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Bioavailability and bacterial degradation of rectally administered 2-chloro-2′-deoxyadenosine*

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Abstract: 2-Chloro-2′-deoxyadenosine (CdA) is a new drug for the treatment of hairy cell leukemia and other lymphoproliferative diseases. It is generally administered as a continuous intravenous infusion during 5–7 days. The oral bioavailability is only 50%. The bioavailability after rectal administration was investigated in two patients with chronic lymphocytic leukemia. Five milligrams per square metre was given i.v. as a 2-h infusion and 24 h later the same dose was administered rectally in a gel formulation. The mean bioavailability was only 21% due to deglycosylation of CdA to 2-chloroadenine (CAde). To further elucidate the factors which are important for the rectal availability of CdA, the *in vitro* stability of CdA in bacterial cultures was tested. *Clostridium perfringens* and *Escherichia coli* as well as whole feces rapidly deglycosylated CdA to CAde while *Bacteroides fragilis*, *Enterococcus faecalis* as well as saliva only degraded CdA slowly or not at all. It is concluded that, due to bacterial degradation, rectal administration of CdA has no advantage over oral administration.

Keywords: *Cladribine; pharmacokinetics; rectal administration; humans; bioavailability; 2-chloroadenine.*

Introduction

CdA (2-chloro-2′-deoxyadenosine, Cladribine, Fig. 1) is a recently-developed nucleoside analogue with very promising activity in lymphoproliferative disorders [1]. Only one treatment course is needed to achieve a complete response in most patients with hairy cell leukemia. In chronic lymphocytic leukemia, however, repeated courses are needed for complete response [2, 3]. In this setting, the recommended mode of administration, continuous infusion over 7 days is not ideal, and alternate modes of administration, like the oral route and subcutaneous injection, have therefore been investigated [4]. The bioavailability of oral CdA was only 50% and further studies in order to improve the bioavailability of non-parenterally administered CdA are warranted. Rectal administration has improved the bioavailability of 6-mercaptopurine significantly [5]. The aim of the present study was therefore to investigate whether the bioavailability of CdA could be improved by rectal administration.

Materials and Methods

Materials

CdA was synthesized by Dr Zygmunt Kazimierczuk (the Foundation for Development of Diagnostics and Therapy, Warsaw, Poland) and a sterile solution 2 mg ml^{-1} in 9 mg ml^{-1} saline was prepared at the pharmacy of Huddinge Hospital. The gel for rectal administration was prepared by dissolving CdA 2.04 mg ml^{-1} in 9 mg ml^{-1} saline and then add 25 mg ml^{-1} hydroxyethyl-cellulose (Natrosol®) while stirring. The CdA concentration was adjusted to 2.00 mg ml^{-1}. CdA was stable in this formulation at 4 and 25°C for at least 2 weeks.

Patients

The study was approved by the ethics committee at the Karolinska institute (93/62)

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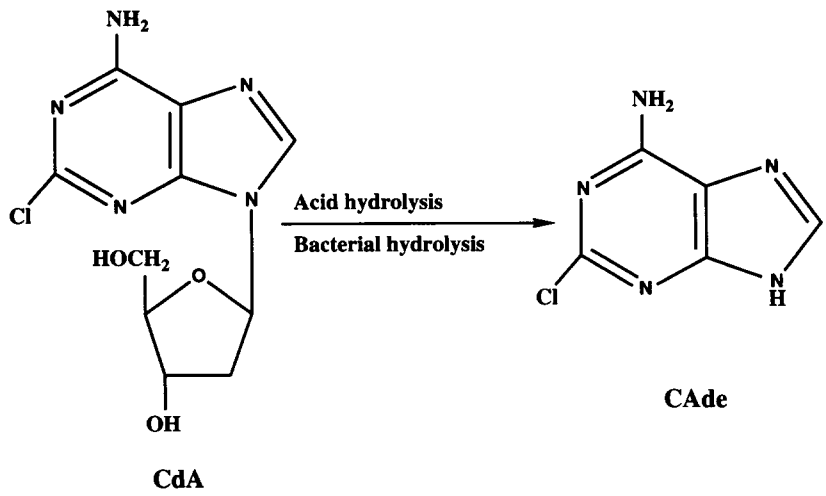

Figure 1
The chemical structure of CdA and its degeneration product 2-chloroadenine.

and by the Swedish Medical Product Agency (93/324). Two male patients, 50 and 73 years old with chronic lymphocytic leukemia gave their oral informed consent to participate in the study. They were given 5 mg m^{-2} CdA as a 2 h i.v. infusion and 24 h later 5 mg m^{-2} rectally using a syringe with an adapter for rectal drug application.

