ELSEVIER

Pharmacological Research 53 (2006) 49–61

Pharmacological research

www.elsevier.com/locate/yphrs

In vivo drug-response in patients with leukemic non-Hodgkin's lymphomas is associated with in vitro chemosensitivity and gene expression profiling

Kai Uwe Chow[a,d,*], Daniel Nowak[a,c], Soo-Zin Kim[a], Bernd Schneider[a], Martina Komor[a], Simone Boehrer[a], Paris S. Mitrou[a], Dieter Hoelzer[a], Eckhart Weidmann[b], Wolf-Karsten Hofmann[c]

[a] *University Hospital, Department of Internal Medicine II, Hematology and Oncology, Theodor-Stern-Kai 7, 60590 Frankfurt/Main, Germany*
[b] *Department of Hematology and Oncology, Community-Hospital "North-West", Steinbacher Hohl 2-26, 60488 Frankfurt, Germany*
[c] *Department of Hematology and Oncology, University Hospital Benjamin Franklin, 12200 Berlin, Germany*
[d] *Medizinisches Versorgungszentrum, Hematology and Oncology, Stresemannallee 3, 60596 Frankfurt/Main, Germany*

Accepted 1 September 2005

Abstract

Only a few approaches are available to address the mechanisms of cell death in vivo which are induced by anticancer treatment in patients with malignancies. In this study in vitro chemosensitivity testing of primary peripheral blood leukemic cells of five patients suffering from different leukemic non-Hodgkin's lymphomas was combined with the analysis of the in vivo rate of apoptosis by flow-cytometry (Annexin V and depolarisation of mitochondrial membrane potential (MMP) by JC-1). Furthermore, changes in expression patterns of apoptosis related proteins during chemotherapeutic treatment were detected by Western Blot. Gene expression profiling (HG-U133A, Affymetrix, Santa Clara, CA) was employed to identify common marker genes of in vivo drug response.

In vitro chemosensitivity was tested using the cytotoxic agents which the patients were scheduled to receive and was strongly correlated with effective reduction of leukemic lymphoma cells in patients resulting in complete remissions in all five cases. Due to the rapid clearance of apoptotic tumor cells in vivo neither the analysis of the in vivo rate of apoptosis and depolarisation of MMP nor the assessment of expression of regulators of apoptosis showed concordant results concerning the drug response. However, assessment of gene expression during therapy could identify a set of 30 genes to significantly discriminate between samples from patients before treatment compared to samples from the same patients after receiving cytotoxic therapy. Among these 30 genes we found a high proportion of genes associated with apoptotic cell death, cell proliferation and cell cycle signalling including complement lysis inhibitor (clusterin/CLU), beta-catenin interacting protein (ICAT), peroxisome proliferator activated receptor alpha (PPARα), TNF alpha converting enzyme (ADAM17/TACE), homeo box A3 (HOX1), inositol polyphosphatase 5-phosphatase type IV (PPI5PIV) and inhibitor of p53 induced apoptosis alpha (IPIA-Alpha/NM23-H6). These results indicate that in vitro chemosensitivity testing and gene expression profiling can successfully be utilised to analyse in vivo drug response in patients with leukemic NHL's and can be used to explore new pathway models of drug-induced cell death in vivo which are independent of different lymphoma subtypes and different treatment regimens.

Keywords: Chemosensitivity; PPAR alpha; ICAT; Clusterin

1. Introduction

Today it is widely established that most, if not all chemotherapeutic agents act by the induction of apoptotic pathways in tumor cells [1,2]. The larger part of studies addressing this issue have been performed utilizing in vitro or ex vivo experimental setups. Only a few studies are available to evaluate the actual mechanisms of cell death in vivo during treatment with anti-cancer therapeutics [3–6]. While previous improvements of anti-cancer therapy were largely based on empirical evaluation of different chemotherapy combination regimens it is a vision of modern cancer research to develop individual therapy concepts based on specific markers and predictive factors of the tumor before the application of an ultimately adapted and customized therapy. Studies on in vitro models [2,7] identified multiple apoptotic pathways that are activated by anticancer therapy such as the CD95/Fas death receptor pathway or intrinsic signalling via

* Corresponding author at: Medizinisches Versorgungszentrum, Hematology and Oncology, Stresemannallee 3, 60596 Frankfurt/Main, Germany.
Tel.: +49 69 69597233; fax: +49 69 69597240.
E-mail address: chow@em.uni-frankfurt.de (K.U. Chow).

1043-6618/$ – see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phrs.2005.09.001

mitochondria leading to alterations of the Bcl-2 family proteins and the activation of caspase cascades. Inhibition of these apoptotic pathways could protect cells from drug induced apoptosis in vitro [7] while enhanced downregulation of inhibitory proteins and enhanced caspase activation could be associated with synergistic anticancer drug combinations [8,9].

