6075.8 Presentation Time 9:45 AM Fellow in Training

Loss of SBDS Results in Dysregulation of Fas at the Plasma Membrane Level

Chhaya S. Ambekar, Hanming Wang, Ken-ichiro Watanabe, Aaron Schimmer, Yigal Dror. Program in Cell Biology, Research Institute and Paedeatric Hematology/Oncology, Hospital for Sick Children, University of Toronto, Toronto, ON, Canada.

BACKGROUND: Shwachman-Diamond syndrome (SDS) is characterized by marrow failure and a marked propensity for myelodysplastic syndrome and leukemia. Approximately 90% of the patients have mutations in the *SBDS* gene, but the function of the gene is unknown. We previously showed that SBDS-deficient hematopoietic and HeLa cells are characterized by accelerated apoptosis and hypersensitivity to Fas stimulation.

OBJECTIVE: To study the mechanism of Fas hypersensitivity in SBDS-deficient cells.

DESIGN/METHODS: We established several cellular SBDS-knockdown models using siRNA (leading to 0-8% SBDS levels by western blotting) and scrambled siRNA control cells. The effect of Fas stimulation was studied by MTT assay, DNA content and annexin V/propidium iodide binding. The contribution of the Fas signaling pathway to the decreased cell growth was determined after caspase inhibition. Western blotting, flow cytometry and confocal microscopy were used to evaluate expression of Fas-signaling proteins and Fas internalization. Fas mRNA expression was measured by real-time PCR.

RESULTS: SBDS knockdown in HeLa cells resulted in prominently decreased cell growth and increased cell death. Stimulation of the Fas signaling pathway by Fas ligand and CH-11 caused marked cytotoxicity. In contrast, no prominent hypersensitivity was found to TNF-alpha, interferon-gamma, radiation and other DNA damaging agents, transcription and protein synthesis inhibition. Interestingly, suppression of the Fas pathway by caspase 8 inhibitor completely rescued the cells. Although the plasma membrane Fas expression was prominently increased in the SBDS-knockdown cells, the total level of Fas protein and mRNA did not differ in the SBDS-knockdown cells. In contrast to Fas, the pathway factors Fadd, caspase 8 and caspase 3 were not increased, and the pathway inhibitors: Erk and Xiap were not decreased in untreated and CH-11-treated *SBDS*-knockdown cells. Internalization of Fas was not affected in the *SBDS*-knockdown cells.

CONCLUSIONS: These results suggest that SBDS plays an important role in regulating apoptosis. Loss of SBDS results in abnormal redistribution of Fas at the cellular level and specific localization to the plasma membrane, where it can bind its ligand and sensitize the cells to stimulation by Fas ligand.

Poster Session II

Sunday, May 6 11:00 AM-3:00 PM Exhibit Hall E

6314.1 Poster Board 500 Fellow in Training

Population Pharmacokinetics of Doxorubicin in Infants and Children with Malignant Diseases: Any Clue To Identify Children at Risk of Cardiac Toxicity?

Yaron Finkelstein, Alejandro A. Nava-Ocampo, Tal Schechter, Edith St. Pierre, Scott Walker, Gideon Koren. Division of Clinical Pharmacology and Toxicology, Hospital for Sick Children, Toronto, ON, Canada.

BACKGROUND: Doxorubicin is an anthracycline glycoside, commonly used in the treatment of pediatric malignancies. The clinical use of doxorubicin is limited by its irreversible dose related cardiotoxic effects, which may develop in up to 65% of survivors of childhood leukemia. Population PK analysis facilitates the evaluation of a limited number of data points, avoiding the common ethical dilemmas and technical issues surrounding sampling infants and young children.

OBJECTIVE: We evaluated plasma drug concentrations of doxorubicin in infants and children undergoing anticancer treatment in order to describe a simplified PK model, and discuss its potential applicability in facilitating future evaluation of pediatric patients at high risk of cardiac toxicity following doxorubicin therapy.

DESIGN/METHODS: We performed a population pharmacokinetic (PK) analysis of doxorubicin, in eleven children receiving intravenous doxorubicin. Blood samples were drawn in heparinized syringes at different time intervals (from time 0 to 60 hours after the end of the infusion). Plasma drug concentrations of doxorubicin were measured by solid-phase extraction and high performance liquid chromatography (HPLC) with fluorescent detection. Data were analyzed by means of a two-compartment intravascular pharmacokinetic model implemented in the compartmental module of SAAM II.

RESULTS: The total dose per cycle of doxorubicin ranged from 0.76 to 2.95 mg/kg, equivalent to 16.25 to 61.0 mg/m2, depending on patient's age, weight and cancer type. Of 11 patients studied, plasma doxorubicin concentration-time courses of 9 children best fitted a 2-compartment intravenous PK model and were included in the population PK analysis. The resulting population parameters were k12 0.78 ± 0.46 1/h, k21 0.17 ± 0.088 1/h, kel 0.22 ± 0.10 1/h, Vd 9.6 ± 6.8 ml/kg, Cl 1,297.5 ± 893.1 ml/kg/h, and half-life of 7.7 ± 11.3 h (ranging from 2.1 to 39.8 h).

