CD4 Count and Biochemical Parameters in HIV Positive Individuals of Western Nepal

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Background: The human immunodeficiency virus is one of the most prime rising infections with multiple impacts on persons, families, communities, society and the entire country. The number of HIV infection is increasing every year in Nepal and estimated numbers of people with HIV were 372735, male (20232) and female (17051) in 2016. Objectives: To see the status of CD4 count and biochemical parameters in HIV positive individuals and correlation of CD4 with liver enzymes.

Materials and method: Data were collected from 146 HIV seropositive individuals at ART center, Western regional Hospital, Pokhara, Nepal. The blood samples were collected and analysed for CD4 count and biochemical parameters at Manipal Teaching Hospital, Pokhara, Nepal. The data were analysed using SPSS 16.

Result: The mean±SD age of HIV infected subjects were 37.74±13.28 and female (55.6%) were infected more in compare to males (44.4%). The mean±SD of CD4 count showed negative correlation with liver enzymes.

Conclusion: The biochemical parameters were normal in HIV infected individuals and CD4 count showed negative correlation with liver enzymes.

B-003

Olive oil diet and supplementation with omega 3

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The thymus shows important functional changes in response to nutritional disorders. The aim of this work was to analyze the effect of diet containing olive oil, with and without the supplementation with omega 3, on serum and thymus fatty acid profiles of growing rats. Weaning Wistar rats were fed during 10 days with normocaloric diet and fat was provided by olive oil (O group). The other group received the same diet supplemented with 24mg/day of fish oil (OS group). Control group (C) received normocaloric diet (AIN’ 93). Serum and thymus fatty acid profiles were determined by gas chromatography. Statistical analysis used ANOVA and Dunnett as post test. This work was approved by the ethics committee of the University of Buenos Aires and in conformance with the FASEB Statement of Principles for the use of Animals in Research and Education. Results of oleic, linoleic, alfa-linoleic (ALA), EPA and DHA acids expressed as %Area were: SERUM: OLEIC O: 23.44±3.68; OS: 18.31±2.22; C: 10.60±2.01; LINOLEIC O: 12.44±1.65; OS: 12.98±4.13; C: 18.27±2.81; ALA O: 0.30±0.09; OS: 0.32±0.08; C: 0.92±0.34; EPA O: 0.65±0.17; OS: 0.63±0.49; C: 0.80±0.23; DHA: O: 1.57±0.58; OS: 4.00±1.70; C: 1.33±0.19; THYMUS: OLEIC O: 21.54±5.92; OS: 24.40±5.04; C: 18.22±3.23; LINOLEIC O: 5.90±0.56; OS: 6.5±0.61; C: 10.89±2.18; ALA O: 0.27±0.02; OS: 0.30±0.07; EPA O: 0.49±0.28; OS: 0.50±0.13; DHA O: 0.47±0.10; OS: 0.70±0.12; C: 0.52±0.16. Media that did not present a letter (*) in common, were different (p<0.05).

In sera, O and OS groups showed lower ALA and linoleic acids levels and higher oleic acid levels, compared to C. The results suggest that the olive oil exacerbated omega 9 family with diminution of essential fatty acids. OS group presented high levels of EPA and DHA. In thymus, O and OS groups showed lower levels of ALA and linoleic acids than C. OS group only increased DHA. Fish oil supplementation increased DHA levels on serum and thymus, not modifying essential fatty acid low levels. EPA increase only in serum. The results suggest that dietary lipids provoked changes in serum and thymus fatty acids profiles. This work was supported by UBACyT: 2002150100011BA.
Preclinical Investigations of Platelet Function using the Chrono-Log Model 700 Aggregometer

