270

A Scanning Immunoelectron Microscopic Study of Cementum Associated Lipopolysaccharides (LPS). F.J.HUGHES*, D.W.AUGER and F.C.SMALES, The London Hospital Medical College, London E.1.

Previous work (Hughes and Smales, 1985) has suggested that cementum associated LPS is largely confined to the superficial surface of cementum previously exposed to a periodontal pocket. To provide further information a scanning immunoelectron microscopic technique has been used to investigate the distribution of LPS in the pellicle and superficial cementum layers of affected teeth.

Human antiserum to a pooled oral LPS sample was prepared using affinity chromatography. Sections were prepared from teeth extracted due to periodontal disease and were incubated with the antiserum, followed by a goat antihuman Ig system conjugated to colloidal gold. The gold particles were subsequently enhanced with silver, according to the method of Holgate (1983). Sections from teeth unaffected by periodontal disease were treated in the same way as a control group. Scanning electron micrographs were taken of the specimens and antibody binding was detected using x-ray microanalysis (EDAX), mapping silver (Ag) x-ray emissions. The x-ray maps obtained were superimposed on the corresponding SEM image.

Early results have shown antibody binding mainly to retained intact bacteria, with little binding to pellicle and superficial cementum layers. These results suggest that cementum associated LPS may be largely confined to adherent dental plaque on the root surface.

271

Steady State Levels of Leukotrienes B_4, C_4, D_4 and E_4 in Periodontal Tissues. S. Offenbacher*, B.M. Odle, T.E. Van Dyke, Emory University School of Dentistry, Atlanta, Georgia.

Metabolites of the arachidonic acid cascade play a key regulatory role in periodontal inflammation and connective tissue destruction. Previous studies have shown that the cyclooxygenase metabolites and certain lipoxygenase products are elevated in inflamed tissues. These pro-inflammatory mediators include the prostaglandins, thromboxanes and monohydroxyeicosatetraenoic acids (MHETES). In the present investigation we examined the steady-state level of the leukotrienes (LT) B_4, C_4, D_4 and E_4 in periodontal tissues. These compounds are of interest since LTB_4 is a potent chemotactic agent and secretagogue for neutrophils and LTC_4, D_4 and E_4 are vasoactive compounds. Inflamed granulomotous tissues and non-inflamed (distal wedge) periodontal tissues were obtained from nine periodontitis patients during routine periodontal surgery. The tissues were pulverized in liquid nitrogen, extracted with organic solvents, purified by silicic acid chromatography and fractionated by C_{18} reverse phase HPLC. The identity of authentic LTB_4, C_4, D_4 and E_4 within the tissue samples were confirmed by the following criteria: 1) co-elution with authentic standards, 2) characteristic UV spectra, 3) hypochromatic shift when treated with soybean lipoxygenase (LTC_4, D_4 and E_4 only), 4) neutrophil chemotaxis (LTB_4 only), 5) GC/MS (LTB_4 only). Quantitation of all 4 leukotrienes was performed by spectrophotometric comparison to PGB_2 peak, which served as the internal standard. Inflamed periodontal tissues contained 6.5 + 2.1 ng/mg LTB_4 (mean + SEM). Higher amounts of LTC_4 (53.8 + 15.1 ng/mg), LTD_4 (141.6 + 34.7 ng/mg) and LTE_4 (142.0 + 29.3 ng/mg) were observed. There were no statistical differences in paired comparisons of inflamed vs. non-inflamed tissue samples within each individual. However, in one individual with documented progressive longitudinal attachment loss (> 3 mm), the levels of all LTs were elevated 2-3 orders of magnitude. Thus, inflamed periodontal tissues contain relatively high levels of leukotrienes which may play a key role in local regulation of neutrophil function and inflammation. This work is supported by NIH Grant DE05967.

272

Development of A Three-Dimensional Collagen Matrix Synthesized by Human Diploid Fibroblasts In Vitro. E.E. QWARNSTROM*, R.C. PAGE, Dept.of Pathology/Ctr.for Res. in Oral Biology, Univ.of Wash., Seattle WA.

Attachment of gingival fibroblasts to two opposing vertical surfaces and development of a continuous matrix were examined with light and electron microscopy. Demineralized fibronectin-coated 200μ slices of human tooth root defining a 1mm-wide space were put on confluent cell layers. Cultures grown under standard conditions with ascorbic acid (50μg/ml/day) added, were followed up to 13 weeks(w). TEM of cross-sections showed close association of cell membrane with collagen bundles during growth of cells up the vertical surfaces. A fibrillar network which stained with ruthenium red developed across the space. Immunocytochemistry showed the presence of hyaluronic acid, chondroitin sulfate, dermatan sulfate, and fibronectin within the matrix at early times. After 6w the matrix, primarily made up of collagen types I, III, and V, occupied the entire space between the vertical surfaces. Cells within the mature network frequently showed a stellate appearance with extended processes in contact with matrix material, and were characterized by an irregular nucleus, a well-developed Golgi apparatus, extensive RER, and an abundance of microfilaments. Cell processes were seen to enclose bundles of collagen fibers and to mediate cell-cell contact, occasionally via desmosome-like structures. Presence of a fibrin clot between the vertical surfaces allowed the cells to grow directly up into the space and apparently increased matrix formation. Ongoing studies on the effects of interleukin-I on the matrix show breakdown of the early fibrillar network and release of sulfated proteoglycans. The geometry and maturation of the space-filling matrix, and the effects of various factors present during inflammation and wound healing on its development, suggest that this is a useful model for studies on various aspects of connective tissue regeneration. Supported by NIH grant DE07063.