Blood sampling

Blood samples were drawn in heparinized glass tubes from a separate venous access before and 60, 120, 135, 150, 165 and 180 min, and 4, 6, 9, 12, 20 and 24 h after the i.v. infusion. Heparinized blood was also collected 5, 15, 30, 45, 60, 90, 120 and 180 min, and 4, 6, 9, 12, 20 and 24 h after the rectal application. The samples were kept on ice until processed. Plasma collected by centrifugation (7 min, 550*g*, 4°C) and stored at −20°C until analysis.

Drug assay

The plasma concentration of CdA and the deglycosylated metabolite 2-chloroadenine (CAde, Fig. 1) was determined by reversed-phase high-performance liquid chromatography using a solid phase extraction [6]. The limit of detection was 1 nM and 2 nM for CdA and CAde, respectively. The relative standard deviation was less than 10% for both compounds.

Pharmacokinetic calculations

The AUC was calculated by trapezoidal rule and extrapolation to infinity using log-linear regression analysis of at least four point of the elimination phase (Siphar software, Societé Simed, Creteil, France). The residual area of CdA and CAde from first dose was subtracted from the AUC of second dose.

Bacterial strains and in vitro *study*

Pure cultures of *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* NCTC 370 were grown aerobically and pure cultures of *Bacteroides fragilis* ATCC 25285, *Clostridium Perfringens* ATCC 13124, and *Lactobacillus fermentum* 8378 were grown aerobically to approximately 10^6 CFU ml^{-1} in Brain Heart Infusion broth (Oxoid, Basingstoke, Hants, UK). Ten per cent 2.0 M phosphate buffer, pH 7.2, was added to all bacterial suspensions before CdA incubation. Saliva from five healthy donors was mixed with 10% 2.0 M phosphate buffer pH 7.2 and mixed fecal samples from three healthy donors was suspended 1:5 in 0.2 M phosphate buffer, pH 7.2. CdA was added to each vial to a final concentration of 200 or 20 μg ml^{-1}. Duplicate samples were incubated at 37°C during gentle shaking for 10, 20, 30, 60 and 120 min. CdA incubated in buffer and saliva-, faecal-, and bacterial suspensions without CdA were run consecutively as controls. The reactions were stopped by boiling for 2 min. CdA was stable at 100°C (data not shown). Samples were centrifuged at 2000*g* in 15 min and the supernatant was stored at −20°C until assayed. The pH was never <5.5 at any time in any of the vials. The CdA and CAde concentration in the supernatant was determined with the same method as plasma samples.

Results

The bioavailability of CdA after rectal administration was 30.4 and 10.6% in the two patients tested (Fig. 2). The concentration of CAde, however, was considerably higher after rectal administration (AUC 266 and 1284 nMh) compared to the i.v. 2 h infusion (AUC 108 and 253 nMh). The residual area after the first dose was 12.5 and 4.5% for CdA and 4.7 and 0.1% for CAde. *In vitro*, CdA was rapidly deglycosylated to CAde by *C. perfringens* and *E. coli* while *B. fragilis* and *E. faecalis* degraded CdA only to a minor extent. *L. fermentum* exerted an intermediate activity (Fig. 3a and b). The process was more rapid at a low CdA concentration of 20 μg ml^{-1} as compared to 200 μg ml^{-1}, indicating that the degradation is saturable. The major product of degradation is CAde (Fig. 3c and d) but in cultures of *C. perfingens*, however, there was also a clear degradation of CAde to an as yet unknown metabolite(s).

Human saliva did not degrade CdA significantly (<5% during 2 h, Fig. 4a) while <10% of CdA remained unchanged after 60 min when 200 μg ml^{-1} was incubated in human faeces (Fig. 4b).

