′-Chloro-cytidine	≥50	No effect	No effect
2′,5′-Anhydro-araC	≥50	No effect	Marginal effect (≥5 μmol/l)

DNA synthesis

Inhibition of DNA synthesis by the different cytosine nucleosides is shown in Table 2. The concentration dependence of DNA synthesis inhibition is shown in Figure 2. No inhibitory activity was seen for 5′-Cl-cytidine at a concentration of 50 μmol/l; 2′,5′-anhydro-araC expressed only marginal effects on DNA synthesis at the same concentration.

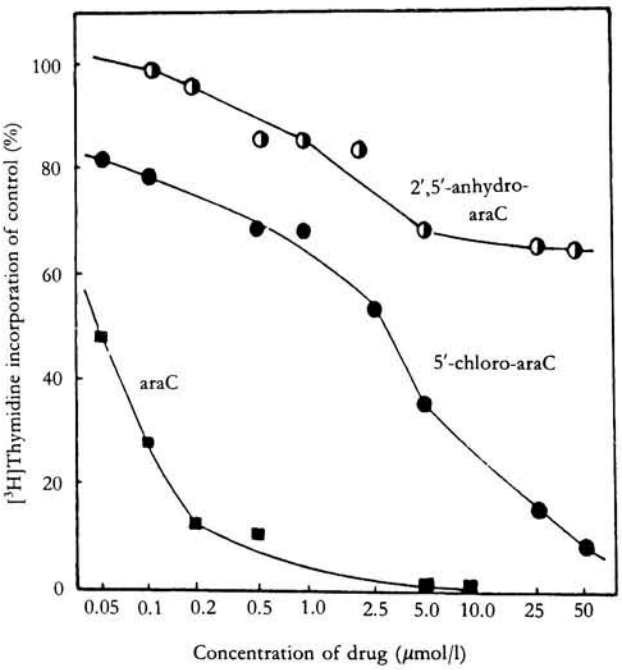

Figure 2. Inhibition of DNA synthesis in L1210 cells by various araC analogs.

Inhibition of cell growth in suspension culture and in the soft agar assay

The inhibition of cell growth of murine leukemia L1210 cells in suspension culture is shown in Table 2. In this test system the activity of araC was 13 times higher than the activity of 5'-chloro-araC. Results obtained in the soft agar assay however indicate that the activity of araC is only 3 times higher than the activity of its 5'-chloro derivative. No effect on cell growth was seen for 5'-chloro-cytidine and 2',5'-anhydro-araC in either experimental system.

Determination of the rate constants of the transformation reaction of 5'-chloro-araC to 2',5'-anhydro-araC

The results of the transformation of 5'-chloro-araC in alkaline conditions is an inactive product, 2',5'-anhydro-araC.[8] The rate characteristics of this reaction were determined by HPLC (Figure 3). It was found that the half-life time of 5'-chloro-araC in physiological conditions (37°C, pH 7.2) is 220 days, suggesting that this transformation is very slow in the organism unless some enzyme increases the rate of the reaction. The results of the rate-constant k_v determinations are shown in Table 3 and the pH–k_v relationship is documented in Figure 4.

Table 3. The rate constants of transformation of 5'-chloro-araC to 2',5'-anhydro-araC at different pH and temperature

Temperature (°C)	pH	k (s^{-1})	n	$t_{1/2}$ (days)
24	7.2	$(1.1 \pm 0.3) \times 10^{-8}$	29	729
	7.6	$(1.3 \pm 0.5) \times 10^{-8}$	31	641
	8.0	$(1.5 \pm 0.5) \times 10^{-8}$	51	524
	8.3	$(1.8 \pm 0.7) \times 10^{-8}$	64	451
	8.6	$(2.3 \pm 0.9) \times 10^{-8}$	67	343
	9.1	$(4.9 \pm 0.6) \times 10^{-8}$	36	163
	9.5	$(1.7 \pm 0.4) \times 10^{-7}$	64	46
	10.0	$(4.2 \pm 0.6) \times 10^{-7}$	42	19
	10.5	$(1.3 \pm 0.1) \times 10^{-6}$	30	6
		$(1.4 \pm 0.3) \times 10^{-6}$	12	6
	11.0	$(4.0 \pm 0.6) \times 10^{-6}$	16	2
37	7.2	$(3.7 \pm 0.7) \times 10^{-6}$	65	220