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The Chrono-Log Model 700 Aggregometer has been used to evaluate in vivo effects on platelet function in humans and preclinical species. We describe two in vitro investigations where the Chrono-Log Aggregometer was used to help characterize suspected platelet dysfunction observed during preclinical in vivo studies. The first investigation was to determine if in vitro exposure to Compound A in monkey or human platelet-rich plasma (PRP) could produce inhibitory effects on platelet function. Blood (anticoagulated with sodium citrate) was collected from 4 monkey and 5 human donors. PRP and platelet poor plasma (PPP) were prepared by centrifugation. Platelet counts for PRP were performed and adjusted to a count of ~250,000/µL with respective PPP for all samples. Compound A was diluted to 0.9% sodium chloride and added to PRP samples to achieve final concentrations ranging from 0.3 µM to 5 µM. Verapamil was prepared as a stock solution and diluted to obtain a final concentration of 0.175 mg/mL (356 µM) in PRP for use as a positive control. Platelet aggregation for each sample was measured on the Chrono-Log 700 using collagen as agonist. Results demonstrated a dose-dependent inhibition of collagen-induced platelet aggregation (%) with Compound A at concentrations of ≥1 µM (monkeys) and 5.0 µM (humans) when incubated with monkey and human PRP. These in vitro findings correlated with macroscopic observations of hemorrhage and petechiae in an in vivo monkey toxicology study at comparable systemic exposure and informed on potential translatability to humans. In contrast, Compound B had a potential platelet activation liability based on decreased platelet counts observed in an in vivo monkey study. We evaluated the ability of Compound B to elicet platelet activation in vitro using monkey PRP. Blood was collected from 4 stock monkeys, and PRP and PPP were prepared as described previously. Compound B (10 µL) was added to PRP samples to achieve final concentrations ranging from 0.001 µM to 0.5 µM and platelet aggregation (%) was measured for 10 minutes. Collagen (10 µL) was added to one tube of PRP from each monkey and served as a control for normal platelet aggregation. Addition of Compound B alone to monkey PRP caused 30% and 70% aggregation at concentrations of 0.1 µM and 0.5 µM, respectively. These findings provided pharmacokinetic thresholds for the desired pharmacologic effect relative to the platelet activation liability. The Chrono-Log 700 Aggregometer is proven to be a valuable tool for characterizing effects on platelet function in preclinical pharmaceutical development.

Evaluation of Two Methods for Measurement of NT proANP in a Mouse Model of Heart Failure.


Atrial natriuretic peptide (ANP) is a cardiac prohormone known to be released in response to cardiac muscle wall stretch. When secreted, ANP cleaves into the active peptide and a more stable inactive fragment, N-terminal proatrial natriuretic peptide (NT-proANP) that is routinely used in clinical medicine and also as a potential translational biomarker for drug or surgically-induced cardiac hypertrophy in preclinical safety studies. The objective of this evaluation was to compare the use of two commercially available assays for NT-proANP in a mouse model of heart failure. Transverse aortic constriction (TAC) in the mouse is commonly used in preclinical studies of heart failure that induces initial compensatory cardiac hypertrophy that transitions to heart failure and is considered a clinically translational animal model to evaluate the effects of new drug candidates on cardiac hypertrophy. Briefly, NT-proANP was measured in plasma samples from experimental control (sham) and TAC mice using the Meso Scale Discovery™ (Rockville, Maryland) rat NT-proANP kit (MSD) and the human-based EIA Biomedica™ (Vienna, Austria) proANP (1-98). All procedures were approved by the Institutional Animal Care and Use Committee of GlaxoSmithKline and were performed according to the guidelines of the Animal Welfare Act. The Biomedica assay is the validated method that has been used routinely to monitor for effects on NT-proANP in the TAC model. The MSD method was validated for use in rat GLP safety studies but had not been qualified for use with mouse plasma. Prior to use of the MSD method for the TAC model samples, we confirmed that the assay demonstrated acceptable precision (CV≤20%), dilutional linearity (R²=0.99) and long-term stability (2 months at -80°C). NT-proANP results for TAC mouse samples for both methods demonstrated comparable (4-fold) increases relative to sham control samples. These NT-proANP increases in TAC mice correlated with increases in the effects of new drug candidates on cardiac hypertrophy. Briefly, NT-proANP was measured in plasma samples from experimental control (sham) and TAC mice using the Meso Scale Discovery™ (Rockville, Maryland) rat NT-proANP kit (MSD) and the human-based EIA Biomedica™ (Vienna, Austria) proANP (1-98). All procedures were approved by the Institutional Animal Care and Use Committee of GlaxoSmithKline and were performed according to the guidelines of the Animal Welfare Act. The Biomedica assay is the validated method that has been used routinely to monitor for effects on NT-proANP in the TAC model. The MSD method was validated for use in rat GLP safety studies but had not been qualified for use with mouse plasma. Prior to use of the MSD method for the TAC model samples, we confirmed that the assay demonstrated acceptable precision (CV≤20%), dilutional linearity (R²=0.99) and long-term stability (2 months at -80°C). NT-proANP results for TAC mouse samples for both methods demonstrated comparable (4-fold) increases relative to sham control samples. These NT-proANP increases in TAC mice correlated with increases in...
Performance of Sysmex XN-1000V™ Automated Hematology Analyzer Compared to the Siemens ADVIA ® 120 in Mice and Rats, Part I: Comparison of automated complete blood counts and reticulocyte parameters.