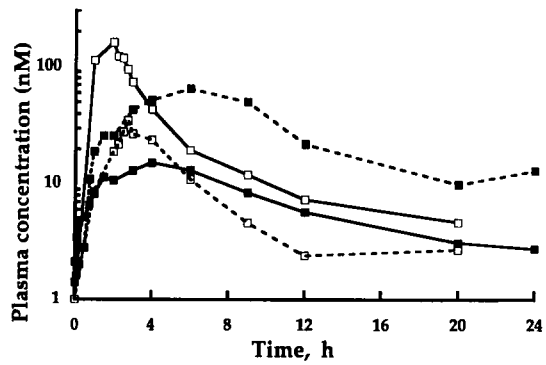

Figure 2
CdA (———) and 2-chloroadenine (– – – –) concentration in plasma after a 2 h intravenous infusion (□) and rectal administration of 5 mg m^{-2} CdA (■).

Discussion

CdA is a major new drug for the treatment of lymphoproliferative neoplasms [1]. It is the treatment of choice for hairy cell leukemia inducing complete remission in 80% of patients with only one week of treatment and has very

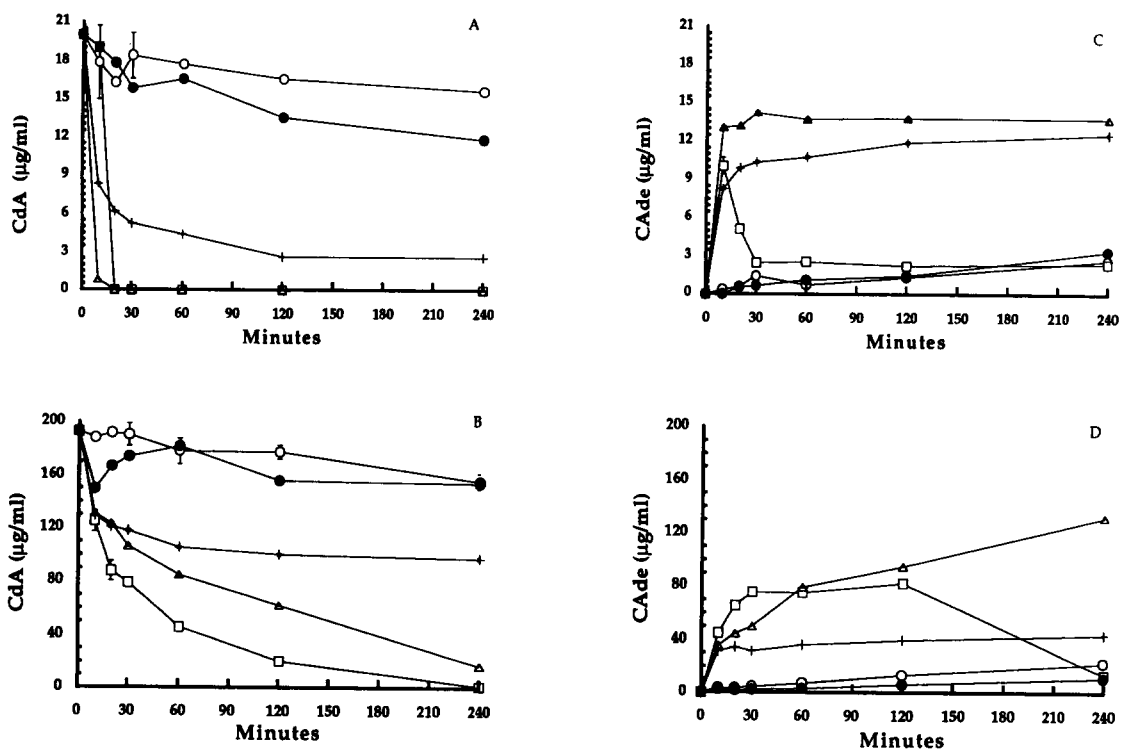

Figure 3
The concentration CdA in cultures of intestinal bacteria at 20 (A), 200 μg ml^{-1} (B) and concentration of 2-chloroadenine in cultures of intestinal bacteria incubated in 20 (C) and 200 μg ml^{-1} (D) CdA. *B. Fragilis* (○), *C. Perfringens* (□), *L. Fermentum* (+), *E. Coli* (△) and *E. Faecalis* (●).

