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LIVEL CELL TRANSPLANTATION IN AN EXPERIMENTAL MODEL OF INBORM ERROR OF METABOLISM.

Maria L. Vega, Javier Alvarez-Cienfuegos, Cesar Benito, Fernando Pardo, Lourdes Sainz, Maria D. Lozano, Ignacio Villa-Elizaga. Departments of Pediatrics and Surgery. University of Navara. Clinica Universitaria. Pamplona. Spain.

Hepatocyte transplantation is an attractive alternative in the treatment of congenital errors of metabolism. Liver cells grafting offers the ability of immunomodulate and cryopreserve the hepatocytes. The aim of this work was to evaluate the efficacy of singeneic liver cell transplantation in a rat mutant unable to synthetize ascorbic acid (AsA) in hte liver (ODS od/od). A total of 4 non-deficient rats (ODS +/+) were used as donors. Hepatocyte isolation was carried out according collagenase perfusion. The proportion of cells excluding trypan blue (0.2%) was counted; viability ranging from 90% to 93%. Hepatocyte transplantation was performed by injection into the splenic pulp of recipient animals of 0.5 - 1.0 ml of cell suspension containing 90 million viable hepatocytes. The recipient animals (ODS od/od rats) were divided into two groups. Group I (n=8) which was transplanted into the spleen; Group II (n =4) which was not transplanted; a control group of ODS +/+ rats (Group III; n=5) was assessed. The deficient animals were fed a standard rat diet and water containing 3g/l of AsA (Fuso, Osaka, Japan) during 4 weeks before transplantation and grafted 1 week later. The survival in the transplanted group (Group I) was 63% at 5 postoperative week and 50% in the non-transplanted group (Group II). Hematoxylin-eosin staining revealed the existence of hepatocytes in spleens of transplanted animals. In spite of the improvement in the survival, all animals developed signs of AsA deficiency.

This study confirms previous works for considering that an enzyme deficiency could be corrected by cellular transplant. Further studies are necessary to prove the usefulness of liver cell transplantation and the timing of grafting.

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OSTEOGENESIS IMPERFECTA (OI):
COLLAGEN METABOLISM OF OSTEOBLASTS IN VITRO

Martin Mörike,Ulrich Vetter,Rolf Brenner,Walter Teller
University of Ulm, Dept.Pediatrics I, 7900 Ulm,Germany

OI is characterized by disorders in collagen I metabolism. Biochemical aspects of OI have been studied mostly with skin fibroblasts although bone is the most affected tissue in OI. We studied collagen metabolism of osteoblasts in culture (OBC) derived from 30 OI patients (types I-IV). All OI OBC produced low lewels of collagen. Qualitative abnormal collagen I as determined by PAGE was found among all OI types. In single cases procollagen processing was slow. Many OI OBC showed a decreased pericellular accumulation of collagen I (coll.I).

	OI type I	II	III	IV
-total number	4	3	14	9
-abnormal coll.I	2	2	9	4
-slow processing	1	0	5	3
-decreased coll I. accumulation	1	2	10	5

Conclusion: Decreased synthesis and pericellular accumulation of collagen was found associated with, but also irrespective of, obvious structural defects in collagen I and may play a crucial role in the phenotype of OI osteoblasts.

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Multifunction Role of Osteonectin/SPARC during Human Embryonic and Fetal Development

S.Mundlos, T.Reichert† B.Zabel; Department of Pediatrics and Pathology*, University of Mainz, 6500 Mainz, Germany.

The temporal and spatial distribution of osteonectin/SPARC was investigated during different stages of human development by in situ hybridization and histochemistry Specific mRNA was associated with(a) tissues exhibiting high rates of matrix production (skin, vessels, tendons fetal mesenchyme), (b) cells involved in the process of mineralization (osteoblasts, chondrocytes, odontoblasts), (c)production of basement membranes (glomeruli and (d) steroid synthesis (adrenal gland, Leydig cells) In the growth plate, expression was found in the upper hypertrophic and proliferative but not in the mineralized zone. Histochemistry detected osteonectin extracellularly in mineralized tissues, whereas others showed intracellular staining only.

The localization of osteonectin in bone, cartilage and teeth is consistent with its proposed role in mineralization. However, the function-specific distribution in non-mineralized tissues suggests a multi-functional role of this protein.

