M400

Truncation of Osteoprotegerin in an Isolated Case of Juvenile Paget's Disease. J. R. Lennard-Jones, J. De Paola, A. Califano, B. L. Lum, A. Gottlieb, R. Altman, P. H. Sivee, P. Valone. 1Stanford University School of Medicine, Palo Alto, CA, USA, 2Robert Wood Johnson Medical School, New Brunswick, NJ, USA, 3University of Miami School of Medicine, Miami, FL, USA, 4Tian Pharmaceuticals, Inc., South San Francisco, CA, USA.

Gallium maltolate is a novel orally available formulation of gallium being developed as an agent for the treatment of metabolic bone disorders. Gallium when given by low dose subcutaneous gallium nitrate has demonstrated anti-resorptive activity in Paget's disease of bone. This study evaluated the pharmacokinetics of oral gallium maltolate administered at three dose levels as a single and as three consecutive daily doses in patients with advanced Paget's disease of bone or primary hyperparathyroidism. Gallium maltolate (200, 400, or 600 mg) was administered as a single dose and 14 days later, as three consecutive daily doses. Serial gallium ion serum concentrations were measured over 350 hours for the single dose only. An alteration in diastolic function was observed in PDB patients with respect to age-matched controls. E/A ratio was significantly reduced (0.79 +/- 0.23 vs 1.11 +/-0.29, p=0.008), while DT (249 +/- 70 vs 182 +/- 17, p=0.009) and IVRT (119 +/- 30 vs 89 +/-12, p=0.009) were significantly increased in PDB patients than in controls. These differences were greatest in the subgroup with more extensive polyostotic skeletal disease only when age-matched controls. In conclusion, results from the present study suggest that in the early phases of PDB together with an increase in ventricular mass there is a trend towards a diastolic dysfunction that could be influenced by the extension of the disease and by the degree of bone turnover.

Disclosures: G. Martini, None.

M403

Echocardiographic Assessment of Cardiac Function in Paget's Disease of Bone. G. Marinii, A. Paladuzza, E. Cordioli, L. Gennari, R. Valentii, D. Merlotti, B. Galli, F. Cipolli, F. Inostra, N. Del Canto, R. Nuti. Internal Medicine, Endocrine-Metabolic Sciences and Biochemistry, University of Siena, Siena, Italy.

To identify the mechanisms which influence the development of cardiovascular complications of Paget's disease of bone (PDB), we performed carotid artery doppler ultrasonography and non-invasive assessment of cardiac size and function by clinical criteria and electrocardiography and echocardiography in 15 patients with Paget's disease of bone and in 20 control subjects matched by sex, age and body mass index. The PDB patients were divided into two groups on the basis of the degree of skeletal involvement (6 patients with widespread and 9 polyostotic disease). Left ventricular mass was calculated using the formula of Devereux and Reichek, modified with Penn convention. Relative wall thickness (RWT) was calculated as the ratio between the end-diastolic thickness of interventricular septum and posterior wall, and half of left ventricular diastolic diameter. Diastolic ventricular function was assessed by measuring the peak E- and A-wave velocities (E/A) and interventricular relaxation time (IVRT) and E-wave deceleration time (DT). Serum calcium, phosphate, creatinine, osteoprotegerin (OPG), RANKL and bone specific alkaline phosphatase were also determined. In conclusion, results from the present study confirm that P32R11 mutation affecting the ubiquitin-binding domain of SQSTM1 is a common cause of familial and sporadic Paget's disease of bone in subjects of Italian descent.

Disclosures: L. Gennari, None.

M404

Pharmacokinetics of Oral Gallium Maltolate Administered in a Single or Multiple Dose Schedule in Patients with Paget's Disease of Bone or Primary Hyperparathyroidism. Pilot Study. B. L. Lum, A. Gottlieb, R. Altman, P. H. Sivee, P. Valone. 1Stanford University School of Medicine, Palo Alto, CA, USA, 2Robert Wood Johnson Medical School, New Brunswick, NJ, USA, 3University of Miami School of Medicine, Miami, FL, USA, 4Titan Pharmaceuticals, Inc., South San Francisco, CA, USA.

