469

OSTEOPENIA IN CEREBRAL PALSY, *N.J. Shaw, *C.P. White, **W.D. Fraser, *L. Rosenbloom (introduced by C.S. Smith), *Institute of Child Health, Royal Liverpool Children's Hospital Alder Hey, and **Department of Chemical Pathology, Royal Liverpool University Hospital, England.

Children with cerebral palsy are recognised to be at risk of osteopenia but it's extent and aetiology are unclear. Nine children aged 2 to 13 years with cerebral palsy who were non-ambulant had measurements of bone mineral density of the lumbar spine. Assessments of 25 OH vitamin D status, parathyroid hormone activity and urinary calcium excretion were also performed.

All had evidence of severe osteopenia with bone mineral density standard deviation scores ranging from -1.7 to -4.2. Three children had gross hypercalciuria. Parathyroid hormone activity was not increased. Although 25 OH vitamin D_2 levels were universally low, all but one had adequate 25 OH vitamin D_3 levels.

Three children with recurrent long bone fractures who have been treated over a 12 to 18 month period with bisphosphonate drugs have shown a marked increase in bone mineral density ranging from 16 to 40%. It appears that osteopenia in severe cerebral palsy is common but there is no consistent abnormality of vitamin D or parathyroid hormone status. Bisphosphonates may well be a useful therapeutic option in such children with recurrent fractures.

470

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NEPHROCALCINOSIS (NC) DUE TO HYPERPARATHYROIDISM IN X-LINKED HYPOPHOSHATEMIC RICKETS (XLHR)-RESULTS OF A RETROSPECTIVE STUDY IN 155 CHILDREN AND ADOLESCENTS.

Nephrocalcinosis is a major complication in patients with XLHR. In a multicentre retrospective study on 155 children and adolescents we found in 62/133 patients signs of NC by ultrasound,23 were /=4y. of age.With high oral phosphate load hyperphosphaturia and hypercoxaluria occurs,but hypercalciuria due to secondary hyperparathyroidism may also develop.A subgroup(`noD´)of 8 prepubertal(0.7-4.4y.,median: 1.95y.)and 2 pubertal children were treated with oral phosphate(18-173mg/kg,median:71mg/kg)but very low vit.D2( 7500U/day).They were compared with 14XLHR children (=`D´,age:0.5-4.1y.,median:1.9y.)given 1,25(OH)2D(max.dose:19-75ng/kg,median:38ng/kg)and oral phosphate(max. 64-150mg/kg,median:94mg/kg)daily.Results:`noD´:hypercalcemia(2.55mmol /l)were found in 8/10 more than once,PTH1-84(normal:5-55pg/ml)were elevated in 8/10(37-397pg/ml,median:90pg/ml).NC was confirmed in 6/9. Group`D´:hypercalcemia were found in 8/14 more than once,PTH1-84 were elevated in 1/14(13-80pg/ml,median:35pg/ml).NC occurs in 7/11. Conclusions:1)NC might occur in patients with XLHR even in cases with insufficient low vit.D therapy, but usual oral phosphate load(70mg/kg) 2)Hyperparathyroidism and hypercalcemia are frequent findings in patients with inadequate low doses of vit.D.3)Combination of 1-OH-vit.D derivates (20-40ng/kg*d)and high oral phosphate prevents hyperparathyroidism.

471

SPORTING AND CALCIUM/PHOSPHORUS METABOLISM : BONE MINERAL DENSITY AND SPECIFIC HORMONAL PATHWAYS COMPARISON IN ADOLESCENT GIRLS. A.Marchi, M.Gualea, A.Pisati, V.Perrone, A.Cella, L.Bachella, P.Baiardi, Departement of Pediatrics, Interdisciplinar Center of Sport Medicine, Fondazione Clinica del Lavoro, University of Pavia, Pavia, Italy .

A team of 10 volley player eumenorrheic adolescent girls and an age-matched control group were tested in order to investigate an hypotetic relationship between Bone Mineral Density (BMD) and specific hormonal pathways : estradiol (E2), testosterone, Δ4-androstenedione, dehydroepiandrosterone sulphate (DEHAS), osteocalcina. Some bone rearrangement factors i.e. procollagen III and 1,25-OH2 cholecalcipherol were also determinated. BMD of the lumber spine was quantitated by Dual Energy X-Ray Absorbtionmetry method (DEXA). Hormones were evaluated by RIA. No significant difference was found in BMD evaluations between the two groups; also tested correlations were not statistically significative. However negative links between BMD and osteocalcina, procollagen III and 1,25-OH2 cholecalcipherol were found; positive links between BMD and remaining parameters. Because of the intriguing of these results, further investigations are in progress in order to assess : 1) the real meaning of negative link BMD vs 1,25-OH2 cholecalcipherol; 2) the influence of different sport activities on calcium/phosphorus metabolism.

472

EVIDENCE THAT IGF 2 INTERFERES WITH THE LYSOSOMAL ENZYME ACTIVITIES OF CARTILAGE CELLS IN VITRO. S. Poiraudeau, J. Bourguignon, M. Pagano and M.T. Corvol, INSERM U 30, Hop. Enfants Malades, Paris, France.