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PROLIFERATION AND COLLAGEN BIOSYNTHESIS OF CHONDROCYTES AND OSTEOBLASTS IN LETHAL SKELETAL DYSPLASIAS

Rolf Brenner,Andreas Nerlich,Frank Kirchner, Martin Mörike,Walter Teller - 1st Department of Pediatrics,University of Ulm,D-7900 Ulm,Germany

We studied in vitro proliferation and collagen biosynthesis of chondrocytes and osteoblasts in thanatophoric dysplasia (TD), chondroectodermal dysplasia (CED), short rib polydaktyly-syndrome type III (SRP-III), short rib syndrome type Beemer (SR-Beemer) and osteogenesis imperfecta type II (OI-II). In TD morphologically proliferation zone is markedly reduced and in 2 out of 3 cases studied clonal growth of articular chondrocytes in methylcellulose was not or only slightly stimulated by IGF-I (1,25 ng/ml:0%; 31% of control) and IGF-II (1,25 ng/ml:10%; 22% of control) but almost normally by TGF-ß (1,25 ng/ml: 153%; 63% of control). In CED and SR-Beemer we found persistent hypertrophic cartilage islands in metaphyseal bone and in vitro elevated sensitivity of chondrocyte proliferation to TGF-ß (1,25 ng/ml:376%; 213% of control). In OI-II osteoblasts synthesised electrophoretically slower migrating collagen α1(I)-chains indicating posttranslational overmodification.

We conclude that both, defects in matrix synthesis and regulation of chondrocyte proliferation play an important role in the pathogenesis of lethal skeletal dysplasias.

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POSTNATAL DEVELOPMENT OF PYRUVATE OXIDATION IN SKELETAL MUSCLE OF THE RAT

[1]Wolfgang Sperl, [1]Rob Sengers, [1]Frans Trijbels, [1]Wim Ruitenbeek, [2]Ruud De Graaf, spn. by Heribert Berger
[1]Departments of Paediatrics and [2]Medical Statistics, University of Nijmegen, The Netherlands

Age dependency of enzymes involved in the energy generating system was investigated in 600 g supernatants of quadriceps muscle from rats of different ages. (1-^{14}C) pyruvate oxidation rates (± ADP) increased significantly from low early values in the neonatal period (321 ± 113 nmol/h/mg prot.) to nearly adult values at the end of the suckling period (859 ± 199 nmol/h/mg prot.) ($p < 0.01$). Pyruvate dehydrogenase complex (PDHC), citrate synthase and cytochrome c oxidase showed similar patterns of development. Immunoblot studies of PDHC indicated that E1α, the regulatory subunit of the multienzyme complex, is the most rapidly increasing protein with age. This study demonstrates a clear increase in mitochondrial oxidative capacity in rat muscle up until weaning which is in accordance with the known differentiation pattern of skeletal muscle fibers.

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THREE FORMS OF CARNITINE PALMITOYL TRANSFERASE (CPT) II DEFICIENCY: (1) FATAL AT BIRTH OR (2) AT AGE 20 YEARS WITH CARDIAC FAILURE, OR (3) LIFE-COMPATIBLE WITH MODERATE MUSCLE SYMPTOMS. George Hug, Kevin Bove, Monica Tsoras, Michael Ralston, Mary Ryan, Edgar Ballard and Shirley Soukup. Children's Hospital, Cincinnati, OH 45229, USA.

Three unrelated patients had Vmax normal for CPT I but reduced for CPT II, and normal Km for both. Palmitoyl CoA synthase, carnitine acetyl transferase, 4 acyl CoA dehydrogenases were normal. The patients: (1) A girl died at 5 days of encephalo-cardiomyopathy. Lipid was high in heart, liver, muscle; in these and in fibroblasts, CPT II was < 6% or not detectable. Total Carnitine was low, acyl carnitine high (Hug et al.Pediatr Res 25:115A;1989). (2) A male without muscle symptoms died at 20 years of dilated cardiomyopathy, as did his half-brother (of a different father). In heart, lipid was not excessive, carnitine was 50% and CPT II was 19%. (3) A male at 20 years had symptoms as in muscle CPT deficiency (DiMauro et al.Science 182:929;1973). Liver and heart were normal clinically. CPT II was 6% in muscle and fibroblasts. - Deficient CPT II and normal CPT I in the same patient indicate that the 2 enzymes differ. Whether different phenotypes in CPT deficiency reflect differences in tissue distribution and in extent of the defect must be studied in multiple tissues obtained at biopsy or autopsy.