ABSTRACTS[1]

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DIFFERENTIATION OF 3T3-L1 PRE-ADIPOCYTES IS ASSOCIATED WITH DOWN-REGULATION OF IGF-I GENE EXPRESSION. ML Adamo, L Wang, and J Talamantez, Department of Biochemistry, MSC 7760, The University of Texas Health Science Center at San Antonio, San Antonio, TX.

3T3-L1 differentiation is stimulated by a cocktail containing either a high dose of insulin or a low dose of IGF-I (Smith et al, JBC, 1988), suggesting that exogenous insulin or IGF-I act through the IGF-I receptor to stimulate differentiation. However, the role of endogenous IGF-I in 3T3-L1 differentiation is less clear. One previous report (Boney et al, Endo., 1994) found that IGF-I mRNA was expressed at low levels in 3T3-L1 cells that could only be detected by RT-PCR, and was the same in undifferentiated cells treated with IGF-I and in cells that had been stimulated to differentiate with a cocktail including IGF-I. However, endogenous IGF-I expression has been detected in primary pre-adipocytes and in mature primary adipocytes, and in Ob1771 cells (e.g., Boney et al, Gaskins et al, Endo., 1990 and Doglio et al, EMBO J, 1987). Moreover, levels of IGF-I mRNA are potently induced during Ob1771 cell differentiation (Doglio et al), and are increased in primary cultures of differentiating adipocytes, where neutralizing antibody to IGF-I inhibits DNA synthesis and biochemical differentiation (Boney et al, Nogues et al Intl J Obesity, 1993). Thus it has been suggested that 3T3-L1 differentiation does not require endogenous IGF-I. We have re-examined this issue by measuring IGF-I mRNA during 3T3-L1 differentiation. Confluent 3T3-L1 cells (ATCC) were treated with 0.1 uM dexamethasone (DEX), 0.3 mM 1-methyl-3-isobutylxanthine (IBMX) and 6 ug/ml bovine insulin for 48 hours in complete medium. The cells were then refed with complete medium containing insulin alone at 48 and 120 hours. IGF-I mRNA was easily detected in confluent pre-adipocytes. Upon addition of differentiation cocktail, IGF-I mRNA declined to about 20% of the levels in non-differentiating cells by 12 hours, and remained at this low level compared to undifferentiated cells throughout the 192 hour observation period. Differentiation occurred in the cultures in which IGF-I was inhibited as evidenced by the ~ 3-fold increase in cell number in the differentiating cultures that occurred between 24 and 48 hours and the large induction of glycerol-3-phosphate dehydrogenase activity observed beginning at 48 hours. The decrease in IGF-I mRNA is temporally correlated with the entry into S phase that results in the limited cell division of differentiating 3T3-L1 cells (Tang and Lane, Genes Develop., 1999). Thus, unlike differentiation of primary pre-adipocytes and Ob 1771 cells, endogenous IGF-I either plays no role in 3T3-L1 differentiation, or provides a signal to 3T3-L1 pre-adipocytes that must be inhibited in order for the limited clonal expansion followed by terminal differentiation to occur.

OVEREXPRESSION OF PARATHYROID HORMONE-RELATED PROTEIN BY THE HUMAN INTESTINAL EPITHELIAL CELL LINE LOVO INHIBITS CELL PROLIFERATION. P K Seitz, M Falzon and C. W. Cooper, Department of Pharmacology, University of Texas Medical Branch, Galveston, TX

Parathyroid hormone (PTH)-related protein (PTHrP), has structural homology with PTH, binds to the PTH/PTHrP plasma membrane receptor, and can cause Humoral Hypercalcemia of Malignancy. However, PTHrP acts in an autocrine/paracrine manner rather than as a hormone and appears important in regulating growth and development of a wide variety of tissues. Recent evidence suggests that PTHrP also may act in an intracrine manner via nuclear internalization mediated by a nuclear localization sequence (NLS) located at amino acids 86-106. Previously, we showed that the human colon cell line LoVo secreted PTHrP and that neutralization with antisera to PTHrP (1-34) increased cell growth. Here, we have established over- and under-expressing clones of LoVo cells by transfecting parental cells with cDNA encoding for full length PTHrP in both the sense (+P) and antisense (-P) orientation. Orientation of the insert was confirmed by northern analysis using probes that recognize sense and antisense mRNA transcripts. Clones transfected with empty vector (V) served as control. To assess a possible nuclear site of action, we used site-directed mutagenesis to delete amino acid residues 88-91 or both 88-91 & 102-106 and then transfected cells with the mutant PTHrP cDNA in the sense orientation to establish single (M1) and double (DM) mutant lines. Secretion of PTHrP was determined with an immunoradiometric sandwich assay kit. To analyze cell growth, cells were plated in 24 well multiplates and counted with a Coulter Counter 1, 4 and 6 days later. DNA synthesis rate was determined by pulsing cells for 2 hr with ^{3}H-thymidine at 4 days of culture and examining incorporation of ^{3}H. All sense transfected cells (+P, M1 and DM) secreted at least 10 times as much PTHrP as V transfected control cells. The +P cells grew only half as fast as V cells, while -P cells grew twice as fast as controls ($p < .001$). Also, the +P cells incorporated only half as much ^{3}H-thymidine as V cells, while -P cells incorporated 50% more ($p < .001$). In cells bearing a mutated NLS, M1 or DM, the suppressive effect on growth seen in the +P cells was negated. The present findings with sense and antisense PTHrP cDNA transfected LoVo cells support our earlier conclusion that PTHrP inhibits the growth of LoVo cells and further show that the growth effect is mediated by a nuclear action that results in decreased DNA synthesis. The findings support the idea that PTHrP may be an important growth regulator of gut epithelial cells.