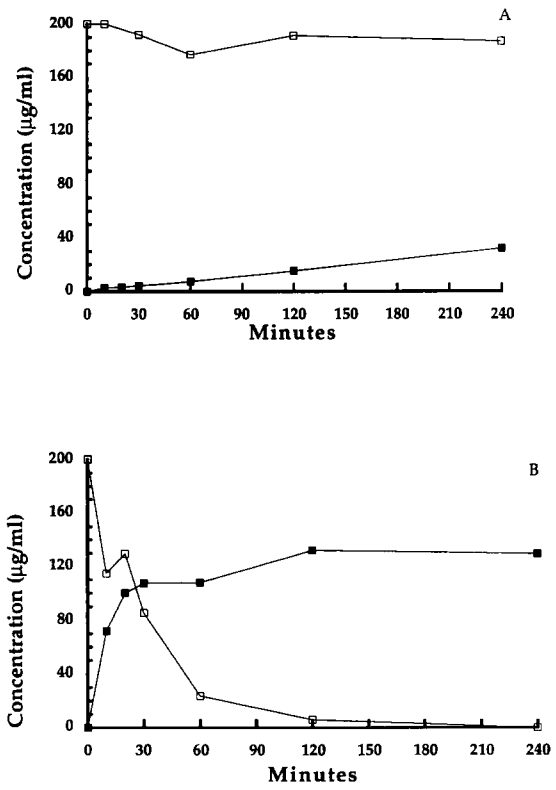

Figure 4
The concentration of CdA (□) and 2-chloroadenine (■) in saliva (A) and human faeces (B) incubated with 200 μg ml^{-1} CdA.

promising activity in chronic lymphocytic leukemia. From *in vitro* data it is apparent that a continuous exposure is important for its cytotoxic effect [7]. Early pharmacokinetic investigations revealed a rapid distribution phase, and due to the low sensitivity of the RIA method used, plasma CdA levels were undetectable beyond 2 h from the end of a 2 h infusion [8]. The overwhelming bulk of experience with this drug is therefore gained using continuous i.v. infusion which has remained the only recommended mode of administration. We could, however, show that there is a slow elimination phase with a $t_{½}$ of 7–10 h [4, 9], which was recently confirmed by others [10, 11, 12]. Furthermore, CdA is a prodrug and intracellular phosphorylation to nucleotides is required for cytotoxic activity [13]. The retention of these nucleotide metabolites in leukemic cells *in vivo* is very long, with a half-life of 15–30 h [14]. Thus, there is a large bulk of data supporting the use of intermittent administration of CdA. This statement is also supported by several recent clinical trials in hairy cell and chronic lymphocytic leukemia [2, 15, 16]. To further improve the feasibility of treatment with CdA, we have investigated alternate routes of administration. While s.c. injection has a bioavailability of 100%, oral administration yields only 50% bioavailability [4, 17]. There are a number of possible explanations for the limited bioavailability of oral CdA.

CdA is unstable in acid. Less than 20% of CdA remains intact after 1 h in HCl pH 1 (data not shown). However, the importance of this is uncertain because the absorption of orally administered CdA is rapid, $T_{max} \approx 20$ min [4] and very little improvement of the bioavailability was found when patients were pretreated with omeprazole, an acid secretion blocking agent [17].

Preliminary, unpublished data from this laboratory show that the first pass effect of CdA in perfused isolated rat liver is important, approximately 50% with CAde as the major degradation product (F. Albertioni *et al.*, in preparation).

Another purine analogue, 6-mercaptopurine is subject to extensive first pass metabolism and its oral bioavailability is 16% [18]. With rectal administration, avoiding the portal blood flow, the bioavailability was improved to ≈80% [5].

However, despite avoiding the first pass effect and the acid environment of the stomach by rectal administration of CdA, the bioavailability is only about 20%. We had planned to include at least six patients in the study. However, when the results from the first two patients, presented here, emerged, it was decided to terminate the study because it was obvious that this mode of administration was not superior to the more feasible oral administration. One plausible explanation for the low bioavailability is that CdA is degraded to CAde already in the rectum. By studying the effect on CdA of mixed faecal suspension and of five different bacterial strains representing the main bacterial groups in the lower intestinal tract, we can show that this is indeed a likely explanation. The high concentration of CAde in plasma after rectal administration also supports this statement. The concentration of CAde in plasma after rectal administration is also considerably higher than after oral administration (F. Albertioni *et al.*, in preparation). An alternate explanation could be a limited absorption of CdA through the rectal mucosa. The surface area of the rectal mucosa

is small compared to the stomach or the small intestine. However, the high plasma concentration of CAde does not favour this explanation.

Conclusion

The low (21%) and variable bioavailability of rectal CdA is probably due to bacterial degradation to CAde in the rectum.

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