Available online at www.sciencedirect.com
ScienceDirect
Cellular Immunology 244 (2006) 121–124
Cellular Immunology
www.elsevier.com/locate/ycimm

The role of ABC-transporter gene polymorphisms in chemotherapy induced immunosuppression, a retrospective study in childhood acute lymphoblastic leukaemia

Dániel J. Erdélyi [a,b,*], Enikő Kámory [c], Anna Zalka [c], Ágnes F. Semsei [a], Béla Csókay [c], Hajnalka Andrikovics [d], Attila Tordai [d], Gábor Borgulya [b], Edina Magyarosy [e], Ilona Galántai [f], György Fekete [b], András Falus [a], Csaba Szalai [e,g], Gábor T. Kovács [b]

[a] *Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary*
[b] *2nd Department of Paediatrics, Semmelweis University, Budapest, Hungary*
[c] *Laborigo Molecular Diagnostics Ltd., Budapest, Hungary*
[d] *Department of Molecular Diagnostics, National Medical Center, Budapest, Hungary*
[e] *Heim Pál Children Hospital, Budapest, Hungary*
[f] *Madarász Street Children Hospital, Budapest, Hungary*
[g] *Immunogenomics Research Group, Hungarian Academy of Sciences, Budapest, Hungary*

Received 3 February 2007; accepted 6 February 2007
Available online 16 April 2007

Abstract

We examined the association of functional *ABCB1* (*MDR1*) and *ABCG2* (*BCRP*) polymorphisms with acute side effects of chemotherapy. Analyses were performed on clinical data from 138 patients treated with the ALL-BFM-95 protocol implying several substrates of these transporters. *ABCB1* 3435T>C, 2677G>T/A 1236C>T and *ABCG2* 421C>A genotypes were determined. A higher proportion of *ABCB1* 3435TT patients suffered excessive infectious complications than those harbouring at least one C allele (OR = 2.5, $p = 0.03$) during the whole half-year-long intensive phase of chemotherapy. Weaker associations were calculated when *ABCB1* 1236T-2677T-3435T haplotype homozygotes were tested against the remaining part of the population (OR = 2.3, $p = 0.09$). During the reinduction phase of therapy, the occurrence of severe leukocytopenia was similar among *ABCB1* genotype groups. The frequency of any toxicities were not shown to differ according to the *ABCG2* 421C>A genotype. Our data suggest that the *ABCB1* 3435T>C genotype is associated with the infectious complications of the applied chemotherapy regimen.

Keywords: Pharmacogenetics; Cytostatic drugs; Myelotoxicity; Infection

1. Introduction

The *ABCB1* (ATP-binding cassette superfamily member B1, alias *MDR1*) and the *ABCG2* (*BCRP*) transporters are highly expressed at the pharmacokinetic barriers of the body serving as defence against xenobiotics. In addition, *ABCG2* plays a crucial role in protecting haematopoetic stem sells. Some functional single nucleotide polymorphisms (SNPs) were reported in these genes: *ABCB1* 3435T>C and linked SNPs were associated with altered gene-expression while *ABCG2* 421C>A with different transport-function [1–4].

Very good outcomes can be achieved with recent treatment protocols in childhood acute lympoblastic leukaemia (ALL); nevertheless approximately 20% of patients die [5]. The outcome could be further improved by identifying

* Corresponding author. Address: Department of Genetics, Cell- and Immunobiology, Semmelweis University, 1089 Budapest, Nagyvárad tér 4, Hungary. Fax: +36 1 303 6968.

0008-8749/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.cellimm.2007.02.007

patient groups which can tolerate higher doses of chemotherapy. In case of most cytostatic drugs, myelotoxicity and consequential immunosuppression are the dose-limiting adverse effects. Therefore, we organized a retrospective study to assess the impact of *ABCB1* and *ABCG2* polymorphisms on these side effects.

