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FUNCTIONAL AND GENOME-WIDE ANALYSIS OF ACQUIRED RESISTANCE TO TRAIL/APO2L MEDIATED APOPTOSIS OF HL60 LEUKEMIA CELLS

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Background. Acute leukemia comprises malignant diseases of clonal character, to which specific treatment remains limited. Apoptosis induced by death receptor activation (i.e. by tumor necrosis factor-related apoptosis inducing ligand, TRAIL/APO2L) is a potential anti-tumor therapeutic mechanism. TRAIL, a member of the TNF family of death ligands, appears to specifically and efficiently kill tumor cells of diverse origin while sparing normal tissues. The TRAIL receptor family consists of five receptors: two death receptors (DR4/TRAIL-R1, DR5/TRAIL-R2), two decoy receptors (DcR1/TRAIL-R3, DcR2/TRAIL-R4), and osteoprotegerin (OPG). *Aims.* Functional analysis of individual TRAIL receptors in HL60 myeloid leukemia cells and analysis of the molecular basis of TRAIL resistance. MATERIALS AND *Methods.* TRAIL-resistant cells were selected from the original HL60 population using pressure of recombinant His-tagged TRAIL (200-2000 ng/mL). The expression of TRAIL receptors and CD14 were analyzed by flow cytometry using fluorochrome labeled antibodies and/or by real-time RT-PCR. Percentage of apoptotic cells was measured by flow cytometry using Annexin-V-FITC/Propidium iodide apoptosis detection kit. The contribution of individual TRAIL receptors on the transmission of apoptotic signal was measured using blocking antibodies to TRAIL receptors. The TRAIL resistance related genome aberrations were analyzed by genome-wide loss of heterozygosity (LOH) screening with marker density of 10cM and comparative genomic hybridization (CGH) assay. *Results.* The blockage of DR4 receptor significantly reduced the number of apoptotic HL60 cells compared to untreated controls. The blockage of DR5 receptor also inhibited TRAIL-induced cell death but the results did not reach statistical significance. Combination of anti-DR4 and anti-DR5 antibodies almost completely abrogated TRAIL-induced HL60 cell death and significantly reduced apoptosis compared to control or anti-DR4 antibody alone ($p<0.01$). Blocking of decoy receptors (DcR1, DcR2) or OPG of HL60 TRAIL-sensitive and TRAIL-resistant cell lines did not significantly affect the apoptotic signaling. Two distinct HL60 TRAIL-resistant phenotypes were identified based on the expression of TRAIL-receptors and CD14. *Phenotype-1* (n=4) was characterized by the decreased expression of TRAIL receptors DR4, DR5, DcR1, and DcR2, CD14 and unchanged expression of OPG as compared to control TRAIL-sensitive HL60 cells. *Phenotype-2* (n=3) was characterized by the decreased expression of DR5 receptor, increased expression of CD14, and undetectable expression of OPG compared to control TRAIL-sensitive HL60 cells. Using LOH assay we identified two genotypes. The first exhibiting deletion on the short arm of chromosome 1p22 and monosomy of chromosome 18, and the second had deletions/uniparental disomy on the short arm of chromosomes 2, 3, 6, and 14. The identified genotypes corresponded to TRAIL-resistant *phenotype-1* and *phenotype-2*, respectively. CGH assay confirmed the loss of genomic material of whole chromosome 18. Further, the CGH detected a gain of genomic material at 1q21-23 of TRAIL-resistant *phenotype-1* while the *phenotype-2* cells did not show genomic defects of chromosome 1. *Summary/Conclusions.* In HL60 cells TRAIL-specific apoptotic signal is transduced predominantly through TRAIL receptor DR4. Decoy receptors, including OPG, did not play a role in TRAIL resistance. The identified TRAIL-resistant phenotypes are associated with distinct genomic conditions. Supported by: IGA MZ NR8317-4 and GAUK 50/2004/c.

