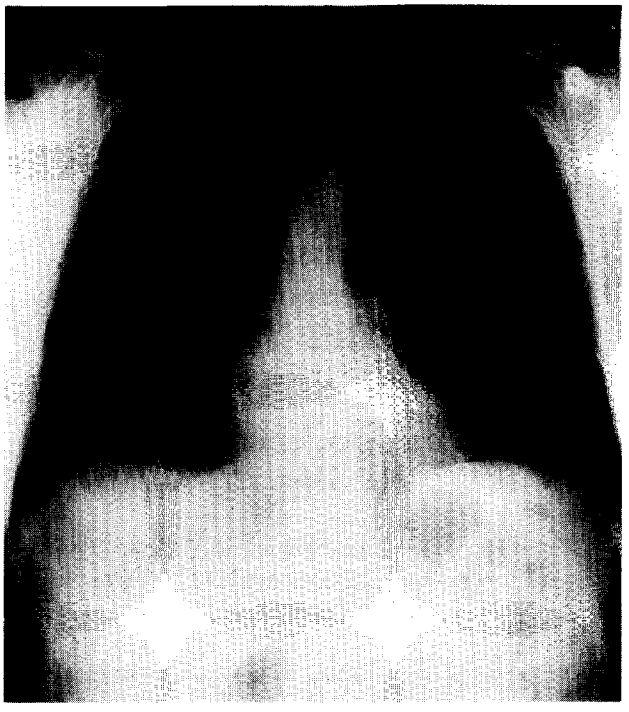

Figure 1. Chest X-ray: left-sided pneumothorax with shrinkage of the mass.

complicated by pneumothoraces, even if there is a clear predominance with metastatic osteogenic and childhood sarcomas, germ cell tumours and lung cancer [4]. The mechanisms advocated depend upon the underlying tumour. In lung cancer, spontaneous pneumothorax may be consecutive, either to various pathological alterations secondary to smoking, or to the progression of the tumour [1, 3]. In sarcomas and germ cell tumours, spontaneous pneumothoraces are more likely induced by tumour necrosis or spontaneous haemorrhages [5]. It can also occur some months or years after irradiation or chemotherapy, in association with treatment, pathological changes rendering the lung more prone to develop spontaneous pneumothorax [2, 6].

Our case fits well with a rapid chemotherapy-induced tumour shrinkage secondary to necrosis. Rosen and colleagues first noted that the risk of spontaneous pneumothorax was higher with efficient chemotherapy [7]. The average time from the initiation of the chemotherapy to pneumothorax development ranges from 1 to 32 days. Several mechanisms have been proposed to explain chemotherapy-induced spontaneous pneumothorax: an underlying co-existent emphysema [4, 9]; the rupture of a peripheral chemosensitive tumour, resulting in a leakage of air into the pleural space [6]; the enlargement of a rapidly necrotising tumour; the combination of necrosis and chemotherapy-induced impairment of repair processes (e.g. doxorubicin) [8–10]; the elevation of intrathoracic pressure due to drug-related emesis [4].

Spontaneous pneumothorax is a well known, but rare, complication occurring during chemotherapy for cancer. This complication should be kept in mind, especially in tumours known to be chemosensitive. Indeed, it may be a sign of tumour response and explain the appearance of a shortness of breath and pulmonary pain shortly after chemotherapy without conferring a bad prognosis to the patient.

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2-Chlorodeoxyadenosine Inhibits Activity of Adenosine Deaminase and S-Adenosylhomocysteine Hydrolase in Patients With Chronic Lymphocytic Leukaemia

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2-CHLORODEOXYADENOSINE (2-CdA) is a new and effective drug for indolent lymphoid malignancies. However, mech-

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anisms which link 2-CdA action and tumour cell death are not yet fully clarified. 2-CdA is rapidly taken up by the target cells and phosphorylated by cytosolic deoxycytidine kinase (dCK). Its triphosphate is a potent inhibitor of human ribonucleotide reductase and a good substrate for human DNA polymerases [1]. However, the phosphorylation of 2-CdA is clearly not the only event responsible for its cytotoxic effect. The 2-CdA phosphorylation in hairy cell leukaemia (HCL) is not higher than in chronic lymphocytic leukaemia (CLL), despite a better response to 2-CdA therapy. Using different cell lines, it has been shown that a wide range of cell sensitivity to 2-CdA could not be explained by different levels of 2-CdA nucleotide [2, 3]. 2-CdA has also been shown to inhibit the growth of myeloid progenitor cells in which the levels of dCK are low [4]. Finally, we have recently shown that *in vitro*, the inhibitory effect of 2-CdA results from suppression of adenosine deaminase (ADA) and S-adenosylhomocysteine hydrolase (SAHH) activity [5]. A similar effect has been observed in lymphoma cells obtained from a patient with central nervous system involvement [6]. Here, we examined the influence of 2-CdA on the activity of ADA and SAHH in CLL patients.