CURRENT TRENDS IN ANTIMICROBIAL SUSCEPTIBILITIES OF BURN WOUND PATHOGENS GD Motykie, LF McCoy, E Carino, P Edgar, DN Herndon, JP Heggers, Department of Microbiology, University of Texas Medical Branch and Shriners Hospital for Children, Galveston, Texas.

Purpose: The antimicrobial spectrum necessary for treating burn wounds is ever changing due to the genetic adaptation of pathogens that are becoming ever more resistant to current antimicrobial armamentariums. The purpose of this study was to assess the changes in burn wound pathogen susceptibilities over a two-year period.
Methods: The susceptibilities of burn wound pathogens to aminoglycosides, cephalosporins, carbapenems, monobactams and quinolones were retrospectively reviewed for the time period of January 1, 1998 to January 1, 2000.
Results: The susceptibilities of 7814 burn wound pathogens (4562 gram-positive and 3252 gram-negative pathogens) were reviewed for the two-year study period. Overall, there was approximately a 50% decrease in the susceptibility of gram-negative pathogens to antimicrobials from 1998 to 2000. In 2000, only *Enterococcus faecalis* and *S. hominis* remained susceptible to imipenem and meropenem. The susceptibility of *Stenotrophomonas maltophilia* to bactrim and levofloxacin dropped from 86% to 64% and 100% to 18% respectively. Vancomycin remained the drug of choice for treating gram-positive organisms and it was the only antibiotic to maintain 100% effectiveness against both enterococci and staphylococci.
Conclusions: Gram-negative pathogens are becoming a formidable challenge to treat in burn wound patients. The susceptibilities of these organisms should be targeted for strict surveillance in order to prevent their continued acquired resistance to newer antimicrobials.

ST JOHN'S WORT CAUSES BLEEDING IN CHICK EMBRYOS, JN Garcia, A Covington, SF Chopin. Texas A&M University-Corpus Christi, Corpus Christi, TX

St. John's wort is a herbal remedy sold over the counter as a treatment for depression. Although its efficacy as an anti-depressant is unproven, sales of St. John's wort have been increasing. There have been no literature reports of its effect in developing systems. This project investigates the effect of St. John's wort on chick development.

Five groups of fertile chick eggs were injected with a single dose of St. John's wort (Frontier Certified Organic St. John's Wort) delivered in 0.1 ml physiological saline prior to incubation. Dose determination was based on weight equivalencies between a 70 g human and a 60 mg chick egg. The experimental groups received St. John's wort in doses equivalent to human dosages of 1, 1.25, 1.5, 1.75, and 2 g: 0.8 mg (n = 39), 1.1 mg (n = 39) 1.3 mg (n = 37), 1.5 mg (n = 36) and 1.7 mg (n = 38). Control eggs (n = 19) received 0.1 ml physiological saline. The eggs were incubated for 10 days, then examined for viability and malformations.

Treatment with St. John's wort did not affect the viability of the embryos: experimental groups were 79%, 69%, 65%, 89%, and 76% viable (lowest to highest dosages), compared to 74% in the controls. However, there were statistically significant (Fisher's Exact Test/ Chi Square) increases in both the total number of abnormalities ($p < 0.05$) and the number of embryos having abnormal subcutaneous bleeding ($p < 0.05$) increased with increasing dosages. Control embryos exhibited 29% total abnormalities; total abnormalities in treated embryos were 55%, 74%, 83%, 75%, and 72% from lowest to highest dosages. Control embryos exhibited 21% abnormal bleeding compared to 52%, 70%, 83%, 75% and 72% in the experimental groups.

Abnormal bleeding in embryos can present a serious developmental obstacle with profound sequelae, including ischemia in rapidly developing organs, particularly the central nervous system. Although the bleeding may not halt development, the ischemia could prevent the normal maturation and functioning of the affected organs. These results indicate that St. John's wort should be avoided during pregnancy and by individual taking anti-coagulants.

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