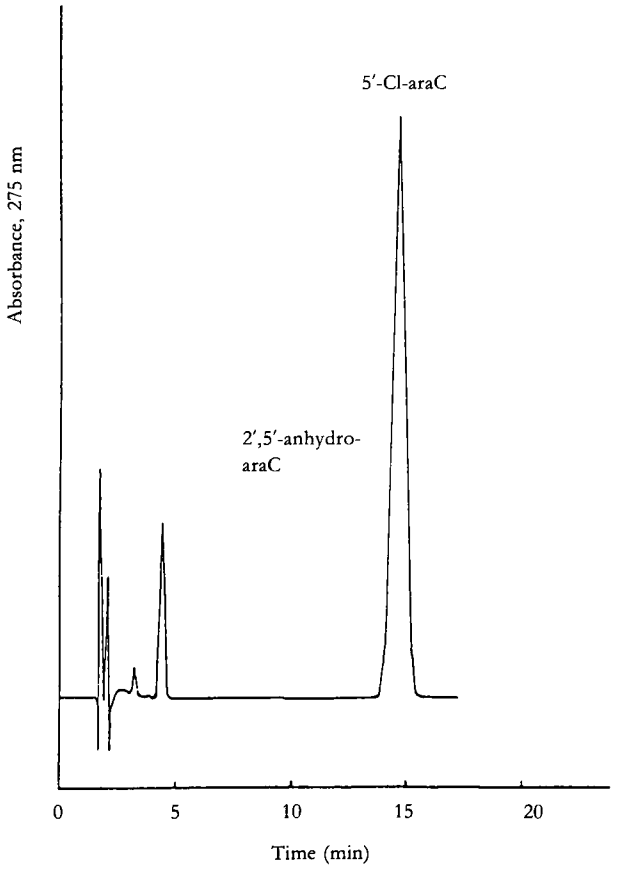

Figure 3. HPLC determination of 5'-chloro-araC and 2',5'-anhydro-araC.

Phosphorylation of 5'-chloro-araC to araCTP

The phosphorylation of 5'-chloro-araC to araCTP is given in Tables 4 and 5. Using HPLC (Figure 5) it was found that the 5'-chloro derivative of the nucleoside also may be phosphorylated and that the hydroxyl group in the 5' position of the nucleoside

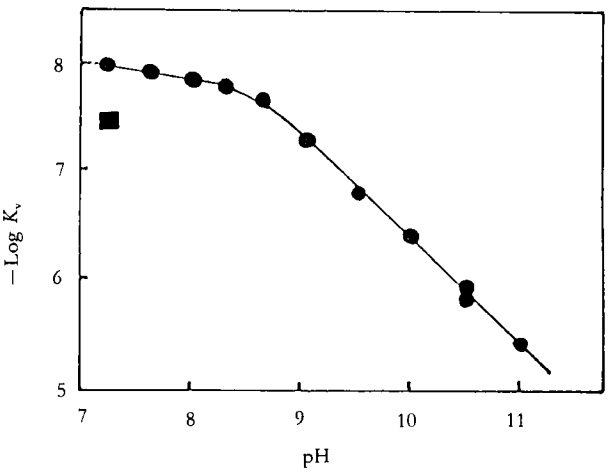

Figure 4. Rate-constant values of the transformation of 5'-chloro-araC to 2',5'-anhydro-araC at various pH and temperature. ●, 24°C; ■, 37°C.

Table 4. AraCTP accumulation from 5'-chloro-araC and araC (10 mmol/l in 2.5% of DMSO) in K562 cells

Incubation time (h)	AraCTP accumulation (μmol/l)		
	5'-Chloro-araC	AraC	%[a]
3	25 ± 3	157 ± 9 178 ± 7[b]	16
6	30 ± 5	238 ± 13	12
12	72 ± 5	303 ± 21	24

[a] Percentage of araCTP formed from 5'-chloro-araC in comparison to araCTP formed from araC.
[b] AraCTP formation from araC without 2.5% of dimethylsulfoxide.