In cartilage cells as well as in other cell types, IGF2 is considered as a growth factor mainly mimicking the effect of IGF1 through IGF1-receptor. Since cartilage cells contain both types I and II IGF-receptors, it is still unknown wether IGF2 may have specific effects mediated through the IGF2-Mannose-6-Phosphate receptor (IGF2/M6P-R). This bifunctional protein also binds glycosylated proteins such as newly synthesized acid protease enzymes being responsible of their targeting from the Golgi to the lysosomes. Our purpose was to investigate the possibility for IGF2, by comparison with IGF1, to interact with the storage of chondrocyte lysosomal enzymes.Cultured chondrocytes from prepubertal, fetal or adult rabbits were used and their content of acid phosphatase, cathepsin B and L activities was quantified by using a colorimetric reaction with appropriate substrates. In basal conditions, the acid protease activities localized by histochemistry and electron microscopy, were observed in the RER, in the Golgi vesicles and in the lysosomes of the chondrocytes.The addition of IGF2 into the culture medium during 60 min., significantly reduced chondrocyte acid protease activities in a dose dependent manner with maximum effect at 10-9M. There was respectively 25%, 33% and 21% decrease of acid phosphatase, cathepsin B and L activities as compared with chondrocyte activities evaluated in basal conditions (100%). By contrast, the addition of [1-34]-Parathyroid hormone (PTH), significantly increased these protease activities in a dose and time dependency. The maximum effect of [1-34]-PTH was observed at 10-8M with 40%, 89% and 60% increased activities above the respective basal levels. IGF2 inhibited the PTH effect and still decreased the chondrocyte acid protease content below control values. Such an inhibiting effect was not observed with similar concentrations of IGF1. In addition, the number of chondrocyte IGF2/M6P specific binding sites was significantly increased in cells treated with PTH as compared with non treated ones. Such a stimulating effect was not observed in the presence of [3-34]-PTH nor [1-34]\[3-34] PTH mixture. Finaly, the number of IGF1 binding sites was not increased by [1-34]-PTH. These data are in favour of a new role for IGF2 in cartilage, concerning the degradation of chondrocyte proteins through the mediation of IGF2/M6P-R.

● 473

VGR-1/BMP-6 INDUCES OSTEOGENIC DIFFERENTIATION IN MESENCHYMAL CELLS. S.E. Gitelman, M. Kirk, A.J. Kahn, R. Derynck, Departments of Pediatrics and Growth and Development, UCSF, San Francisco, CA 94143, USA

Recent studies implicate the transforming growth factor beta (TGF-β) superfamily as playing a major role in bone formation. Nothing is currently known about the function of vgr-1 (also called Bone Morphogenetic Protein-6, or BMP-6), a TGF-β-like protein that localizes to hypertrophic cartilage. To determine if this factor enhances chondrocytic differentiation and/or stimulates osteogenesis, we have utilized two pluripotent mesenchymal cell lines, C3H-10T1/2 and ROB-C26. Stable transfectants that over-express vgr-1 mRNA were created with each cell line. Such over-expression does not alter the growth rate of the two cell lines. Vgr-1 over-expression results in up to a 9-fold increase in alkaline phosphatase(ALP) activity in C26 cells, but has no significant effect on 10T1/2 cells. In the presence of 10^{-6}M retinoic acid (RA), an osteoinductive agent, C26 and 10T1/2 parental cells as well as the 10T1/2 vgr-1 over-expressors exhibit a stimulation in enzyme activity; by contrast, C26 vgr-1 over-expressors consistently show a decrease in ALP activity relative to untreated vgr-1 over-expressors. To determine if vgr-1's osteoinductive effects are mediated through a feedback mechanism involving changes in the extracellular matrix(ECM), we have grown parental and vgr-1-transfected C26 cells on plastic, then removed the over-lying cells, and replated parental cells back onto the various underlying residual ECMs. The ECM from vgr-1 transfectants induces up to 4-fold greater ALP activity than the ECM of parental cells; such induction was not seen with ECM from RA-treated C26 vgr-1 over-expressors. These results suggest that: 1)vgr-1 induces osteogenic differentiation, and that this effect is mediated through changes in the ECM compostition; 2)vgr-1 over-expression inhibits RA induction of alkaline phosphatase activity in C26 but not in 10T1/2 cells. Ongoing studies will determine vgr-1's effects on other markers of both bone and cartilage differentiation in these cell lines, through *in vitro* and *in vivo* analyses.

474

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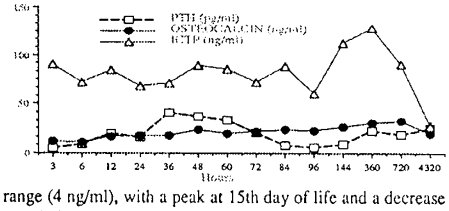
ICTP: A NEW INDEX OF BONE RESORPTION IN NEWBORN.

Cross-linked carboxyterminal telopeptide of type I collagen (ICTP) is a new marker of bone metabolism, liberated during the degradation of type I collagen (the only collagen type found in the mineralized bone). In order to evaluate more completely the bone turnover in the neonatal period, we have studied 94 healthy full-term newborns, measuring the serum concentration of ICTP, as index of bone resorption, of osteocalcin and alkalyne phosphatase (AP), as indices of bone formation, and of PTH (PTH intact 1-84). All these parameters were determined at 3rd, 6th and 12th hour of life, every 12 hours during the first 6 days and at 15th day, 1st and 6th month. As observed in the figure,



PTH shows a peak in the 2nd day of life. Osteocalcin increases progressively during the first month. ICTP levels in newborns are up to 31-fold higher (range 60.2-127.8 ng/ml) than adult normal range (4 ng/ml), with a peak at 15th day of life and a decrease after the 1st month. No correlation was found between ICTP and AP. High ICTP levels seem to indicate an elevated osteoclastic activity in bone remodeling of the neonatal age. ICTP determination seems to be useful for a more complete evaluation of bone metabolism in newborns.