Gallium maltolate is a novel orally available formulation of gallium being developed as an agent for the treatment of metabolic bone disorders. Gallium when given by low dose subcutaneous gallium nitrate has demonstrated anti-resorptive activity in Paget's disease of bone. This study evaluated the pharmacokinetics of oral gallium maltolate administered at three dose levels as a single and as three consecutive daily doses in patients with advanced Paget's disease of bone or primary hyperparathyroidism. Gallium maltolate (200, 400, or 600 mg) was administered as a single dose and 14 days later, as three consecutive daily doses. Serial gallium ion serum concentrations were measured over 350 hours for the single dose and over 480 hours for the multiple dose schedule. Samples were assayed for total gallium ion concentration using inductively coupled plasma mass spectrometry. Eight of 12 subjects were male. The median age was 83 years (range: 55-88 years). Ten of the 12 subjects completed the intended course of treatment. One serious adverse event, possibly related to drug study, was reported during the study. This subject had an episode of congestive heart failure after discharge from the study unit, while in the washout period. No deaths were observed. Adequate pharmacokinetic data were available for 9 subjects during the single dose and for 7 subjects during the multiple dose portion of the study. Serum gallium ion concentrations increased in linear fashion with increasing dose over the dose range examined. For single or multiple doses, a three-fold increase in gallium concentrations resulted in an increase of the mean serum total gallium ion Cmax and AUC total values by 2.8-fold and 3.0-fold, respectively. Apparent clearance (CL/F), T1/2, Tmax, and terminal phase apparent volume of distribution (Vz/F) were similar across all three doses of oral gallium maltolate. Steady-state...
concentrations were not achieved during the three-dose schedule. At the 600 mg/day, total serum gallium ion Cmax following the third dose averaged 1126 ng/mL (S.D. 45–533) and concentrations observed 24 hours following this dose ranged from 420 to 934 ng/mL. These concentrations are similar to those expected to inhibit bone resorption in vivo. Thus gallium maltolate achieves serum gallium ion concentrations that may be therapeutic. Further testing of this agent in Paget’s and other metabolic diseases is warranted.

Disclosures: P.H. Sayre, Titan Pharmaceuticals, Inc. 3.

M405

Clinical manifestations of primary hyperparathyroidism (PHPT) have changed dramatically over the past several decades. Today, most patients have no signs or symptoms typically associated with this condition; however, parathyroid carcinomas are recognized. Parathyroid carcinoma is suspected. We describe two cases of benign PHPT in which the presumptive clinical diagnosis, pathology revealed oxyphil adenoma. Most parathyroid adenomas are composed primarily of chief cells, and oxyphil cells are usually non-functional. Oxyphil adenoma must be in the differential diagnosis of PHPT patients with severe biochemical and clinical abnormalities and large tumors that mimic parathyroid carcinoma.

In summary, 2 patients presented with marked hypercalcemia, striking PTH elevations, large tumors, and clinical symptoms and overt skeletal disease. Although parathyroidectomy would have been the presumptive clinical diagnosis, pathology revealed oxyphil adenoma. Most parathyroid adenomas are composed primarily of chief cells, and oxyphil cells are usually non-functional. Oxyphil adenoma must be in the differential diagnosis of PHPT patients with severe biochemical and clinical abnormalities and large tumors that mimic parathyroid carcinoma.

Disclosures: J.B. Fleischer. None.

M406

The majority of patients with primary hyperparathyroidism (pHPT) recurrently produce kidney stones, while the rest have other clinical manifestations (malignant bone disease, acute pancreatitis, depression). The aim of this study was to examine the association between the clinical symptoms and the location of adenoma.

This was a retrospective evaluation in the records of 91 patients (10 males, 81 females, mean age: 61.9 years (20-70 y) operated for primary hyperparathyroidism between 1995 and 2000. Kidney stones were presented in 55 cases and other clinical symptoms in 35 cases. The diagnosis of pHPT was proved by surgery and histology. The adenoma was accurately located by operation.

The adenoma of patients with kidney stones was located in 50 cases (91 %), (chi²=67.5, p<0.0005) in the left inferior parathyroid gland and in 2 patients in the left superior one, while in 3 patients suffered from multiple hyperplasia. In patients without kidney stones the adenoma was localized in the right inferior parathyroid gland in 24 cases (49 %), (chi²=43.9, p<0.0001), however, in 5-3 patients it was detected in the left or right superior parathyroid gland. Less frequently multiple hyperplasia (3 patients) and ectopic location (2 patients) was also observed.

These results raise the possibility that the clinical manifestation of the pHPT with or without kidney stones could be influenced by the location of the adenoma. Would it be possible that the biologic effects of parathyroid hormone sourced from one or another fraction of the four glands could be different or different biologically active fragments are produced? From a practical point of view: in patients with kidney stones due to pHPT the left inferior parathyroid gland should be the first target of choice for the surgeon if the preoperative procedures leave any debate for the exact location of the adenoma.

Disclosures: E. Capuron. None.