Table 5. AraCTP accumulation from 5'-chloro-araC and araC (100 mmol/l in 2.5% of DMSO) in K562 cells

Incubation time (h)	AraCTP accumulation (μmol/l)		
	5'-Chloro-araC	AraC	%[a]
3	75 ± 6	129 ± 8 125 ± 7[b]	58
6	75 ± 7	251 ± 18	30
12	87 ± 7	533 ± 22	16

[a] Percentage of araCTP formed from 5'-chloro-araC in comparison to araCTP formed from araC.
[b] AraCTP formation from araC without 2.5% of dimethylsulfoxide.

sugar moiety does not represent an absolute condition for the monophosphate formation. The rate of the phosphorylation of 5'-chloro-araC to araC monophosphate (araCMP), probably by deoxycytidine kinase, is lower than that of araC. Consequently, the formation of araCTP from araCMP formed from the 5'-chloro derivative is lower than the araCTP formation originating from araC. This is in good agreement with the efficacy data of both compounds against different colorectal cancer cell lines obtained in the Bactec system (Table 6).

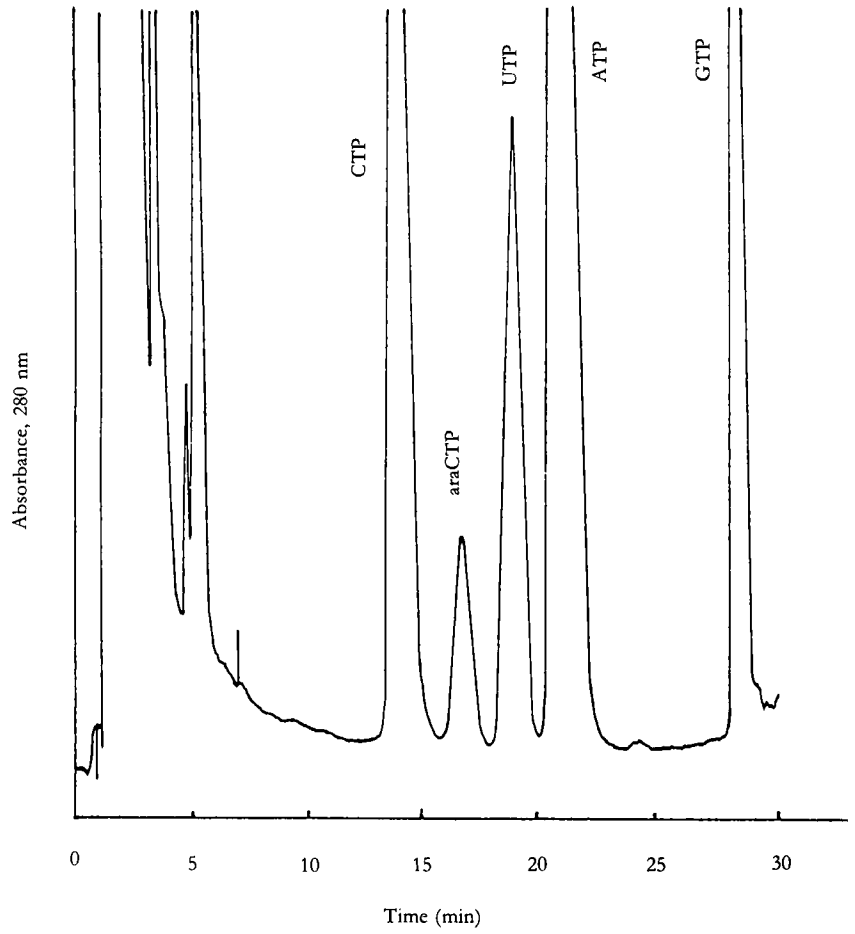

Figure 5. HPLC determination of araCTP formed from 5'-chloro-araC.

Table 6. Effect of continuous incubation of araC and 5'-chloro-araC (10 mg/l)[a] on the growth of colorectal cancer cells (Bactec system)

Cell line	Cell growth (%)[b]	
	AraC	5'-Chloro-araC
OM-1	27	85
Ht-29	14	85
COLO 320 DM	13	76

[a] 0.04 mmol/l.
[b] The Bactec system results' variation ranged from 2 to 10%.

