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Short Communication

Liquid chromatographic study of acid stability of 2-chloro-2′-*arabino*-fluoro-2′-deoxyadenosine, 2-chloro-2′-deoxyadenosine and related analogues*

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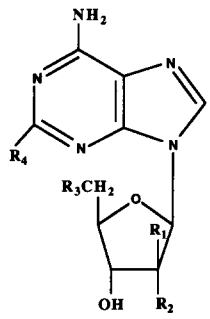
Introduction

Purine nucleosides and their analogues have often been used in the cancer therapy. Naturally occurring deoxynucleosides (dAdo, Fig. 1) cause the death of lymphocytes in hereditary adenosine deaminase (ADA) deficiency. 2-Chloro-2′-deoxyadenosine (CdA; Fig. 1), the nucleoside analogue resistant to deamination by ADA appeared to be effective in the treatment of hairy cell leukemia, chronic lymphocytic leukemia, low grade lymphomas, childhood acute myelogenous leukemia [1, 2] and it has also currently been in phase I trials in the treatment of solid tumours [3]. Oral administration of CdA is an attractive option because of its feasibility and cost effectiveness of the overall therapy. However, the oral bioavailability of CdA was estimated to be only approximately 50% [4], partly due to the acid instability of the glycosidic linkage.



Compound	R_1	R_2	R_3	R_4
dAdo	H	H	OH	H
CdA	H	H	OH	Cl
F-ara A	OH	H	OH	F
2-Cl-Ado	H	OH	OH	Cl
5′-Cl-5′-dAdo	H	OH	Cl	H
CAFdA	F	H	OH	Cl

Figure 1
Structural formulae of compounds studied.

The acid-catalysed hydrolysis of purine nucleosides is thought to proceed by an A-1 mechanism in which the protonated nucleoside dissociates in the rate-controlling step to a glycosyl carbonium ion and free purine [5]. Removal of the 2′- or 3′-, and to a lower extent also 5′-hydroxyl enhances the rate of hydrolysis. On the other hand, the introduction of an electronegative fluorine atom at position C-2′ decreases the rate of hydrolysis due to a destabilization of the resulting oxocarbonium ion [6]. Recently, the 2′-*arabino*-fluoro derivative of CdA, termed 2-chloro-2′-*arabino*-fluoro-2′-deoxyadenosine (CAFdA; Fig. 1), was found to be much more stable than CdA at pH 2.0. CAFdA was shown to be directly toxic to human quiescent lymphocytes and mono-

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cytes and it was regarded to be a potential substitute for CdA as an effective oral drug [7].

There is no report in the literature on the rate constants of the acid-catalysed hydrolysis of CdA and CAFdA. The present study was undertaken to compare the stability of CAFdA, CdA, dAdo and other analogues including 2-Cl-Ado, 5′-Cl-5′-dAdo and clinically used F-araA under conditions which roughly approximate the human stomach environment (pH 1.0, 37°C). Reversed-phase liquid chromatography (R-PLC) was utilized since it allows a selective quantification of both studied compounds and degradation products in one analysis.

Experimental

Materials

CdA and 2-chloroadenine (2-Cl-Ade) were provided by Dr Zygmunt Kazimierczuk (Foundation for the Development of Diagnostics and Therapy, Warsaw, Poland). CAFdA was a gift from Dr Howard Cottam (University of California, San Diego, CA, USA) and F-araA was a gift from Dr Ze've Shaked (Berlex, Alameda, CA, USA). The nucleosides dAdo, 2-Cl-Ado and 5′-Cl-5′-dAdo were obtained from the Sigma Chemical Company (St Louis, MO, USA). Methanol was of HPLC grade (J.T. Baker, Deventer, the Netherlands). Analytical-reagent grade potassium dihydrogen phosphate and potassium hydroxide (KOH) were purchased from Merck (Darmstadt, Germany). The base adenine (Ade) was from the Sigma Chemical Company.

Liquid chromatography

The chromatographic system consisted of a CM400 pump (Milton Roy, LDC Division, USA), a CMA-240 Carnegie autosampler (Carnegie Medicine, Stockholm, Sweden) and a Milton Roy variable wavelength detector (Milton Roy, LDC Division, USA). A Macintosh Classic computer (Apple Inc., Chicago, IL, USA) equipped with Chromac 3.1. software (Drew Ltd, London, UK) was used for collecting the LC data. The analyses were performed isocratically on a high speed C_{18} column (80 × 4.6 mm, 3 μm, Perkin–Elmer, Norwalk, CT, USA) at 265 nm. An aqueous mobile phase consisting of a potassium phosphate buffer (pH 6.8; 10 mM)–methanol (80:20, v/v) at the flow rate of 1 ml min^{-1} was used. The pH was adjusted with a few drops of potassium hydroxide before methanol was added using a PHM 62 standard pH meter (Radiometer, Copenhagen, Denmark). The capacity factors, k', were calculated as $(t_r - t_0)/t_0$, where t_r is the retention time of an individual compound and t_0 is the retention time of an unretained compound which was determined as the time from injection to the first distortion of the baseline.