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BACKGROUND: Side-by-side studies comparing the recently released Sysmex XN-1000V™ to the Siemens ADVIA® 120 will provide insight into performance and capabilities for use in toxicological and efficacy studies for drug discovery or nonclinical development studies. The Sysmex XN-1000V, an automated veterinary hematology analyzer, utilizes laser light, impedance, fluorescent stains and flow-cytometry to analyze whole blood determining CBC, reticulocyte, and WBC counts. OBJECTIVE: The purpose of this study was to evaluate the Sysmex XN-1000V for determining complete blood counts (CBC), red cell indices, and reticulocyte counts in whole blood from mice and rats compared to the Siemens ADVIA 120. METHODS: Whole blood samples were collected from healthy untreated CD-1 mice and Sprague-Dawley rats from two distinct cohorts. Group 1 consisted of 40 rats and 85 mice. Group 2 was collected approximately 6 months later and consisted of 20 rats and 30 mice. Blood samples were analyzed within 4 hours of collection using both the Sysmex XN-1000V and the Siemens Advia 120 automated hematology analyzers with multi-species software. Analysis included CBC, total WBC and reticulocyte counts. Data from each collection were analyzed separately and then combined for further analysis to increase the N and assess variation between analysis events. Both methods for platelet analysis on the Sysmex XN-1000V (impedance for Group 1 and fluorescence for Group 2) were tested in comparison to the Advia 120 light scattering mode. Method correlation data from all samples were determined using EP Evulator (Data Innovations LLC) for regression statistics including random error (Stan- der Error of the Estimate - SEE) and systematic error (absolute and percent bias). RESULTS: Measured parameters from individual cohort analyses (WBC, RBC, HGB, MCV, absolute and relative reticulocyte counts) showed very good to excellent correlation for both species (R≥0.91) with the majority of these parameters displaying excellent correlation (R>0.95). Hematocrit showed good correlation for both species (R=0.89). While mice showed excellent consistency and correlation for reticulocyte counts (R=0.96-0.97), MCH and MCHC showed a little more variability within acceptable ranges. Rats showed significant variability between groups for these parameters. Platelets correlated consistently for both species with mouse R=0.90 or 0.92 and rats R=0.78 or 0.79. Upon combined data analysis from the two cohorts, N was increased to 60 rats and 115 mice. Measured parameters retained high correlation values for both species (R≥0.91). Reticulocyte counts in mice also showed excellent correlation (R=0.96). Both species maintained acceptable correlation on the combined data for HCT, MCHC, MCH and PLT (R≥0.71). Bias overall was ≤10.9% with the exception of platelets in mice (22.6%) and reticulocyte counts (32.4% in rats and R=17% in mice). CONCLUSIONS: The Sysmex XN-1000V showed overall consistent, comparable performance to the Siemens Advia 120 (low bias and acceptable to very good correlation) for CBC and reticulocyte analysis for laboratory mice and rats. While there were some differences between groups of animals collected at different time periods, the correlation values for all parameters overall were in the acceptable to excellent range indicating that the Sysmex XN-1000V is a reliable platform for hematology analysis in rodents.

Analysis of serum HDL subclass from rats with non-alcoholic fatty liver disease induced by high-fat and high-cholesterol diet.

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Background: Non-alcoholic fatty liver (NAFL) associates with obesity, insulin resistance, hypertension, and dyslipidemia, and can progress to non-alcoholic steatohepatitis (NASH) characterized by hepatocyte injury, inflammation and fibrosis. However, mechanisms and risk factors involved in the progression to NASH remain poorly understood. Dietary cholesterol is well known to induce NAFL/NASH in animal models, and increase HDL-cholesterol (HDL-C) levels, especially along with apoE-rich HDL subclass. However, it is unknown whether this HDL subclass is involved in the development of NASH. Therefore, we compared HDL subclass between dietary-induced rat models of NAFL or NASH.

