tion recovered more rapid and more close to the normal value in radioisotope renal scan. *Conclusions.* The results presented here suggest that MSCs are capable of healing and functional restoring of damaged kidney by ischemic injury. So MSCs may be useful for cell therapy of renal failure.

1259

IMMUNOREACTIVITY TO ANTI-FIBRONECTIN AND ANTI-LAMININ POLYCLONAL ANTIBODIES IN PARAFFIN EMBEDDED MICE BONE MARROW ARE DEPENDENT ON HISTOLOGICAL PROCESSING

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Immunohistochemistry (IH) is an useful tool to study tissues and organs and it has been widely used in researchs or to supplement classical morphologic diagnosis including pathological conditions of the hemopoietic system. However, applicability of IH in bone marrow analyses presents some technical limitations, because some antigens are *masked* during tissue processing (fixation, decalcification and paraffin embedding) making the applicability of this methodology unfeasible. Bone marrow microenvironment contains cells of different tissues (bone, hemopoietic tissue and stromal elements) and several extracellular matrix (ECM) substances, mainly glycoproteins, proteoglycans and cytokines. The composition of bone marrow ECM is topographically variable and is associated to the development of different lineages of blood cells, suggesting the existence of specific interactions between ECM, stem cells and stromal elements. In previous studies, we have shown that bone marrow from mice submitted to protein malnutrition underwent structural changes with decrease in cellularity and increase of glycoproteins extracted from ECM. The aim of this work was to evaluate the influence of fixative and time of fixation in the antigenic preservation of extracellular matrix glycoproteins fibronectin and laminin, and their distribution in bone marrow of mice. Esternum of well nourished Swiss mice, 2 to 3 months old, were fixed with 3 different fixatives: Methacarn (1 hour), 10% neutral buffered formalin pH 7,2 (1 hour, 6 hour or 24 hours) and 4% buffered paraformaldeyde pH 7,2 (24 hours). Decalcified using 5% nitric acid (3 hours) or 10% buffered EDTA, pH 7,2 (7 days) and then processed routinely with standard dehydration and embedding in paraffin. Tissue sections (5 micron thick) mounted on silane coated slides were dewaxed, rehydrated, and brought to phospathe buffered saline. Endogenous peroxidase activity was blocked by incubation for 30 minutes in 3% hydrogen peroxide. Sections were incubated with primary antibodies against fibronectin (1:400) and laminin (1:25) overnight at 0-4°C. After washes in PBS, slides were incubated with biotinylated secondary antibody for 30 minutes, washed in PBS and incubated with a streptavidin-biotin complex coupled to peroxidase for 30 min. Peroxidase activity was revealed with diaminobenzidine. Slides were counterstained with Harris hematoxylin. No immunoreactivity, for both anti-laminin and anti-fibronectin antibodies, was detected in any specimen fixed in 4% paraformaldeyde (24 hs) and decalcified with EDTA. Sections fixed in Methacarn showed strong background reaction for laminin immunostaing and a false nuclear pattern for fibronectin immunostaining. Among the used conditions, adequate morphological and antigenic preservation of fibronectin and laminin were achieved on sections fixed in 10% buffered formalin during one hour and decalcified in 5% nitric acid. Tissue processing stages can significantly influence on immunoreactivity of antibodies against fibronectin and laminin. This way, sections fixed in 10% buffered formalin during one hour and decalcified in 5% nitric acid were selected to compare bone marrow ECM glycoproteins distribution *in situ* of nourished and malnourished mice.

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1260

VORICONAZOLE (VCZ) PROBABLY DOES NOT AFFECT THE PHARMACOKINETICS OF METHOTREXATE (MTX)