Determination of the ionization constants (pK_a)

The pK_a values of CAFdA and other analogues were determined spectrophotometrically as described in a previous study [8].

Acid stability study

Stock solutions of the studied compounds were diluted 20 times in a prewarmed (37°C) solution of hydrochloric acid (0.1 M and 1 M HCl) to yield a concentration of 5 μM and the acid hydrolysis was followed at 37°C. Aliquots of 15 μl were withdrawn, neutralized with 0.1 M KOH (20 μl), chilled on ice and analysed by LC. Each kinetic analysis was performed in duplicate. Natural logarithms of

Table 1
Apparent first-order rate constants, and half-lives for acid hydrolyses of protonated nucleoside analogues at 37°C in 0.1 M HCl and 1.0 M HCl. Results (k, s^{-1}) are the average of two experiments (with 4 to 8 time points in each experiment)

Compound	pK_a	k (s^{-1}) 0.1 M HCl	$t_{1/2}$ (h)	k (s^{-1}) 1 M HCl	$t_{1/2}$ (h)
dAdo	3.48*	7.14×10^{-4}	0.27	6.14×10^{-3}	0.031
CdA	1.28*	4.42×10^{-4}	0.44	4.23×10^{-3}	0.046
F-araA	0.79*	2.03×10^{-6}	94.8	1.12×10^{-5}	17.2
2-Cl-Ado	0.9*	5.78×10^{-7}	333.0	6.23×10^{-6}	30.9
5′-Cl-5-dAdo	3.80*	3.43×10^{-7}	561.2	3.52×10^{-6}	54.7
CAFdA	1.75	8.91×10^{-8}	2160.5	3.15×10^{-7}	611.1

*Data taken from [8].