Discussion

The derivative of the cytotoxic agent araC with the substituted hydroxyl group at the 5' position by the chlorine atom—5'-chloro-araC—is more lipophilic than the parent compound.[5] The presence of an electronegative chlorine atom in the sugar moiety furthermore influences the dissociation (p*K* value, Table 1). Since both physico-chemical parameters (lipophilicity and p*K*) that modify certain biological properties, such as bioavailability, transport and the stability towards biodegradation,[5] may be advantageous in the 5'-chloro-5'-deoxy analog of araC, this compound appears interesting for further evaluation. It was found that this compound[8] may be transformed to another araC analog—2',5'-anhydro-araC. Experiments of DNA synthesis inhibition in the murine leukemia cell line L1210 showed a lower inhibition by 5'-chloro-araC in comparison with araC and a marginal effect of both 2',5'-anhydro-araC and 5'-Cl-cytidine. The results with 5'-Cl-cytidine furthermore indicated that a part of the nucleoside moiety responsible for the cytotoxic activity of 5'-chloro-araC is not the chlorine atom at the 5' position.

Similar results as in the DNA synthesis inhibition experiments were obtained when the inhibition of cell growth was determined in suspension culture and in the soft agar assay. An interesting finding is that the ratio of anticancer drug activity of araC and 5'-chloro-araC in suspension culture is about 13:1 but in the soft agar assay the activity of both compounds differs only by a factor of 3. This variation may be explained by the time necessary for both kinds of experiments. The duration of the suspension culture test is approximately 3–6 days, whereas the soft agar assay lasts about 10–12 days. A plausible explanation would be that in the soft agar assay, 5'-chloro-araC is phosphorylated to a greater extent, thus demonstrating a stronger growth inhibitory effect than in the suspension culture test.

The stability of 5'-chloro-araC in solution was examined at different pH and temperature conditions using HPLC. The values of the rate constant of the transformation reaction to 2',5'-anhydro-araC suggest that the electronegative chlorine group is capable of being displaced by RO^- in a nucleoside sugar moiety more easily in alkaline conditions and at higher temperatures. It was found that under physiological conditions, 5'-chloro-araC (37°C, pH 7.2) has a high stability with a half-time of transformation to 2',5'-anhydro-araC of 220 days.

The course of rate-constant changes indicated that the transformation reaction has pseudomonomolecular character at a pH higher than 9 and that the character of this reaction changes at a pH lower than 9. The transformation of 5'-chloro-araC to its 2',5'-anhydro product is so slow that this would not influence or modify the biological activity of 5'-chloro-araC in different test systems.

It has been indicated in the literature,[7] that the presence of 5'-hydroxyl is a basic condition for the biological activity of araC analogs. For assessment of the cytotoxic effect of 5'-chloro-araC we tried to measure the araCTP formation from 5'-chloro-araC and compared it with the araCTP formation from araC. We found that araCTP is being formed from 5'-chloro-araC, thus the absence of the hydroxyl group at the 5' position influenced only the amount of araCTP formation but not the mechanism of action of this compound which remains the same as for araC. The slower formation of araCTP from 5'-chloro-araC in relation to araC explains the results of a lower activity of 5'-chloro-araC in DNA inhibition in suspension culture, in the soft agar assay as well as in the Bactec system experiments.

We have previously shown[5] that 5'-chloro-araC is characterized by a better transport through intestinal cells than araC. Therefore the analog may be a suitable candidate for an oral drug formulation. Due to its lipophilicity this compound would probably have a bigger distribution volume in the human organism and may serve as a depot for araCTP formation. However, this potential advantage of 5'-chloro-araC cannot be seen in experimental systems where distribution and elimination of drug as factors important for its action are eliminated. The lower anticancer activity of this drug in the various test systems is likely to be related to the slower araCTP formation and not to reflect the real activity in an *in vivo* system.

These results indicate that 5'-chloro-araC de-

serves further investigation as a potential new anticancer compound.