273

Antibodies to Procollagenase Block Collagen Breakdown in Human Skin Fibroblast Cultures. H. BIRKEDAL-HANSEN*, B. BIRKEDAL-HANSEN, R. E. TAYLOR, and L. J. WINDSOR. Dept. Oral Biol., Inst. Dent. Res., Univ. Alabama at Birmingham.

Previous studies have shown that human skin fibroblasts (HSF) secrete procollagenase when properly stimulated. The purpose of this study was to determine the role played by this enzyme in cell-mediated collagen breakdown. HSF (ATCC, CRL 1224, Le Mor) were seeded on airdried films of reconstituted collagen fibrils formed by heat gelation of 150 ul aliquots of a neutral solution of [^{3}H]-acetyl rat tail tendon type I collagen (2.4 mg/ml) in 24-well cluster dishes. The cells were allowed to attach overnight in MEM-10% FCS and then incubated in serum-free medium (MEM). The rate of dissolution of the collagen coating was measured h by h by the release of radioactivity. Under these conditions the cells secreted high levels of procollagenase (1-2 ug/10^5 cells/day) and dissolved the substrate coating in 6-8 h when activated by 1 ug/ml trypsin. Addition of monospecific affinity purified rabbit Ig raised against HSF procollagenase completely blocked the breakdown whereas preimmune Ig and Ig which failed to bind to the collagenase-sepharose affinity column did not. A murine monoclonal Ig raised against the same procollagenase and selected on the basis of its inhibitory properties (clone VI-8) also completely blocked collagen breakdown whereas anti-procollagenase Ig secreted by two non-inhibitory clones (X-2a and III-7) did not. On the other hand, addition to the cultures of lysosomal protease inhibitors (antipain, leupeptin, pepstatin and E-64) in concentrations of 0.1-1.0 mM did not influence the rate of collagen breakdown. These findings show that the dissolution of reconstituted collagen fibrils in vitro by live fibroblasts is mediated by a collagenase-dependent pathway and that lysosomal proteases do not play a rate limiting role, if any, in the process.

Supported by NIH grants DE 02670, DE 05817 and DE 06028 and by a gift from Procter and Gamble.

274

Effect of F on Rat Serum Insulin Levels In Vivo. A.R. Shahed, W. Zhang and D.W. Allmann*, Dept. of Biochemistry, Indiana Univ. School of Med., Indianapolis, IN 46223.

Several investigators have shown that an acute administration of NaF in rats can induce a hyperglycemia. The current study was undertaken to determine if acute injections of NaF would alter the serum insulin levels since LIN et al., Horm. Metab. Res 8, 353-358, 1976 had shown that NaF could inhibit the synthesis and release of insulin from isolated pancreatic islets and that a lowering of serum insulin could result in hyperglycemia. Male Wistar rats (8 per group) were fasted for 16-24 hours prior to receiving an ip injection of NaCl (control) or NaF (0.5 to 20 mg F/kg body weight). The rats were anesthesized and blood collected by heart puncture 30 min after the injection of NaCl or NaF. The serum was analyzed for insulin (radio-immunoassay), glucose, and fluoride. There was a significant increase in serum glucose (120% increase), and serum fluoride (10 to 700 fold) 30 min after the injection of NaF. The serum insulin levels were also significantly reduced 30 min after injection of NaF. The insulin levels were 60%, 66%, 75% and 45% of the control values after injection of 0.5, 1.6, 5, and 20 mg F/kg. The data for the first time showed that acute administration of fluoride may inhibit insulin release in 24 hr fasted rats as reflected by significantly lower serum insulin level. It is concluded that the NaF induced hyperglycemia could be due in part to the reduction of serum insulin which would decrease the uptake of glucose by muscle and adipose. It should be noted that the lowest dose of NaF (0.5 mg/kg) is in the range of fluoride ingestion observed following a topical application of APF gel. Thus it is conceivable that normal ingestion of F following an APF application could alter several metabolic processes. This research was supported in part by a NIDR grant 04387, Grace M. Showalter Trust fund and DRTC - AM 20542.

275

Plasma Enzyme Levels During The Absorption Of F. N.L. BIRDSONG-WHITFORD* and G.M. WHITFORD, The Medical College of Georgia, Augusta, GA, USA.