18 CLL patients were included in the study (mean age, 58 years; range, 35–84 years). 8 patients had Rai disease stage IV, 3 had stage III, 4 had stage II, and 3 patients had stage I. 4 patients had Coombs positive haemolytic anaemia before 2-CdA treatment. The drug was administered at a dose of 0.1 mg/kg/day by 2-h infusion for 7 consecutive days. 10 patients received 2-CdA as the first-line therapy. 8 patients had received other therapies prior to 2-CdA (Leukeran, COP or CHOP regimens), but all were refractory to those modalities. The homogenated erythrocyte lysates, obtained from the patients one day before, on the day of completion, and 2 weeks after 2-CdA administration, were used as a source of SAHH, ADA and 5′-NT. Their activity was assayed by radiochemical TLC analysis as previously reported [5]. Statistical analysis was performed using Student's *t*-test. The response criteria recommended by the National Cancer Institute-Sponsored Working Group were used [7]. The overall objective response to 2-CdA was 78%, including 4 patients who achieved complete remission (22%) and 10 patients with partial response (56%). 4 patients did not respond to 2-CdA. Upon drug administration, a marked decrease of ADA activity in the lysate of erythrocytes was observed in 15 out of 18 patients (Figure 1). Patients who responded to 2-CdA therapy were found to have more pronounced inhibition of ADA than non-responders (by 57% and 28%, respectively). The overall ADA activity before 2-CdA administration (mean, 62.9; range 22.1–128.0 nmol/mg Hb/h) was significantly higher as compared with the enzyme's activity on the day of completion (mean, 42.4; range, 18.9–85.1 nmol/mg Hb/h; $P < 0.003$) and 2 weeks after 2-CdA discontinuation (mean, 29.7; range, 14.1–51.2 nmol/mg Hb/h; $P < 0.001$) (Figure 1). Upon 2-CdA administration, a significant decrease of SAHH activity was also observed (Figure 2). Its overall activity in erythrocyte lysates before 2-CdA treatment was higher (mean, 10.8; range 3.1–37.5 nmol/mg Hb/h) compared with activity observed on the day of completion (mean, 8.3; range, 2.9–24.6 nmol/mg Hb/h; $P < 0.03$) and 2 weeks after the drug discontinuation

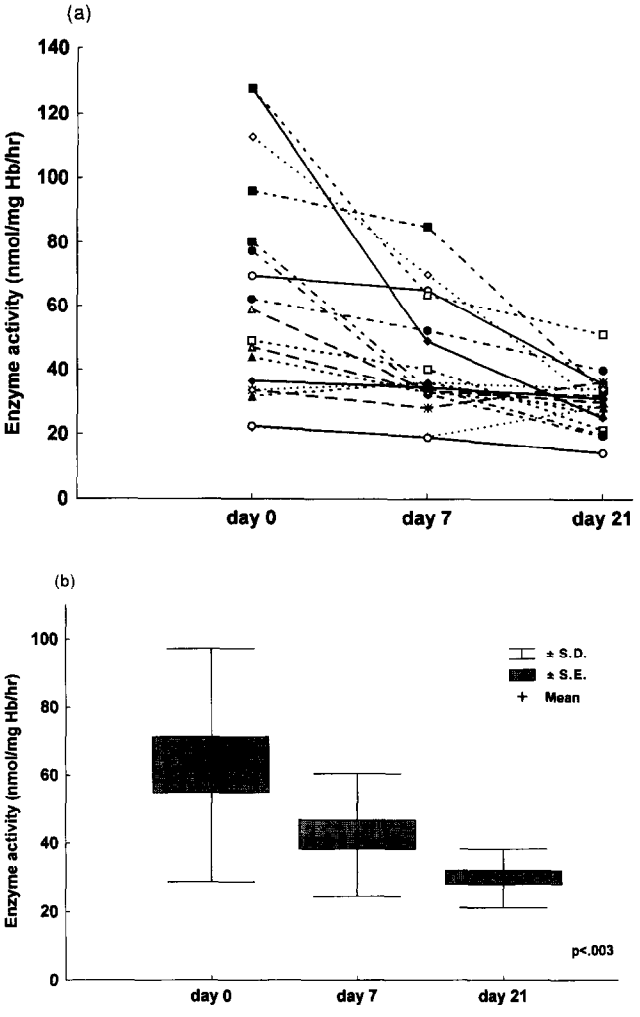

Figure 1. 2-CdA-related fluctuations of adenosine deaminase activity in erythrocyte lysate of each chronic lymphocytic leukaemia patient (a) and in the whole group of 18 patients studied (b). (Day 0 indicates the day before drug administration; day 7 indicates the day of completion of the treatment; day 21 indicates the time 2 weeks after 2-CdA discontinuation).

(mean, 6.6; range, 2.7–16.9 nmol/mg Hb/h; $P < 0.01$) (Figure 2). While 2-CdA non-responders were found to have only minimal fluctuations of SAHH activity, it was found to be reduced by 49.5% in patients who responded to 2-CdA treatment (Figure 2). Similar changes of ADA and SAHH activity were also observed in lysates of lymphocytes obtained from these patients, while 5′-NT activity did not change markedly, neither during the course nor after the 2-CdA therapy (data not shown).

