870 STUDIES WITH MELPHALAN (PAM) IN PEDIATRIC CANCER CELLS.

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We have given marrow (BM) - ablative doses of PAM followed by BM autografting to children with neuroblastoma (NBL), Ewing's sarcoma (ES) and osteosarcoma (OS). To examine in vitro effects of this drug, we exposed fresh and cultured NBL (6 pts), ES (4), OS (5) and normal BM (9) cell colonies (CFU) to PAM in concentrations (conc) from 10^{-1} to 10^{-7} molar in a 14 day clonigenic assay. The T/2 of PAM measured by high pressure liquid chromatography was about 45 minutes. All CFU showed linear responses to PAM, correlating with both increasing drug conc and exposure duration (up to 8 hours). Fresh cells were significantly more sensitive than cultured cells. NBL-CFU were most sensitive, followed by OS, BM and ES in decreasing order. Repeat dosing up to 4 doses of PAM further inhibited CFU formation. We conclude: (1) NBL and OS CFU are more sensitive to PAM than normal BM or ES CFU; (2) PAM breakdown products apparently remain cytotoxic to tumor cells; (3) prolonged or repeated exposure may increase in vivo efficacy of PAM. We are currently correlating in vitro and in vivo conc of the parent drug and its metabolites with their cytotoxic effects.

● 871 PLATELET COUNT AND PLATELET-ASSOCIATED IgG IN 123 CONSECUTIVE PREGNANCIES: RELATIONSHIP OF MATERNAL FACTORS TO NEONATAL PLATELET COUNT

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Automated cell counting has brought to our attention the increased incidence of thrombocytopenia (TP) in pregnant women. This observation prompted us to evaluate 123 consecutive pregnancies for maternal and neonatal platelet counts (PC) and for maternal platelet-associated IgG (PAIg). PAIg was measured by solid phase RIA with Protein A. Thirty-two women (26%) were TP (PC: 43K-149K, mean±SD=121K±25) during the prenatal period (6), the perinatal period (12), or throughout pregnancy (14). Twenty-five (78%) TP women had elevated levels of PAIg (3.57-38.81ng/10^6platelets, mean±SD=9.10±8.14) at least once during pregnancy when compared with 23 non-pregnant women of child-bearing age (0.58-3.40, mean±SD=2.10±0.67). Fifty-one (56%) of the 91 women who were never TP had elevated PAIg (3.55-32.39, mean±SD=6.68±4.20). Two (7%) of 30 infants born to TP women were TP at the time of delivery (initial PC: 83K,110K). Perinatal PAIg was elevated in one of these mothers and normal in the other. Six (7%) of 81 infants born to women who were never TP were TP (initial PC: 109K-149K). Perinatal PAIg was elevated in 2 of these mothers, normal in 3, and not done in 1. These studies indicate that: 1) There is a high incidence (26%) of TP in pregnant women, 2) There is a high incidence of elevated PAIg in pregnant women, whether they are never TP (56%) or TP (78%), 3) Seven percent of neonates are born TP, and 4) Maternal PC and PAIg do not predict which infants will be born with low platelet counts.

872 HUMAN POLYMORPHONUCLEAR LEUKOCYTES OF THE BONE MARROW, CIRCULATION AND MARGINATED POOL: FUNCTION AND GRANULE PROTEIN CONTENT.

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Polymorphonuclear leukocytes (PMNLs) demonstrate altered function during acute infections and after administration of corticosteroids. We questioned whether such changes are due to population shifts from functionally different compartments of the granulocyte pool. Ten volunteers were given epinephrine 0.3 ml s.c. to induce demargination. PMNLs obtained before and after epinephrine injection were compared for adherence to nylon wool fiber, chemotaxis under agarose, luminol enhanced chemiluminescence, and total content and release of the granule proteins lactoferrin (LF) and β-glucuronidase (β-glu). Similar experiments were carried out using hydrocortisone (HC) to induce PMNL egress from the bone marrow. Epinephrine induced a significant neutrophilia of mature PMNLs, but there was no change in function or granule protein content. HC induced a neutrophilia with significant number of immature PMNLs (bands). These PMNLs demonstrated less adherence, increased chemiluminescence to zymosan stimulation and increased β-glu and LF release to stimulation with phorbol myristate acetate. Total LF and β-glu content was unchanged. There was no correlation between change in function and magnitude of HC induced neutrophilia or the "left shift". We conclude that the functional changes of PMNLs after HC infusion are due to an effect of HC upon PMNLs and not a result of a population shift from bone marrow PMNLs.

● 873 EARLY LIFE THREATENING THROMBOCYTOPENIA UNRELATED TO BONE MARROW SUPPRESSION SECONDARY TO ACTINOMYCIN-D (ACT-D) IN FIVE PEDIATRIC SOLID TUMOR PATIENTS SUCCESSFULLY TREATED WITH PREDNISONE THERAPY.

