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Calorimetric study as a potential test for choosing treatment of B-cell chronic lymphocytic leukemia

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Abstract

Differential scanning calorimetry (DSC) and complementary techniques were utilized to evaluate the sensitivity of B-cell chronic lymphocytic leukemia (B-CLL) cell samples *in vitro* exposed to cladribine or fludarabine in combination with mafosfamide. Mafosfamide, the active *in vitro* form of cyclophosphamide with both purine analogs produced the cytotoxic effect on mononuclear cell probes, however, to a different degree. Our results indicated that higher sensitivity of examined leukemic cell samples to the used drug combinations was usually accompanied by a marked decrease or even a complete loss of thermal transition at $95 \pm 3\,^{\circ}C$ in DSC scans of nuclear preparations as well as by more significant reduction of cell viability, higher extent of DNA damage estimated by the comet assay and by dropping/disappearance of anti-apoptotic protein Mcl-1 in comparison with untreated cells. We have also observed that the reduction of transition at $95 \pm 3\,^{\circ}C$ in thermal scans of nuclear preparations isolated from blood of B-CLL randomized patients who showed response to cladribine or fludarabine combined with cyclophosphamide, i.e., CC and FC, respectively, corresponded with the decrease or disappearance of anti-apoptotic proteins Bcl-2 and/or Mcl 1. In conclusion, these *in vitro* and *in vivo* studies revealed that quick DSC technique, usually supplemented by other methods, is a potent tool to distinguish efficacy of B-CLL treatment and could be helpful in choosing the most effective manner of treatment for this type of leukemia.

Keywords: B-CLL; Purine analogs; Mafosfamide/cyclophosphamide; DSC; Comet assay; Anti-apoptotic proteins

1. Introduction

The clinical course of B-CLL is very heterogenous [1]. Despite decades of clinical trials and research into the treatment, this cancer has remained largely incurable. The biological mechanism(s) underlying high variability of B-CLL in clinical behaviour remain still unclear. The clonal excess of $CD19^+/CD5^+$ B lymphocyte population is mainly caused by an accumulation of neoplastic cells. Longevity of leukemic cells is caused by their arrest in the G_0/G_1 phase of cell cycle and by inhibition of apoptosis [2–4].

Very recently published results, highlighting the role of cell signalling involved B-cell receptor (BCR) and mitogen activated kinase (MAPK)/NFκB activation as well as other extracellular signals in pathology and prognosis of B-CLL [5–7]. It seems that the balance of these signalling molecules is important in determining of B-cell fate. Advances in understanding of the cell biology and molecular defects in B-CLL may contribute significantly to the changing concept of therapy in this cancer [8,9]. Conventional chemotherapy used for B-CLL treatment has improved by an introduc-

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tion of some purine nucleoside analogs (such as fludarabine, cladribine, 2′-deoxycoformycin) [5,10–12]. Purine analogs used alone or in combination with cyclophosphamide have become an increasing popular approach for the patients' treatment compared with conventional therapy. Their application yields high response rates, high complete remission rate and longer progression-free survival [12]. The evidence indicates that in leukemia cells these agents exert their cytotoxic effect by induction of apoptosis [4,13,14]. Purine analogs interfere with DNA synthesis through inhibition of DNA polymerase and ribonucleotide reductase [5,15]. In addition, these compounds reveal deleterious effects on normal resting lymphocytes. New insights into pathophysiology of B-CLL and prognostic markers, especially biological factors (cytogenetic abnormalities, *IgVH* mutation status, CD-38 and ZAP-70 expression, serum markers) are beginning to change the concept of this disease treatment [5,8,9,16].

The aim of this study was to compare the differential scanning calorimetry profiles of B-CLL nuclear fraction isolated from peripheral blood of randomized patients before and during treatment with FC (fludarabine + cyclophosphamide) or CC (cladribine + cyclophosphamide). The leukemic cells obtained from blood of several B-CLL patients before treatment were also exposed *in vitro* to combinations of the used purine analogs with mafosfamide. We assessed the cytotoxicity *in vitro* of tested drug combinations by applying DSC as well as conventional techniques (viability test, comet assay, Mcl-1 and/or Bcl-2 expression). The application of DSC – the useful method to study the enthalpy changes in biological macromolecules (proteins, DNA), gave opportunity to observe structural changes in leukemic nuclear fraction as a function of temperature [17–20]. Our previous data [20] revealed the differences in thermal profiles between normal and leukemic nuclear preparations. In addition, we suggested that the thermal transitions at about 93 °C zone could be a marker of advanced status of disease. We have also noticed that thermal profiles of leukemic cell nuclei obtained from peripheral blood of B-CLL patients responding to the used therapy are characterized by the changed course of thermal profiles compared to unsensitive ones.