CONCLUSIONS: A 2-compartment PK model was successfully used to describe the plasma levels of doxorubicin in pediatric cancer patients. This is a relatively simple and clinically applicable model, compared to other, very complex models, suggested by others.

6314.2 Poster Board 501

Different Rates of Clinical Trial Enrollment between Adolescents and Young Adults Aged 15 to 21 Years-Old and Children under 15 Years-Old with Cancer at a Children's Hospital

Peter H. Shaw. Pediatrics - Division of Hematology/Oncology, Children's Hospital of Pittsburgh, Pittsburgh, PA.

BACKGROUND: Over the last 30 years significant strides have been made in disease-free survival rates in children with cancer. However, similar gains in survival have not been seen in the adolescent and young adult population, aged 15-21 years old. The reasons for this lack of progress are multi-factorial, but it is clear that greater cure rates are directly correlated with higher rates of clinical trial participation.

OBJECTIVE: Our objective was to see if this older age group had different rates of clinical trial enrollment than our patients under the age of fifteen. We also studied the reasons why our non-enrolled patients were not on clinical trials.

DESIGN/METHODS: We retrospectively analyzed the clinical data on all patients with new oncology diagnoses at Children's Hospital of Pittsburgh (CHP) over a five-year period from July 2001 through June 2006.

RESULTS: 640 new oncology patients were seen at CHP over this time. 501 (78%) were under the age of 15 at the time of diagnosis and 139 patients (22%) were aged 15 to 21. Overall 229 patients (36%) were treated on a clinical trial. This included 191 patients (38%) in the younger age group and only 38 patients (27%) in the older group, which was significantly lower (p=0.005). 48% of the patients 15 and older were not enrolled on a clinical trial because one was not available, which was higher than the 41% of the younger patients not enrolled for the same reason (p = 0.03). An additional 14% of the older patients were not enrolled because they were ineligible for an open study versus only 8% in the younger group (p=0.02). Other reasons patients were not treated on clinical trials included the study being suspended or not IRB approved (6% of all patients), parent/patient refusal (2%), physician choice (2%) and other reasons (2%). There were no significant differences between the age groups when these reasons were analyzed.

CONCLUSIONS: A significantly lower proportion of adolescent and young adult patients (aged 15 to 21) are placed on clinical trials than younger patients. The lack of open clinical trials and patient ineligibility were the main reasons for this deficit at our center. One intervention to address this discrepancy is to open more national clinical trials to better address the malignancies in this often overlooked population of pediatric cancer patients.

6314.3 Poster Board 502 Fellow in Training

Sevelamer Hydrochloride: A Novel Treatment of Hyperphosphatemia Associated with Tumor Lysis in Children

Shaker Abdullah, Manuel Diezi, Lillian Sung, Lee Dupuis, Denis Geary, Oussama Abla. Hematology/Oncology, Hospital for Sick Children, Toronto, ON, Canada; Nephrology, Hospital for Sick Children, Toronto, ON, Canada; Hemato-Oncologie Pediatrique, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland.

BACKGROUND: Sevelamer hydrochloride (SH) is an aluminum and calcium-free, oral phosphate-binder used effectively for the treatment of hyperphosphatemia in patients with renal failure undergoing dialysis.

OBJECTIVE: To investigate the efficacy and safety of SH in children with leukemia/lymphoma and hyperphosphatemia secondary to tumor lysis.

DESIGN/METHODS: A retrospective chart review of all children with leukemia/lymphoma diagnosed between November 2002 and April 2004 who received SH during their admission was conducted. We monitored the effects of SH on serum phosphate level, calcium/phosphate product and renal function at hours 24, 48 and 72 from SH initiation. Demographic data and side effects attributed to SH were documented.

RESULTS: Fourteen patients received SH therapy for the treatment of hyperphosphatemia during the study period. Three were excluded from the efficacy but not toxicity analyses due to concurrent use of dialysis. The remaining 11 children had a median age of 10.8 yrs (range 2.6 to 17.7); seven were boys. Seven children had acute lymphoblastic leukemia, one had acute myeloid leukemia and 3 had non-Hodgkin's lymphoma. The most frequently used dose of SH was 400 mg po twice daily. The median duration of SH therapy was 2.5 days (range 1 to 7.5). SH caused a decrease in serum phosphate levels in all patients. Serum phosphate decreased by a mean of 29% at hr 24, 40% at hr 48 and 48% at hr 72; calcium/phosphate product decreased by a mean of 29%, 42% and 44% at hrs 24, 48 and 72, respectively. A 50% decrease in serum creatinine was obtained in 5 children receiving SH and without rasburicase. The only toxicity attributed to SH was mild vomiting in 3 patients.

CONCLUSIONS: SH seems to be effective and tolerable for the treatment of hyperphosphatemia associated with tumor lysis syndrome. Prospective randomized studies are needed to confirm the efficacy of SH in children with this metabolic complication.