Methods: Wister Kyoto (WKY) and SHRS/SP5/Dmcr rats were divided into four groups (n=2-5/group), and fed with stroke-prone (SP): 20.8% crude protein, 4.8% crude lipid, 3.2% crude fiber, 5.0% crude ash, 8.0% moisture, and 58.2% carbohydrate) or high-fat and high-cholesterol (HFC: a mixture of 68% SP diet, 25% palm oil, 5.0% cholesterol, and 2.0% cholic acid) diets. NAFL and NASH were induced in WKY and SHRS/SP5/Dmcr rats by the HFC diet, respectively. After eight weeks of HFC or SP diet feeding, serum HDL subclass was evaluated. Total HDL fractions were isolated from whole sera by the polyethylene glycol precipitation method, and applied into a cat-ion-exchange column (HiTrap SPH, GE healthcare) for separating apoE-rich HDL. RESULTS: On SP diet, total HDL-C and apoE-rich HDL-C levels were lower in SHRS/SP5/Dmcr (48.1 mg/dL and 27.5 mg/dL, respectively) than WKY rats (125.9 ± 12.7 mg/dL and 89.7 ± 7.9 mg/dL, respectively). The HFC diet induced a significant increase in apoE-rich HDL-C and decrease in apoE-poor HDL-C in both rats. ApoE-rich HDL-C levels were significantly lower in SHRS/SP5/Dmcr with NASH compared to WKY with NAFL (58.6 ± 12.9 mg/dL vs. 107.6 ± 8.8 mg/dL) and apoE-rich HDL-C levels were also lower in SHRS/SP5/Dmcr with NASH (10.2 ± 2.5 mg/dL vs. 24.1 ± 8.4 mg/dL). Furthermore, SHRS/SP5/Dmcr with NASH had significantly higher ratio (%) of free cholesterol to total cholesterol (FC/TC ratio) in both apo-rich and apo-poor HDL subclasses (19.7 ± 5.1% and 11.5 ± 1.9%, respectively), compared to WKY with NAFL (11.7 ± 2.3% and 6.0 ± 1.5%, respectively). There was no significant difference in non-HDL-C levels between the rats with NASH or NAFL. Conclusion: The present observations suggest that apoE-rich HDL may protect against free cholesterol accumulation in the liver, and the higher FC/TC ratio in HDL as well as the lower level of HDL may be an important risk factor for the progression to NASH in diet-induced rat model.
Performance of Sysmex XN-1000V™ Automated Hematology Analyzer Compared to the Siemens ADVIA® 120 in Mice and Rats, Part II: Comparison of automated leukocyte differential counts and manual microscopic methods.

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BACKGROUND: The Sysmex XN-V Series™ of automated hematology analyzers was recently introduced with software for use in research animals. Studies comparing the Sysmex XN-1000V™ to the Siemens ADVIA® 120 and manual microscopic methods will provide insight into performance and capabilities for use in nonclinical drug development. The Sysmex XN-1000V is a flow cytometry-based whole blood analyzer that produces complete blood, reticulocyte, and WBC differential counts. A 5-part leukocyte differential count is produced by fluorescent staining of WBCs and laser-based measurements of Side Fluorescent Light (SFL), Side Scatter Light (SSL), and Forward Scatter (FSC). Mononuclear cells are further separated by Sysmex Adaptive Flagging Algorithm for Shape-recognition (SAFLAS) technology.

OBJECTIVE: The purpose of this study was to evaluate the Sysmex XN-1000V for determining differential leukocyte counts in blood from mice and rats compared to the Siemens ADVIA® 120 and manual microscopic reference methods.

METHODS: Blood samples from healthy untreated animals (40 Sprague-Dawley rats and 85 CD-1 mice) were analyzed with the Sysmex XN-1000V and the Siemens ADVIA 120 using multi-species software. Manual differential counts were performed by two technologists counting 200 leukocytes each on separate Wright-stained blood smears for each animal. Manual differential values were averaged for comparison to instrument counts. Each instrument was compared to manual differential counts using percent values. Automated differential counts were evaluated using both absolute and relative WBC differential counts. Method correlation data from these samples were determined using EP Evaluator (Data Innovations LLC). Regression statistics, random error estimates, and systematic error (absolute and percent bias) were determined.

RESULTS: Acceptance criteria included well-separated leukocyte scattergrams from both Sysmex XN-1000V and Siemens Advia 120 platforms. Regression values for automated neutrophil and lymphocyte count comparisons were very good to excellent (R≥0.90) for mice and rats. Eosinophil percent values showed higher variability between species (R≥0.90 for mice and R≥0.80 for rats) however, absolute values showed less agreement for rodents (R≤0.76). Microscopic manual methods correlated well with the automated platforms for both species (R≥0.87) for neutrophils and lymphocytes but monocytes and eosinophils showed less agreement (R≤0.71). Basophils were not observed in these rodent studies. This was likely due to low number of cells included. The analyzers showed similar correlation to manual counts for rodents with rats having higher correlation than mice. Agreement between analyzers was better than between each analyzer and manual counts likely due to the high number of cells processed by the analyzers compared to low number of cells counted manually. Percent bias data was generally ≤25% with the majority of comparisons showing less than 10% bias. Some differential count parameters having inherently low cell numbers (e.g. Mono%, Baso% and #Baso) had much higher systematic differences.

CONCLUSIONS: The Sysmex XN-1000V performed well for samples from laboratory mice and rats for automated differential leukocyte counts. The Sysmex XN-1000V showed comparable performance and acceptable correlation to the Siemens Advia 120 results and to manual microscopic analyses. Based on these results, the Sysmex XN-1000V is suitable for use to support nonclinical studies in drug development.