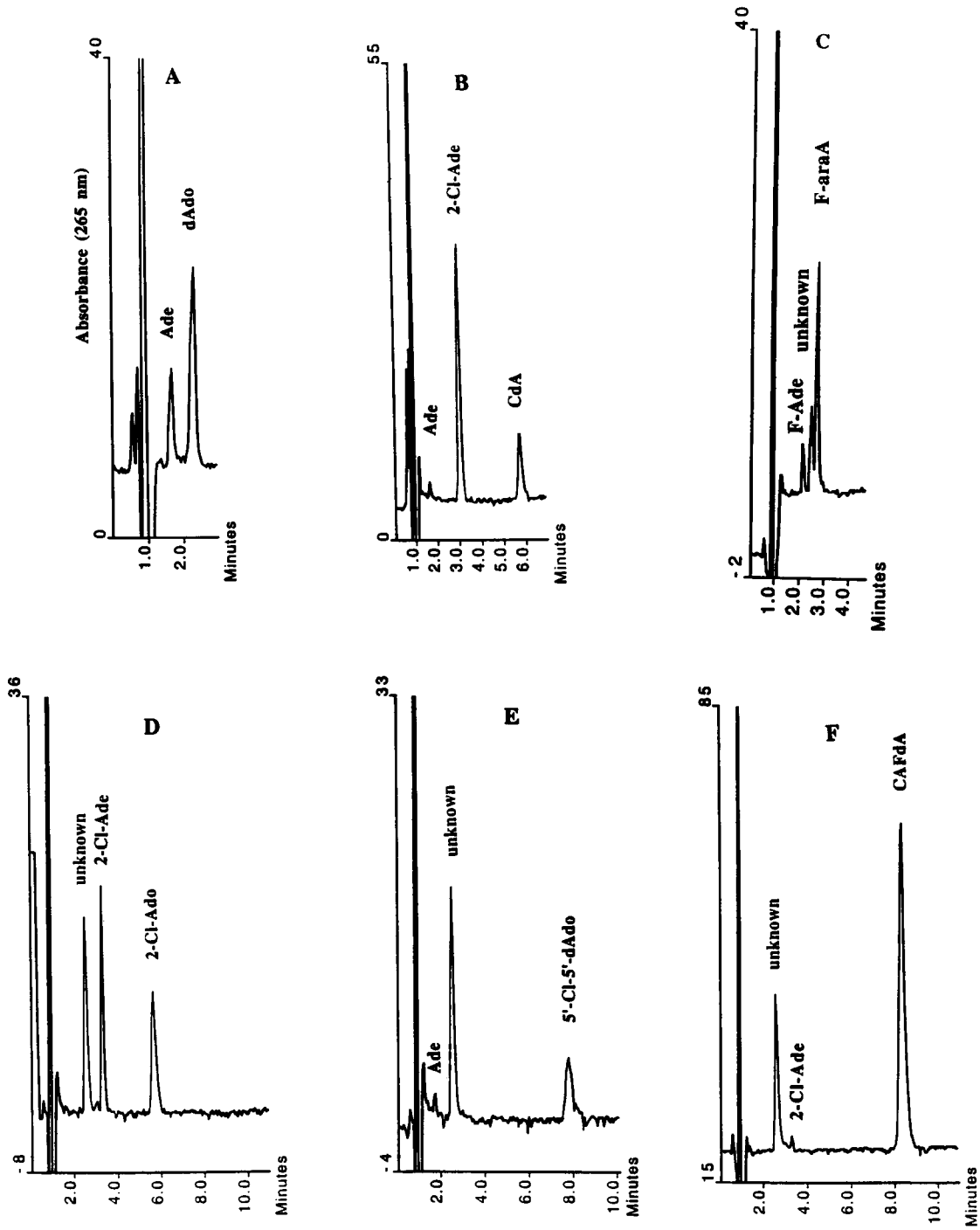

Figure 2
Reversed-phase liquid chromatograms of purine nucleoside analogues and their degradation products in the acid solution (pH 1.0, 37°C): dAdo (A), CdA (B), F-araA (C), 2-Cl-Ado (D), 5′-Cl-5′-dAdo (E), CAFdA (F). Chromatographic conditions as in Experimental.

peak areas of remaining compounds were plotted as a function of time and the slopes of these linear plots corresponded to the apparent first-order rate constants, k, (s^{-1}).

Results and Discussion

The apparent first-order rate constants of acid hydrolysis of purine nucleoside analogues

are presented in Table 1. Since the kinetic analyses were conducted either below or very close to the pK_a of the nucleosides, the constants refer to the acid-catalyzed hydrolyses of the protonated nucleosides.

The degradation rate of dAdo and CdA at pH 1.0 and 37°C were of approximately the same magnitude (7.14×10^{-4} s^{-1} for dAdo and 4.42×10^{-4} s^{-1} for CdA). A similar ratio of degradation rates of structurally related nucleosides 2′, 3′-dideoxyadenosine (ddA) and its 2-chloro analogue (ClddA) at pH 1 and 25°C could be derived from recently reported data (5.1×10^{-3} s^{-1} for ddA vs 2.5×10^{-3} s^{-1} for ClddA) [9, 10]. When analogues with a halogen atom (Cl, F) in the position C-2 of the purine ring were compared (CdA and F-araA), the stabilizing effect of the 2′-*arabino* hydroxyl group was confirmed. The half-life of F-araA was about 216-fold that of CdA (94.8 h for F-araA vs 0.44 h for CdA). On the other hand, only a minor difference in half-lives was observed between F-araA and 2-Cl-Ado (94.8 h for F-araA and 333.0 h for 2-Cl-Ado) with fully hydroxylated sugars, but of different configuration. The 5′-Cl-5′-dAdo with the chlorine atom in the position C-5′ (the half-life of 561.2 h) had a slightly higher stability (1.7-fold) than 2-Cl-Ado with the chlorine in the purine ring. The most stable compound in the studied group turned to be CAFdA. The presence of a fluorine atom at the position C-2′ in the molecule of CAFdA decreased the degradation rate in acid (pH 1.0) compared to CdA by the factor of 5.0×10^3 (8.91×10^{-8} s^{-1} for CAFdA vs 4.42×10^{-4} s^{-1} for CdA).

The stability of studied analogues was also assessed in the solutions of 1 M HCl, since this stronger acid was used as the solvent for the spectrophotometric determination of the pK_a, when $pK_a < 2$ was expected. The degradation of most compounds in 1 M HCl was about 10-times faster than in 0.1 M HCl (Table 1).

Some discrepancies were observed between previously published rate constants of the acid stability of dAdo (1.04×10^{-3} s^{-1}) and the present study (7.14×10^{-4} s^{-1}). While a selective and quantitative LC method was used in the present study, a spectrophotometric absorbance recording after adjusting the pH of the reaction mixture samples of pH 12–13 was performed [11]. Though the solvolysis of the nucleosides under this condition was reported by previous investigators to be negligible it may bring about the observed differences between rate constants determined by different methods.

The LC method used for the study of the acid-catalysed hydrolysis of nucleoside analogues allowed a simultaneous determination of the parent compounds and their degradation products as well (Fig. 2). The purine base Ade was the only degradation product of dAdo (Fig. 2A). In the case of CdA the main product was 2-chloroadenine, but some traces of Ade were also observed (Fig. 2B). The degradation products of F-araA, 2-Cl-Ado, 5′-Cl-5′-dAdo and CAFdA were surprisingly different from those expected. Besides the expected bases, an unknown peak with a capacity factor of 2.75 appeared in all above mentioned solutions, but not in solutions of dAdo and CdA. It did not correspond to any of expected purine bases, either Ade, F-Ade or 2-Cl-Ade (Fig. 2C–F) and its identity has not been clarified in the present study. It seems to be a degradation product of respective bases since the same peak was observed in the profile and acid hydrolysis of 2-Cl-Ade (data not shown). The fact that the acid hydrolysis of dAdo and CdA was only followed during one to two hours may explain the absence of the unknown peak which was found after 24 h of hydrolysis of 2-Cl-Ade.

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