2. Materials and methods

2.1. Patients

Fourteen B-CLL patients (10 men and 4 women; underwent randomization) who had not received treatment previously with a leukocytosis from 100 to 182×10^9/l were studied. Diagnosis for these B-CLL patients was established according to standard clinical, cytological and immunological criteria [21]. The clinical staging of the disease was determined according to the Rai system [22]. The immunophenotypic characteristics of leukemic cells ($CD5^+/CD19^+/CD23^+$, presence of surface immunoglobulin κ or λ chains) were determined. We used monoclonal antibodies manufactured by DAKO (Glostrup, Denmark) and flow cytometry (Coulter, Hialeah, FL) (Table 1). Additionally, *in vitro* studies were performed with B-CLL cells isolated from peripheral blood of 9 patients. The present study was approved by the local ethics committee of Medical University of Lodz (No. RNN/237/03KE) and all the patients' signed consent form.

2.2. Treatment modality

The doses and schedule of the B-CLL patients' treatment were based on previous studies [23,24]. A group of 8 patients received fludarabine at a dose 25 mg/m^2/day i.v. with cyclophosphamide 250 mg/m^2/day i.v. for 3 consecutive days (FC protocol). Six others received cladribine at a dose 0.12 mg/kg/day by 2 h i.v. with cyclophosphamide 250 mg/m^2/day i.v. for 3 consecutive days (CC protocol). Cladribine (Biodrybin) was obtained from the Institute of Biotechnology and Antibiotics Bioton (Warsaw,

Table 1
Drug-induced changes on DSC transition at 95 ± 3 °C of nuclear fraction preparations, expression of Bcl-2 and/or Mcl-1 anti-apoptotic protein and response to treatment of B-CLL proteins treated with cladribine + cyclophosphamide (CC) or fludarabine + cyclophosphamide (FC)

Patient initials	Sex	Protocol	Stage of disease	DSC peak at 95 ± 3 °C	Expression of Bcl-2 and/or Mcl-1 protein	Response to treatment
KC	M	CC	III	Slight decrease	Bcl-2 ↓ Mcl-1 ↓	PR
FK	M	FC	IV	↓	ND	CR
JF	M	CC	IV	↓	Mcl-1 ↓ Bcl-2 no change	PR
EJ	F	FC	IIB	Loss	Mcl-1 loss Bcl-2 ↓	CR
SL	M	FC	II	↓	Mcl-1 loss Bcl-2 ↓	CR
JO	M	FC	IV	No change	Bcl-2 ↑	NR
JW	M	CC	I	No change	Bcl-2 no change	NR
MD	M	CC	IV	No change	Mcl-1 no change	NR
KR	M	FC	IV	↓	Mcl-1 ↓ Bcl-2 ↓	CR
EO	M	FC	IV	↓	Bcl-2 ↓	CR
IS	F	CC	I	Loss	Mcl-1 loss	CR
ES	F	CC	II	↓	Mcl-1 ↓	CR
TD	F	FC	0	↓	Mcl-1 ↓ Bcl-2 ↓	PR
JR	F	FC	I	↓	Mcl-1 ↓ Bcl-2 ↓	CR

The clinical staging of B-CLL was determined according to the Rai et al. [22]. Clinical response to the treatment was estimated by NCI Sponsored Working Group criteria [21]. Complete response (CR) required to the absence of symptoms and organomegaly, normal complete cell counts (absolute neutrophil count > 1.5×10^9/l, hemoglobin concentration >11.0 g/dl, platelet counts >100×10^9/l) and bone marrow with less than 30% lymphocytes for at least 2 months. Partial response (PR) was considered in the case of a spleen, and peripheral blood findings either identical to those of CR or improved over pre-therapy values by at least 50%. ↓ – decrease; ND – not determined; NR – non-responder.