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We studied five pediatric solid tumor patients (age 13-72 mo.),(3 Wilms and 2 Rhabdomyosarcomas) treated with Vincristine and ACT-D (15 mcg/kg x 5 days) with or without Cyclophosphamide or Adriamycin. All five patients developed significant early thrombocytopenia (TCP) while on VAC.

TX	1	3	5	7	9	11	13 days
VAC	252±101	180±78	86±44	39±17	23±10	55±35	97±23 (plt x 10^3)
VAC-PRED	186±35	180±31	243	168±25	190±25	54±59	106±60(plt x 10^3)
P-VALUE	.25	NS	.005	.0005	.0005	NS	NS

There was no significant difference in Absolute Granulocyte Count on day 5 in VAC vs VDRC treatment, 3006±1607/UL vs 3173±1728/UL, P-NS. Lowering the dose (25%) did not decrease the incidence or severity of the TCP. All patients who developed significant TCP while on VAC therapy had BMA revealing markedly increased megakaryocytes suggesting a drug induced peripheral destructive process. All 5 patients were treated with fresh plts (1 U/10 lbs) without a significant rise in their plt count. Therefore, we pretreated 4 patients with Prednisone (2mg/kg/day) starting 2 days prior to VAC therapy for a total of 10 days. The total mean plt count was compared in the steroid treated vs the non-steroid treated group. All patients have remained in complete remission and have not had any steroid induced complications. Prednisone therapy given concurrently with VAC in pediatric patients with ACT-D induced TCP can decrease morbidity and eliminate life-threatening TCP.

TX	1	3	5	7	9	11	13
VAC	252±101	180±78	86±44	39±17	23±10	55±35	97±23
VAC-PRED	252±28	224±14	193±3	176±21	151±69	126±12.4	158±18
P-VALUE	NS	NS	.005	.0005	.0005	.0025	.0005

874 SERUM INHIBITOR OF GRANULOCYTE COLONY FORMATION ASSOCIATED WITH NEUTROPENIA IN TYPE I DYSGAMMAGLOBULINEMIA.

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A sixteen year old white male with X-linked Type I Dysgammaglobulinemia developed persistent neutropenia (total neutrophils < 500/mm^3) which was unresponsive to broad spectrum antibiotics, prednisone, or a single infusion of one liter of fresh frozen plasma. His plasma agglutinated neutrophils in a microagglutination assay. His plasma (or serum) inhibited colony formation in a dose dependent fashion in a granulocyte/macrophage colony forming assay using human bone marrow cells. Complete inhibition was obtained when the patient's serum reached a final concentration of 5%. This effect was similar when either the patient's or normal bone marrow cells were used. Heating the patient's serum to 56°C for 30 minutes did not destroy the inhibitor. Bone marrow cells cultured in a medium containing 5% of the patient's serum and 5% of normal serum were also completely inhibited. Seven total plasma exchange procedures were performed over three weeks during which time the peripheral neutrophil count returned to normal and no serum inhibitor could be demonstrated. Upon completion of the plasma exchange procedures, the peripheral neutrophil count fell within three days to less than 500/mm^3 and the serum inhibitor of colony formation reappeared. Three weeks after the plasma exchange procedures, the neutrophil count spontaneously returned to normal while on broad spectrum antibiotic therapy.

875 THE NEED FOR A MICRO PLATELET MDA ASSAY

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Malondialdehyde (MDA) is a marker for lipid peroxidation of unsaturated fatty acids. The amount of MDA generated by red blood cells (RBC)exposed to a standard H_2O_2 stress reflects the available antioxidant protection. As such it is more specific than the H_2O_2 hemolysis test & provides more information than simultaneous measurement of plasma Vit E & total lipids. However, after blood transfusions, the RBC-MDA assay poorly reflects E sufficiency since for a matter of weeks, it measures donor as well as patient cells. This is illustrated in the data on Baby B presented below. Unfortunately, the smallest sickest infants who are most in need of antioxidant defenses & most in need of antioxidant protection are also most in need of frequent blood transfusions. Because the much shorter life span of the platelet as compared to the RBC makes it preferable as a test cell, we have developed a platelet MDA assay (1.5 ml of blood). Results, as judged by simultaneous RBC-MDA and H_2O_2 assays, suggest the test is clinically feasible & will be useful in defining the state of E nutrition in premature infants.

Amnionitis Nec Day 3	Age Days	Serum E mg/dl	RBC/MDA nM/gHgb	%RBC H_2O_2 Frag	T. Lipid mg/dl
BW 2000g	1	0.6	414	88	469
HAL & IL	3	1.0	415	85	559
↓	8	1.0	575	71	701
↓ PRBC ⟶					
═ Oral Feeds ⟶	15	1.1	215	38	728

HAL-Hyperalimentation, IL-Intralipid, PRBC-Packed RBC Transfusions