Some studies which have compared apoptotic mechanisms during anti-cancer treatment rather showed that in vivo and in vitro responses to chemotherapy differ considerably [3,4,6,10] suggesting that insights gained on apoptosis in vitro may only be transferred to an in vivo setting to a limited extent. On the other hand, approaches mostly assessing the baseline expression of apoptosis-relevant proteins such as Bcl-2 family members [11–13], inhibitor of apoptosis proteins (IAP's) [14,15] or caspases [16] in primary patient material strongly imply that dysregulation of apoptosis is associated with resistance against- and prognosis of anti-cancer therapy. Therefore, in order to finally achieve the above described goal of individually adapted therapies it will be necessary to combine results from in vitro and in vivo analysis.

A major technical problem is that cells undergoing chemotherapy induced apoptosis in vivo are rapidly removed from the peripheral blood. It is a difficult challenge to carry out quantitative analyses such as determination of the rate of apoptotic cell death [10] or reliable measurement of protein expression levels of apoptotic in vivo cells as it is routinely done with in vitro cell lines.

The analysis of gene expression using oligonucleotide microarrays is a powerful new method to analyse cellular responses to chemotherapy in vivo at early stages of apoptosis enabling insights into apoptotic mechanisms in vivo before cells are cleared out of circulation. Although this method has recently been employed in this context [5], we here show for the first time an experimental setup combining in vivo and in vitro analysis with the aim to identify common genes and mechanisms associated with in vivo response to different individual therapies. By assessing gene expression profiles before and after therapy in vivo and comparing ex vivo chemosensitivity analysis with in vivo cell depletion after treatment of patients with low grade non-hodgkin lymphomas we were able to identify 30 genes which were significantly altered in all patients despite of different lymphoma entities and different chemotherapeutic regimens. Of these 30 genes an outstandingly high proportion is associated with apoptosis or cell-cycle control.

2. Patients and methods

2.1. Patients and in vitro assessment of chemosensitivity of untreated primary cells

The study group consisted of five patients suffering from different entities of non-Hodgkin lymphomas treated at Department of Internal Medicine III (Haematology/Oncology) at the Johann Wolfgang Goethe University Hospital, Frankfurt am Main, Germany. The study was performed according to the guidelines and consent of the institutional committee of ethics. Patient characteristics are detailed in Table 1. In vitro chemosensitivity analyses of patients' cells were carried out with the analogue drug combinations and the single drugs contained therein which the patients were to receive as treatment. The applied drug combination regimens have previously been studied extensively in in vitro and ex vivo experimental setups by our group and were identified as synergistic in a number of cell lines and in primary patient material by the determination of median effect plots and combination indices [8,9,17]. The concentrations of drugs applied in the in vitro chemosensitivity assays resemble the averaged IC-30 (inhibiting concentration, IC), respectively the IC-50 concentrations ascertained as experience values for the single drugs in the course of the above mentioned preliminary studies.

2.2. Blood samples, time schedules, cell isolation and culture

Forty millimiters of heparinized blood were drawn from peripheral veins of patients directly before receiving combination chemotherapy (day 0) and on days 1–4 during chemotherapeutic treatment. Mononuclear cells (MNC) were separated by Ficoll density gradient centrifugation (Biochrom, Berlin, Germany) using standard protocols. The malignant cells in all samples represented at least 80–90% of the total MNC as confirmed by flowcytometric analysis. At least 3×10^7 cells per time point (day 0–4) were immediately cryopreserved in liquid nitrogen for subsequent gene expression profiling (described below).

Table 1
Lymphoma entities, scheduled treatment and in vitro drug concentrations

Patient	Diagnosis leukemic NHL	Immunophenotyping	Initial WBC [nl]	Tested drugs in vitro chemosensitivity (in the range of in ng/ml)	Received chemotherapy
1	Immunocytoma	CD19, CD20, CD23	33	Rit (10), Clad (0.05–0.2), Benda (30–100)	Rit, Clad, Benda
2	Mantle cell lymphoma	CD5, CD19, CD20, CD23, CD38, FMC7	23	Rit (10), Clad (0.05–0.2), Benda (30–100)	Rit, Clad, Benda
3	Chronic lymphocytic leukemia	CD5, CD19, CD20, CD23	227	Flud (30–80), Mafo (25–50)	Flud, Ctx
4	Atypical chronic lymphocytic leukemia	CD5, CD19, CD20, CD23, CD38, FMC7	81	Rit (10), Flud (40–80)	Rit, Flud
5	Prolymphocytic leukemia	CD19, CD20	211	Rit (10), Flud (80), Epi (0.3)	Rit, Flud, Epi

Abbreviations: WBC, white blood cell count; NHL, non-Hodgkin-lymphoma; Rit, rituximab; Clad, cladribine; Benda, bendamustin; Flud, fludarabine; Mafo, mafosphamid; Epi, epirubicin; Ctx, cyclophosphamid.