References

1. Chabner BA. Cytosine arabinoside. In: Chabner B, Saunders WB, eds. *Pharmacologic Principles of Cancer Treatment.* Philadelphia: 1982: 387–401.
2. Ho DHW, Freireich EJ. Clinical pharmacology of arabinosylcytosine. In: Sartorelli AC, Johns DG, eds. *Antineoplastic and Immunosuppressive Agents, Part II.* Berlin: Springer 1975: 257–67.
3. Ho DHW. Distribution of kinase and deaminase of 1-β-D-arabinofuranosylcytosine in tissues of man and mouse. *Cancer Res* 1973; **33**: 2816–20.
4. Novotný L, Farghali H, Jankü I, Beránek J. Comparative study of cyclocytidine and arabinosylcytosine disposition in rats. *Neoplasma* 1988; **35**: 707–15.
5. Novotný L, Farghali H, Ryba M, *et al.* Structure-intestinal transport and structure-metabolism correlations of some potential cancerostatic pyrimidine nucleosides in isolated rat jejunum. *Cancer Chemother Pharmacol* 1984; **13**: 195–9.
6. Kára J, Bártová M, Ryba M, *et al.* Sensitivity of some arabinosylcytosine derivatives to enzymatic deamination by cytidine deaminase from mouse kidney. *Collect Czech Chem Commun* 1982; **47**: 2824–30.
7. Chu NY, Fischer GA. A proposed mechanism of action of 1-β-D-arabinofuranosylcytosine as an inhibitor of the growth of leukemic cells. *Biochem Pharmacol* 1962; **11**: 423–30.
8. Hřebabecký H, Brokeš J, Beránek J. Preparation of the 1-(2,5,anhydro-β-D-arabinofuranosyl) derivatives of cytosine and uracil and their cleavage with hydrogen bromide. *Collect Czech Chem Commun* 1982; **47**: 2961–8.
9. Hřebabecký H, Brokeš J, Beránek J. Reaction of nucleosides with thionyl chloride, preparation of the deoxy derivatives of cytidine and adenosine. *Collect Czech Chem Commun* 1980; **45**: 599–605.
10. Brokeš J, Beránek J. Synthesis of 1-β-D-arabinofuranosylcytosine. *Collect Czech Chem Commun* 1974; **39**: 3100–8.
11. Lozzio GB, Lozzio BB. Human chronic myelogenous leukemia cell line with positive Philadelphia chromosome. *Blood* 1975; **45**: 321–34.
12. Plunkett W, Chubb S, Alexander L, Montgomery JA. Comparison of the toxicity and metabolism of 9-β-D-arabinofuranosyl-2-fluoroadenine, and 9-β-D-arabinofuranosyladenine in human lymphoblastoid cells. *Cancer Res* 1980; **40**: 2349–55.
13. Plunket W, Hug V, Keating MJ, Chubb S. Quantitation of 1-β-D-arabinofuranosylcytosine 5'-triphosphate in the leukemic cells from bone marrow and peripheral blood of patients receiving 1-β-D-arabinofuranosylcytosine therapy. *Cancer Res* 1980; **40**: 588–91.
14. Fogh J, Trempe G. New human tumor cell lines. In: Fogh J, ed. *Human Tumor Cells* in vitro. New York: Plenum Press 1975: 115–59.
15. Quinn LA, Moore GE, Morgan RT, Woods LK. Cell lines from human colon carcinoma with unusual cell products, double minutes, and homogeneously staining regions. *Cancer Res* 1979; **39**: 4914–24.
16. Spremulli EN, Scott C, Champbell DE, *et al.* Characterization of two metastatic subpopulations originating from a single human colon carcinoma. *Cancer Res* 1983; **43**: 828–35.
17. Scheithauer W, Clark G, Moyer MP, Von Hoff DD. New screening system for selection of anticancer drugs for treatment of human colorectal cancer. *Cancer Res* 1986; **46**: 2703–8.
18. Novotný L, Reichelová V, Balážová E, Ujházy V. Comparison of some biochemical parameters of arabinosylcytosine and cyclocytidine in L1210 murine leukemia cells. *Neoplasma* 1990; **37**: 13–22.

(Received 28 May 1991; in revised form 8 August 1991; accepted 28 August 1991)