M407

Rapid intraoperative parathyroid hormone (IOPHT) test is used to guide adequacy of resection during surgery for primary hyperparathyroidism (HPT). Commonly, a 50 % decrease versus baseline PTH at 10 min after resection of the suspected parathyroid adenoma indicates "cure". For this rule to be valid, the time interval of 10 min would be stated starting from an instant at which the hormone efflux into circulation totally stopped ("ideal clamping"). Nevertheless, at the "actual" clamping the vessels of the affected gland have partially been clamped before excision, and this situation, modifying the decay curve, may cause false negative results. Our goal was the definition of a "virtual" clamping, for the development of a new monitoring protocol. Forty-four patients with primary hyperplasia or parathyroid adenoma who underwent total parathyroidectomy were studied. By a simulation study on the behaviour of the iPTH disappearance curve, we showed that the shifting in time of the origin of the curve produced a behaviour like that to one obtained in false negatives. Using the time interval between manipulation and clamping (Tmc), 17 patients were tested. Using a 50 % reduction versus clamping in the levels at 10 min after clamping, n 10 patients with Tmc <10 min were correctly classified as being cured, and n 4 with Tmc <10 min were incorrectly classified as not being cured (false negatives); of these four patients, 2 patients were correctly reclassified using the 50 % reduction, versus manipulation only in 4 patients (false positives). n 10 patients with Tmc < 10 min were not correctly reclassified by additional monitoring. Additional monitoring was also required in the remain 3 patients with Tmc > 10 min. In conclusion analysis of PTH kinetics is more accurate in assessing adequacy of resection by taking into account the substantial variations in relative values of clamping to manipulation, as well as their different time intervals.

Disclosures: M. Tommasi. None.

M408
Characterization of the GCMB Promoter by Transient Transfection of Parathyroid Cell Primary Cultures. A. Maret, C. Ding, D. Goldenberg, M. Shamblott, M. A. Levine, 1. Pediatrics, Johns Hopkins University, Baltimore, MD, USA, 2. Otolaryngology-Head and Neck Surgery, Johns Hopkins University, Baltimore, MD, USA, 3. Gynecology-Obstetrics, Johns Hopkins University, Baltimore, OH, USA, 4. Pediatrics, Cleveland Clinic Foundation, Cleveland, OH, USA.

GCMB, a recently discovered transcription factor, is uniquely expressed in developing and mature parathyroid cells and is critical for the development and function of parathyroid glands in mice as well as in humans. In order to investigate the mechanism for transcriptional regulation of this gene, we have cloned and sequenced 2.8 kb of the 5’ untranslated region (UTR) of GCMB gene from a primary parathyroid adenoma subjected to RACE using 4-oximinoquinolizone primers to identify the transcription start site. We generated plasmids in which a luciferase reporter gene was coupled to the 2.8 kb GCMB promoter or nested deletion products. The ability of these sequences to activate luciferase expression was analyzed 48 hours after transient transfection of COS7 cells and primary cultures of bovine parathyroid glands and human parathyroid adenomas using Fugene 6. Analysis of the RACE amplicons revealed a single transcription start site located 247 bp upstream of the translation start site. The promoter contains a conventional TATA box and GC-rich box as well as potential regulatory elements for many transcription factors, including AP2 and Paxx1, within 200 bp of the TSS. The 2.8 kb GCMB promoter region strongly stimulated luciferase activity in bovine (40-fold greater than vector) and human parathyroid gland primary cultures (15 to 100-fold). This stimulated luciferase activity only weakly (5 to 10-fold) when transfect in COS 7 cells. Progressive deletion analysis of GCMB promoter-luciferase constructs indicated that the core minimal promoter is located between nt -186 and +83, which stimulated luciferase activity 20-fold over vector. These results demonstrate the utility of parathyroid primary cell cultures in the analysis of transcriptional regulation of genes expressed in the parathyroid. Moreover, the identification of binding sites in the GCMB promoter for Pax1 and Pax9, which are critical for parathyroid gland development, suggests that these two transcription factors may promote parathyroid cell development via regulation of GCMB expression.

Disclosures: A. Maret. None.

M409
Gene Expression Profiling in Patients with Primary Hyperparathyroidism Before and After Treatment. S. Renne*, L. Stilen*, O. K. Olstad*, K. Brixen*, K. M. Gautvik1, B. Abrahamsen2, 1. Department of Medical Biochemistry, University of Oslo, Oslo, Norway, 2. Department of Endocrinology, Odense University Hospital, Odense, Denmark.

Primary hyperparathyroidism (PHPT) is characterised by increased and sustained secretion of parathyroid hormone (PTH), most frequently due to an adenoma. PTH is known to increase the rate of remodelling where both bone formation and resorption are greatly accelerated, reflecting a hyperactive state of osteoblasts and osteoclasts. The up-regulation of bone turnover in PHTP results in a net catabolic state where a decrease in bone mass is