Effect of Composition and Method of Preparation of Liposomes on Their Stability and Interaction with Murine Monocytes Infected with *Brucella abortus*

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Abstract of:

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The success of the use of liposomes as drug carriers depends on both their formulation and the method of preparation. We have carried out a series of in vitro studies using different formulations and preparation methods, with the aim of obtaining a type of liposome which is efficient in the treatment of brucellosis. On the basis of results obtained in studies of stability at 37ºC in the presence of serum lipoproteins and of the activation of phagocytic cells and antibiotic transport to the interior of monocytes infected with *Brucella abortus,* we conclude that the most suitable vesicles are positively charged, stable plurilamellar vesicles (phosphatidylcholine, 30% cholesterol, and 10% stearylamine). Gentamicin incorporated into these cationic liposomes completely eliminated all of the intracellular *Brucella* organisms (4.6 logs), vhile free gentamicin was capable of reducing the number of intracellular bacteria by only 0.3 log.

Characterization of *Brucella abortus* and *Brucella melitensis* Native Haptens as Outer Membrane O-Type Polysaccharides Independent from the Smooth Lipopolysaccharide

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Abstract of:

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Brucella native haptens 9NHs) extracted with hot water from smooth (S)-type *B. abortus* and *B. melitensis* were purified to high levels of serological actitity and compared with the polysaccharide obtained by acid hydrolysis (PS) of the S lipopolysaccharide (S-LPS). By ^{13}C nuclear magnetic resonance analysis, NHs showed the spectrum of a homopolymer of α-1,2- or α-1,2- plus α-1,3-linked 4-formamido 4-formamido-4,6-dideoxy-D-mannose (*n*-formylperosamine) previously reported for the LPS O chain. However, while PS contained up to 0.6 % 3-deoxy-D-manno-2-octulosonate, this LPS-core market was absent from NH. High performance liquid chromatography and thin-layer chromatography showed heterogeneity in NH purified from whole cells but not in PS. By immunoprecipitation, polysaccharides indistinguishable from NH were demonstrated in extracts obtained with phenol-water, saline at 60ºC, and ether-water treatments, and none of these treatments caused S-LPS hydrolysis detectable with antibodies to the O chain and lipid A. Two lines of evidence showed that NH was in the cell surface. First, NH became biotinylated when *B. abortus* live cells were labelled with biotin-hydrazide, and the examination of cell fractions and electron microscopy sections with streptavidin-peroxidase and streptavidin-coloidal gold, respectively, showed that labelling was extrinsic. Moreover, whereas only traces of NH were found in cytosols, the amount of NH was enriched in cell envelopes and in the outer membrane blebs spontaneously released by brucellae during growth. Interactions between NH and S-LPS were observed in crude cell extracts, and such interactions could be reconstituted by using purified NH and LPS. The results demonstrate that NH is not a hydrolytic product of S-LPS and suggest a model in which LPS-independent O-Type polysaccharides (NH) are intertwined with the O chain in the outer membrane of S-type brucellae.

Outer Membrane Differences between Pathogenic and Environmental *Yersinia enterocolitica* Biogroups Probed with Hydrophobic Permeants and Polycationic Peptides

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Abstract of:

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Sensitivities to polycationic peptides and EDTA were compared in *Yersinia enterocolitica* pathogenic and environmental biogroups. As shown by changes in permeability to the fluorescent hydrophobic probe *N*-phenylnaphthylamine (NPN), the outer membranes (OMs) of pathogenic and environmental strains grown at 26ºC in standard broth were more resistant to poly-L-lysine, poly-L-ornithine, melittin, cecropin PI, polymyxin B, and EDTA than *Escherichia coli* OMs. At 37ºC, OMs of pathogenic biogroups were resistant to EDTA and polycations and OMs of environmental strains were resistant to EDTA whereas *E. coli* OMs were sensitive to both EDTA and polycations. Similar results were found when testing deoxycholate sensitivity after polycation exposure or when isogenic pairs with or without virulence plasmid pYV were compared. With bacteria grown without Ca++ available, OM permeability to NPN was drastically increased in pathogenic but not in enviromental strains or *E. coli*. Under these conditions, OMs of pYV- cells showed small differences in NPN permeability but differences in polycation sensitivity could not be detected by fluorimetry. O:1,6 (environmental type) lipopolysaccharide (LPS), but not O:3 or O:8 LPS, was markedly rough at 37ºC, and this could explain the differences in polycation sensitivity. LPSs from serotypes O:3 and O:8 grown at 37ºC were more permeable to NPN than O:1,6 LPS, and O:8 LPS was resistant to polycation-induced permeabilization. These data suggest that LPSs relate to some but not all the OM differences described. It is hypothesized that the different OM properties of environmental and pathogenic biogroups reflect the adaptation of the latter biogroups to pathogenicity.