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Background and aim: Both MTX & VCZ have many drug interactions. Whether an interaction exists between them is not known. The aim of the study was to explore whether oral VCZ affects the pharmacokinetics of MTX. *Patients and Methods.* With informed parental consent, a prospective study with standard clinical & drug monitoring was performed on 2 children with intermediate-risk, B-cell-precursor acute lymphoblastic leukemia during consolidation chemotherapy. This consisted of 6-mercaptopurine 25 mg/m²/d PO 56 d, MTX 2 g/m²/24h IV q 2 wk x4, leucovorin 15 mg/m² x3 IV per each MTX & MTX 12 mg IT q 2 wk x4. The first child (case) has been on oral VCZ because of proven invasive pulmonary aspergillosis (IPA). The other (control) was infection free. MTX serum levels [μmol/L] were measured by fluorescence polarization immunoassay at 0, 18, 24, 36 & 42h of starting the infusion until achieving a concentration of ≤0.25. Inter- & intra-patient MTX levels were compared by Wilcoxon signed ranks test & Friedman test, respectively. *Results.* A 10.5-yr-old boy developed IPA, controlled by ABLC (29 d). Oral VCZ for 61 d was given thereafter (150 mg bid first d, then 100 mg bid 60 d). Consolidation started while being 17 d into VCZ treatment. Another 5-yr-old boy w/o IPA was undergoing identical consolidation under the same conditions. 3 pairs of MTX infusion running in parallel were evaluated. Baseline MTX levels were always below the detection limit (<0.05) in both. In the VCZ/MTX arm, MTX levels at 18, 24, 36 & 42h were, respectively: 28.62, 19.35, 0.52, 0.18 (1. MTX); 27.62, 24.17, 0.79, 0.25 (2. MTX); 23.13, 21.38, 0.46, 0.16 (3. MTX). The 4. MTX was delivered 5 d off VCZ, yielding a concentration of 21.53, 11.39, 0.32 & 0.11 at those time points, respectively. In the MTX-only arm, the corresponding figures were 24.14, 19.50, 0.62, 0.22 (2. MTX); 27.09, 18.63, 0.50, 0.23 (3. MTX); 26.60, 18.77, 0.50, 0.19 (4. MTX). All MTX levels were in the expected range, with non-significant (NS) inter-patient difference (p=0.7; 0.07; 0.5). However, while the intra-patient difference was not significant (p=0.1) in the control, it was so (p=0.02) in the case because of significant (p=0.02-0.04) depression in levels of the last MTX (off-VCZ) vs the first 3 ones (on-VCZ), within which the intra-patient difference was NS either (p=0.1). Within 1-3 d of every MTX infusion, the first pt developed cheilitis and photosensitivity over exposed body parts, the severity of which was related to intensity of sunshine. This reaction always resolved towards the next MTX. No other side effects were observed, nor IPA did exacerbate under VCZ/MTX. Assessment of VCZ levels is planned. *Conclusions.* Although the off-VCZ levels of MTX differed significantly from those on-VCZ in the case, this could be attributed to the well known intra-patient inter-dose variability in MTX disposition. The bulk of data suggests that oral VCZ seems not to affect MTX pharmacokinetics significantly. However, this should be confirmed on a larger number of pts and/or doses of MTX given during VCZ therapy.

1261

CELL DIFFERENTIATION AND APOPTOSIS OF U-937 LEUKEMIA CELL LINES BY A NEW COMPOUND FROM DENDROSTELLERA LESSERTII

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Recently, we have reported on the activity of 3-hydrogenkwadaphnine (3-HK), purified from Dendrostellera lessertii, to induce differentiation and apoptosis in HL-60 cells upon a single dose treatment at a drug concentration of 2.5-40 nM. Regarding the relatively weaker potency of 3-HK compared to that of the crude extract, we looked for additional compound(s) with similar properties in the crude extract. Herein, we report on the isolation of a second and a more potent compound, with differentiation capability and apoptotic effects. The new compound inhibited growth and proliferation of U937 cells with an IC50 of 1.75 μg/ml. The new compound, at 0.5-2.5 μg/mL inhibited proliferation of U937 cells by more than 70% and their viabilities were decreased by 47±2.1% after 72 h of treatments. The new compound also induced differentiation of U937 to monocyte/macrophage-type cells as became evident through phorbol ester-dependent reduction of NBT, morphological changes as examined by Wright-Giemsa staining and expression of CD11b and CD14 as analyzed by flow cytometry. The results indicated that treatment of U937 cells with the new compound for 3 to 4 days induced apoptosis as assayed qualitatively by acridine orang/ ethidium bromide (Ao/EtBr) double staining, agarose gel electrophoresis and quantitatively by Annexin V technique using flow cytometry. Based on these observations, *D. lessertii* could be a novel candidate for pharmaceutical evaluation.

1262

A TRIAL TO IDENTIFY SIDE EFFECT OF DRUGS AT ULTRA-EARLY STAGE

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Method. Using the M-H Method, living blood cells are taken from a patient, and divided into two layers, upper and lower (i.e., ULRBC(U)