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THE ADMINISTRATION OF REPEATED DOSES OF CYCLOPHOSPHAMIDE, INDUCES CYTOCHROME P450 IN RAT

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Background. Cyclophosphamide is used in high doses as a part of the conditioning regimen prior to stem cell transplantation. It is usually given for two or four consecutive days, primarily to facilitate engraftment of donor cells. Cyclophosphamide is a prodrug that has to be activated in the liver by a 4-hydroxylation reaction catalyzed by cytochrome P450 (CYP) enzymes. Several studies have shown that cyclophosphamide induces its own metabolism, which affects its pharmacokinetics and pharmacodynamics after repeated doses. *Aim.* In the present study, we aimed to investigate the effect of repeated doses of cyclophosphamide on the CYPs in rat. The levels of mRNA, protein, and enzyme activity were investigated. *Methods.* Male Wistar rats were given 4 consecutive doses of CPA (2 dose levels). Plasma and livers were collected to study the pharmacokinetics of cyclophosphamide in plasma and to measure the levels of mRNA (by real time PCR), protein (by western blot) and enzyme activity (by microsomal incubation with cyclophosphamide) of CYPs, respectively. *Results.* mRNAs of CYP2B1 and 2B2 were significantly induced with repeated dosing. Protein levels were also induced and autoinduction of CPA metabolism to 4-hydroxylation was found. *Conclusion.* Repeated dosing of CPA leads to autoinduction of CPA metabolism and induction of CYP2B mRNA and protein in rat. This knowledge may help in optimizing the dosing regime of cyclophosphamide in patients to keep plasma levels within the therapeutic range. It may also help in minimizing drug-drug interactions and hence increase the therapeutic efficacy and reduce side effects of cyclophosphamide in cancer patients.

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OPTIMIZATION OF THERAPY FOR THIOPURINE S-METHYLTRANSFERASE DEFICIENT CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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Background. Thiopurine S-methyltransferase (TPMT) is an enzyme that inactivates thiopurine drugs, such as 6-mercaptopurine (6-MP) and thioguanine, commonly used in therapy for childhood acute lymphoblastic leukemia (ALL). In BFM protocol for childhood ALL, 6-MP is administered during maintenance therapy. Patients with low TPMT activity experience severe hematological toxicity when standard 6-MP doses are used. It is now well established that lower TPMT activity can be due to TPMT gene mutations. Three alleles account for more than 95% of the clinically relevant TPMT variants: TPMT*2, TPMT*3A and TPMT*3C. Wild type has been designed as TPMT*1. TPMT*2 allele contains single G238C mutation, TPMT*3C-A719G mutation, TPMT*3B-G460A mutation and TPMT*3A allele has two mutations (G460A and A719G). *Aim.*The purpose of this study was to determine the relevance of TPMT gene mutations in the management of childhood acute lymphoblastic leukemia (ALL). *Methods.* Blood samples from 100 children with ALL were analyzed for TPMT mutations, using polymerase chain reaction-based assays (PCR-RFLP and ARMS). For 50 patients TPMT variant alleles were determined retrospectively, after completing the standard BFM protocol maintenance therapy. Maintenance therapy period was compared according to patients' TPMT genotypes. For the other 50 patients TPMT variant alleles where determined prospectively. For prospectively detected patients with TPMT variant alleles we introduced therapy protocol modification in a way that if leucopenia was noticed, only the dose of 6-MP was reduced but there were no reduction of Methotrexate (MTX) doses. The number of weeks when full, reduced dose or no 6-MP therapy was given, was determined for each patient during the maintenance therapy. Number of neutropenic fever was also considered as a toxic effect of 6-MP therapy. *Results.* Of 100 patients participating in this study, 89% were homozygous for TPMT*1 (W/W), 10% were heterozygous (W/M): 9% for TPMT*1/*3A, 1% for TPMT*1/*2. One patient was double heterozygous for TPMT*3A/*3B