The biochemical consequences of 2-CdA-induced suppression of ADA and SAHH might be numerous. Inhibition of ADA should lead to increases of intracellular levels of adenosine (Ado) and deoxyadenosine (dAdo), and consequently, to increased levels of ATP and dATP. However, the presence of 2-CdA contributes to a significant fall in intracellular levels of ATP and dATP [8]. This probably depends on complete 2-CdA-induced inhibition of deoxycy-

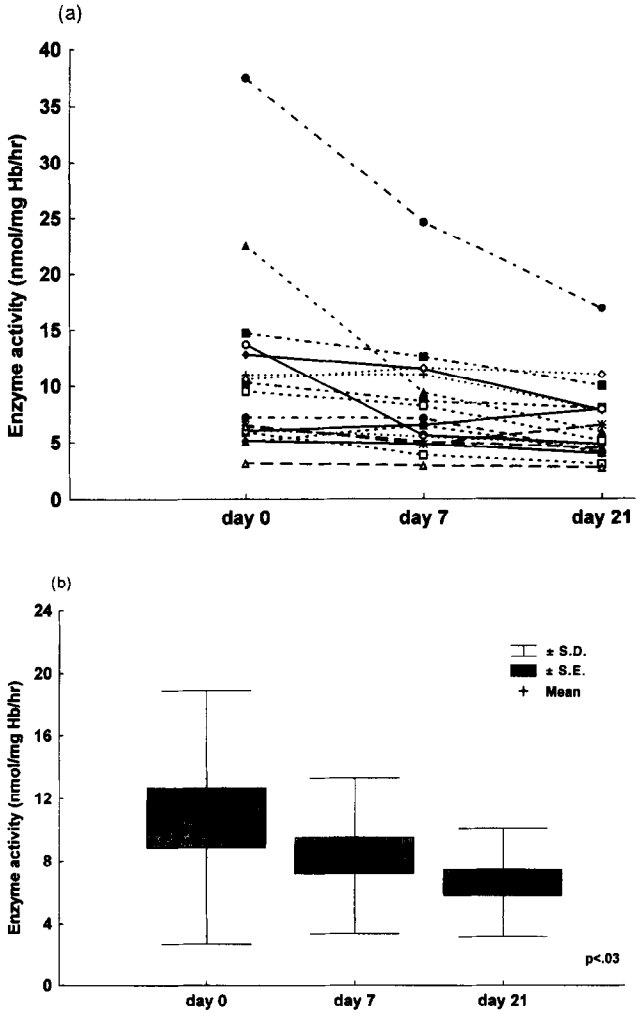

Figure 2. 2-CdA-related fluctuations of S-adenosylhomocysteine hydrolase activity in lysate of erythrocytes of each chronic lymphocytic leukaemia patient (a) and in the whole group of 18 patients studied (b). (Day 0 indicates the day before drug administration; day 7 indicates the day of completion of the treatment; day 21 indicates the time 2 weeks after 2-CdA discontinuation).

tidine kinase activity [6]. However, increased concentrations of Ado and dAdo induce significant inhibition of SAHH activity [9]. Inactivation of SAHH is associated with irreversible binding of NAD [10]. Thus, the observation that depletion of NAD levels is a 2-CdA-related event can be attributed not only to consumption of NAD by activated poly (ADP-ribose) polymerase [1], but also to inactivation of SAHH by non-metabolised dAdo. In addition, inhibition of SAHH results in enhanced intracellular concentration of S-adenosylhomocysteine (SAH), the substrate of the enzyme. SAH acts as a competitive inhibitor of most methylotransferases and suppresses methylation reactions in affected cells. The unmethylated 5'-cap structure may provide unstability and disturbances of splicing as well as binding of mRNA to ribosomes, and may result in decreased translation of the message [11]. Defective methylation of myelin basic proteins may induce demyelination of nerves [12], a frequently observed side-effect complicating therapy with purine analogues [13]. Finally, inactivation of SAHH results in reduced homocysteine formation which is a significant source of methionine in humans and the methyl acceptor in the methionine synthase reaction catalysing the conversion of 5-methyltetrahydrofolate to tetrahydrofolate. Tetrahydrofolate is required for folate-dependent purine and thymidylate synthesis, so depletion of homocysteine would, therefore, indirectly inhibit nucleotide synthesis and cellular proliferation [14]. All these mechanisms have been shown to be critical for cell survival, as an irreversible inhibitor of SAHH was found to inhibit proliferation of normal and transformed cell lines, and suppress T-cell activation [14, 15]

In view of the present study and previous *in vitro* results, we hypothesise that the cytotoxic mechanism of 2-CdA may originate not only in suppression of ribonucleotide reductase and DNA polymerases, but also in the inhibition of dAdo deamination and phosphorylation, with subsequent inactivation of SAHH as a natural consequence of dAdo accumulation. The latter may also serve as a good explanation for the immunosuppressive and neurotoxic function of 2-CdA.

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