Rapidly absorbed substances, such as ionic fluoride (F), achieve relatively high concentrations in the venous plasma draining the stomach and small intestines within minutes after ingestion. The portal vein, which supplies the liver with about 75% of its blood flow, carries this plasma directly to the liver. There is, therefore, the possibility of "first-pass" effects on liver function during the absorption of F. This possibility was evaluated using 15 pentobarbital-anesthetized mongrel dogs that received 100 mM NaF, MFP or NaCl (10 mg/kg body weight) by gastric intubation. Blood samples were collected from gastric, portal and hepatic veins and the femoral artery at 0.25, 0.5, 1, 2, 3 and 4 hrs after the dose. The cannulae were placed through needle punctures and secured to local connective tissue to minimize changes to blood flow rates. Plasma was analyzed for F, LDH (cytoplasmic enzyme), SGOT and SGPT (mitochondrial enzymes). The peak plasma F levels occurred within the first hr after NaF (ca 700μM) while, after MFP, the concentrations continued to increase during the 4 hr period to an average peak value of 156 μM. In general, the enzyme levels of the dogs that were dosed with NaCl or MFP showed statistically insignificant variations over time. In contrast, the enzyme levels in the plasma samples from all vessels in the dogs that received NaF showed significant increases that ranged from 62% to 610% over control (pre-dose) levels after an initial lag period of about 1 hour. The largest concentration changes occurred for LDH and SGOT. For any given enzyme, the rates of increase were not different among the four vessels. It was concluded that local and systemic plasma LDH, SGOT and SGPT levels may be elevated during the absorption of F. Compared to NaF, the increases are less likely to occur when F is given as MFP.

Supported by NIDR Grant 06113.

276

Deleterious Effects of NaF on Gastro Intestinal Tract. A. Fujii*, S. Kobayashi, and T. Tamura, Dept. of Pharmacol., Sch. of Dent. at Matsudo, Nihon Univ., Matsudo, Chiba 271, Japan

We have previously demonstrated that NaF ingestion caused apparent redness on gastrointestinal mucosa in mice and rats. This nature was found to be the dilatation of vein smooth muscle, in which F was acting as a Ca-antagonist. The present investigation was undertaken to clarify further the deleterious effects of NaF by studying the blood flow rate in the stomach mucosa and the free Ca^{++} concentration in blood of rats.

1) Blood flow rate of the rat stomach mucosa was obtained in the following manner: NaF(2%) was given to rats orally with the dose of 300 mg/kg 30 minutes after the initial test of H_2 clearance. H_2 electrode was inserted into the mucosa of stomach and H_2 gas was inhaled through a tracheal cannula. H_2 clearance rate was measured using pH_2 Monitor(M.T. Giken, Tokyo, Japan). Blood flow rate was calculated from $T_{1/2}$ of pH_2 clearance curve. The blood flow change of the control was within 20% of the initial period, however, the experimental group decreased approximately 40% at 10 and 20 minutes, and 70% at 30, 40, 50, and 60 minutes after administration of NaF.

2) In vitro blood free Ca^{++} after the addition of a various amount of NaF was measured in the following manner: Each fresh blood from rats(2 ml) was added NaF to make the final concentrations of NaF of 10, 20, 30, 50, and 100 ppms. Free Ca^{++} levels were measured using ionized Ca analyzer(Radiometer, Copenhagen, Denmark). The free Ca^{++} levels decreased to 90% and 72% of the initial values after three hours of 50- and 100-ppm NaF treatments, respectively.

These results indicate that NaF ingestion causes the dilatation of vein smooth muscle in gastrointestinal mucosa, in which the free Ca^{++} decreased in blood and also the blood flow rate decreased extensively.

277

Urinary Fluoride/Creatinine Ratio Determination in Testing the Fluoride Intake. P.KERTÉSZ*, J.BÁNÓCZY, B.RITLOP and A. BRÓDY. Semmelweis University, Medical School, Budapest Hungary.

In human caries preventive studies the control of fluoride intake is an important task. Determination of the 24 hr urinary fluoride excretion considered one of the best indices of fluoride absorption, is not feasible within the conditions of field studies.

Therefore, the fluoride/creatinine ratio of urinary spot samples was determined, /expressed in μmol/mmol and related to the 24 hr urinary excretion. Urine was collected from 21-, 9-14 yr old girls consuming drinking water with 7 μmol/l fluoride. Linear correlation was found between the 24 hr urinary fluoride and the fluoride/creatinine ratio, r= .6811. The mean value of fluoride/creatinine ratio in groups of children from eight different parts of Hungary, also showed linear correlation with the measured fluoride concentration of the drinking water, r= .9720.

Accordingly, the fluoride intake in the course of a caries preventive field study where the children received fluoridated milk, could be controlled. The fluoride/creatinine ratio seems informative for testing group's fluoride intake in the conditions of field studies.

278

WITHDRAWN