2. Patients and methods

2.1. Study population and sample collection

In a retrospective manner, DNA was collected from 186 ALL children who had undergone chemotherapy due to ALL between 1995 and 2003, aged 1–18 years at diagnosis, treated according to the ALL Berlin–Frankfurt–Münster (BFM) 95 study protocol [5,6] in eight Hungarian paediatric oncology centres. Most children belonged to the Hungarian (Caucasian) population, although approximately 5% were of gypsy origin (estimate based on state population statistics). In most cases DNA was extracted from peripheral blood taken in haematologic remission ($n = 165$). From patients who died before our sample collection period, DNA was obtained from old bone marrow smears ($n = 16$) or from neonatal Guthrie spots ($n = 5$). We extracted genomic DNA from blood using the QIAmp Blood DNA Maxi Kit (Qiagen) and from smears and Guthrie spots with the HighPure PCR Template Preparation Kit (Roche Diagnostics) according to the manufacturers' instructions. Informed consent was requested from patients' parents. The study was approved by the Ethical Committee of the Hungarian Medical Research Council.

In order to have a homogenous therapy group only patients treated in standard risk (SR) and medium risk (MR) arms of the chemotherapy protocol (detailed description in Supplementary Table S1) were enrolled. Forty eight children were excluded from further analyses because of lost clinical documentation ($n = 8$), death before the end of intravenous chemotherapy protocol ($n = 10$), deviations from protocol ($n = 23$), preceding chemotherapy courses ($n = 4$) or coexisting diseases influencing pharmacokinetics or infectious complications ($n = 3$). Biological and clinical parameters of the 138 patients analyzed after exclusions are shown in Supplementary Table S2.

2.2. Clinical data and definitions

Days were counted on which intravenous (i.v.) antibiotic and antifungal drugs were administered from courses started after the first i.v. cytostatic dose until those started within 21 days following the last dose of i.v. chemotherapy. Regarding this parameter, patients were categorized by two ways: according to the median and the upper quartile values of antimicrobial agent usage. We assessed the occurrence of leukocytopenia during the 35-day-long reinduction phase. We have chosen this segment of chemotherapy for the following reasons. *ABCB1* substrate agents (vincristine, anthracyclines, glucocorticoids) were administered during induction and reinduction only. Leukaemic bone marrow infiltration contributes to leukocytopenia during induction, while it is related to cytostatic therapy alone in reinduction phase. The lowest leukocyte count observed throughout this period was graded according to CTCEA v3.0 criteria (http://ctep.info.nih.gov/reporting/index.html).

2.3. Genotyping and statistical analysis

The *ABCB1* 3435T>C, 2677G/T,A and 1236T>C genotypes were determined by a single base extension method based on that published by Gwee et al. [7] with several minor alterations. *ABCG2* 421C>A genotyping was performed using the LightCycler (Roche Diagnostics) allelic discrimination system [8]. Detailed description of these procedures can be found in the online Supplementary material.

Genotype data were analysed as dichotomous variables: homozygotes for the more frequent allele versus those harbouring at least one variant allele. When comparing clinical data between genotype group pairs, univariate and multivariate generalized linear model procedures were performed with logistic design. Multivariate models were using backward stepwise variable selection with inclusion criterion of $p \leqslant 0.05$ and exclusion criterion of $p > 0.1$, including potential confounders such as treating hospital, chemotherapy protocol, anthracycline dose, sex and age of patients. Analyses were performed using Statistica 7.0 (StatSoft Inc.) and SPSS 13.0 (SPSS Inc.) softwares.

3. Results

After exclusions, we analyzed the data of 138 children who had been treated according to ALL BFM 95 SR or MR protocol arms. Our study population is potentially biased as patients who died before our sample collection period are underrepresented. The cohort is in Hardy-Weinberg equilibrium regarding all polymorphic sites (data not shown). Allele frequencies (indicated in Supplementary Table S2) were very similar to other published Caucasian populations with two dominating *ABCB1* haplotypes: 1236C-2677G-3435C (CGC) and 1236T-2677T-3435T (TTT). The distribution of allele frequencies in the adverse reaction group or the whole set of excluded patients did not differ from that of the remainder population (data not shown).

Data regarding antimicrobial use and the frequency of leukocytopenia within each genotype group are described in Supplementary Table S5. Results of the statistical analyses are shown in Table 1.

4. Discussion

After exclusions, we studied the association of ABC-transporter gene variants and the acute immunosuppressive side effect of chemotherapy in 138 children with ALL.