Decreased Anion Exchanger 2 Immunoreactivity in the Liver of Patients With Primary Biliary Cirrhosis

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Abstract of:

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Chloride-bicarbonate anion exchanger 2 (AE2) is expressed in a variety of tissues, including the liver and salivary glands, where it may participate in the generation of hydroionic fluxes into secretions. We have previously reported decreased hepatic levels of AE2 messenger RNA in patients with primary biliary cirrohosis (PBC), a cholestatic condition frequently associated with pluriglandular exocrine failure. Here we investigated the expression of AE2 protein in the liver of PBC patients. Using a monoclonal antibody against an AE2 peptide, immunohistochemistry was performed on liver biopsy specimens from subjects with normal liver (n=7), patients with PBC (n=13), and patients with cirrhosis or cholestasis other than PBC (n=17 and 11, respectively). Immunostaining was graded from 0 to 7, according to its intensity and distribution. AE2 immunoreactivity was observed in normal livers, as previously reported, and in many pathological liver biopsy specimens, being mainly restricted to canaliculi and the luminal membrane of terminal and interlobular bile ducts. Canalicular and ductular scores were significantly reduced in the PBC group compared with each control group (normal liver and cirrhosis or cholestasis other than PBC), whereas no differences in immunoreactivity scores were observed among control groups. When four patients with primary sclerosing cholangitis (PSC) were analyzed, they also differed from those with PBC. These results suggest that PBC is characterized by diminished expression of AE2 in the liver. Reduced levels of this transporter protein might be involved in the pathogenesis of cholestasis in PBC.

A Potencial Experimental Model For the Study of Osteopenia in CCl4 Liver Cirrhotic Rats*

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Abstract of:

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In order to search for an experimental model to further investigate the osteopenia associated to liver cirrhosis (LC), this study has been focused on investigating the occurrence of bone disorders in male rats to which LC histologically confirmed was induced through the validated procedure of CCl_4 inhalation. Length, anteroposterior and lateromedial diameters, densitometry, mechanical stress resistance, hydroxyproline (OHprol) and calcium and phosphate contents were measured in femurs from control (n=10) and liver cirrhosis rats (n=10). It has been found that femurs from liver cirrhosis rats showed a significant reduction ($p < 0.01$) in bone weight (0.254 ± 0.003 vs 0.230 ± 0.004 g/100 g b.w), anteroposterior (4.08 ± 0.06 vs 3.69 ± 0.05 mm) and lateromedial (5.33 ± 0.05 vs 5.08 ± 0.04 mm, $p < 0.05$) diameters, resistance to mechanical stress (405.8 ± 9.5 vs 332.5 ± 9.1 N) and total densitometry (0.416 ± 0.005 vs 0.381 ± 0.004 g/cm^2). However, no significant differences were observed in bone length, calcium, OHprol and phosphate (all expressed as mg/100 mg fresh bone tissue) contents. Therefore, the proteins matrix to mineral contents ratio was not altered. These results indicate that in this model of experimental liver cirrhosis there is osteopenia characterized by bone frailty and reduced thickness, and it could offer an experimental model to stududy bone changes associated to liver cirrhosis.

Avascular Necrosis of the Distal Fibular Epiphysis: A New Condition?

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Abstract of:

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A 5-year-old boy was seen because of a right ankle sprain; radiographs showed a marginal anterior avulsion of the distal fibular epiphysis. After plaster immobilization, the patient continued with pain and subjective instability. New radiographs showed a lytic area in the same zone of the avulsion with surrounding sclerosis and a detached bone fragment. Biopsy showed bone necrosis. Two years later, without any traumatic event the patient began to have a similar pain in the left ankle. Radiographs showed fragmentation of the anterior portion of the distal fibular epiphysis with surrounding sclerosis. Surgical treatment was perfomed by curettage and cancellous bone grafting; biopsy showed connective tissue, mucoid degeneration, and necrotic bone. After excluding any general disease, we believe that this bone necrosis could be considered an epiphyseal idiopathic avascular necrosis in children. Key Words: Avascular necrosis–Fibula–Osteochondroses.

Cytogenetic Analysis of 280 Patients With Multiple Myeloma and Related Disorders: Primary Breakpoints and Clinical Correlations

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Abstract of:

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Cytogenetic analysis of unstimulated short-term bone marrow cell cultures was performed on 280 patients with multiple myeloma and related disorders. In 65% of the cases, an additional short term B-cell stimulated culture was also examined. Chromosomally abnormal clones were found in 31% of the patients, 15% in Waldenström macroglobulinemia, 25% in monoclonal gammopathies, 33% in multiple myeloma, and 50% in plasma cell leukemia. Three primary chromosomal breakpoints were recurrently involved: 14q32, 16q22, and 22q11. Structural rearrangements of chromosome I were the most frequent (26% of the abnormal cases), but always as a secondary change. Rearrangements of band 14q32 were found in 22% of the abnormal cases. Among the multiple myeloma patients who showed an abnormal karyotype, 33 (46%) were hyperdiploid, most frequently with 52-56 chromosomes, 29 patients (40%) were pseudodiploid, and the remaining 12 cases (14%) were hypodiploid. A highly significant relation was observed between the presence of an abnormal karyotype and the following clinical parameters: stage III (P=0.0001), bone marrow plasma cell infiltration greater than 30% (P=0.0001), presence of bone lesions (P=0.0009), and β2-microglobulin levels greater than 4 mg/L (P=0.0001). *Genes Chromosom. Cancer 18:84-93, 1997.* © 1997 Wiley-Liss, Inc.

Ewing's tumor of the spine: report on seven cases including one with a 10-year follw-up

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Abstract of:

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This study analyzed the cases of seven patients who completed the Treatment Protocol of the University Clinic of Navarre for Ewing's tumor of the spine between 1982 and 1993. The surgical procedures aimed at gaining local control and recovering the neurological deficit are discussed. Poor results in the survival rate can be expected, as shown by the clinical study. Only two patients are alive, including one who is free of disease more than 10 years after surgery.