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FATTY ACID COMPOSITION OF PHOSPHOINOSITIDES IN RAT LIVER NODULES

M.Andersson and L.C.Eriksson

Department of Pathology, Karolinska Institute, Huddinge Hospital, Huddinge, Sweden

Rat liver nodules produced by treatment wth carcinogens exhibit elevated proliferation rate and differs from normal liver in several biochemical properties.

The composition of individual phospholipids in nodular tissue is changed with a two-fold increase in phosphatidylinositol (PI) compared to normal liver. Since PIs play a critical role in cell regulatory mechanisms, it is of great importance to understand the action of PIs in nodules. Earlier studies show a connection between cell proliferation, PI-metabolism and arachidonic acid release.

The fatty acid composition of the phosphoinositides in nodules was studied, with special interest focused on arachidonic acid (20:4) and its precursor linoleic acid (18:2). Preliminary results indicate no difference in fatty acid composition in phosphoinositides between nodular and normal liver.

INHIBITION OF COLONIC NEOPLASIA AND CRYPT CELL PRODUCTION RATES BY INTRALUMINAL CALCIUM

G.V.N.Appleton, P.W.Davies, J.B.Bristol and R.C.N.Williamson

University Department of Surgery, Bristol Royal Infirmary, Bristol BS2 8HW, U.K.

Small bowel resection and intrarectal administration of sodium deoxycholate each stimulate cell proliferation and promote carcinogenesis in the large intestine; oral supplements of calcium reduce the mitogenic effect of bile acids on colorectal mucosa. Potential suppression of intestinal adaptation and carcinogenesis by intraluminal calcium was tested in 120 male Sprague-Dawley rats weighing 186±9 g. Rats were randomised to receive azoxymethane s/c 15 mg/kg/week for 6 weeks or vehicle, followed by 80% mid small bowel resection or transection with reanastomosis. Half the animals in each group received supplemental calcium in the drinking water (calcium lactate 24g/1). Crypt cell production rate (CCPR) in descending colon was determined 7 weeks postoperatively in vehicle-treated rats; in the remainder colonic tumour yield was assessed at 26 weeks. Among rats with transection calcium supplements reduced colonic CCPR by 26% from 4.49±0.33 to 3.32+0.40 cells/crypt/hr ($p<0.05$) and more than halved tumour yield from 4.3 to 1.8 tumours/rat ($p=0.0007$). Jejunoileal resection increased both CCPR (by 51 to 61%; $p<0.001$) and tumour yield (by 65 to 105%; $p<0.005$), but again calcium lowered CCPR by 31% (7.23+0.44 vs 4.98±0.70; $p<0.02$) and tumour yield by 46% (6.9 vs 3.7; $p=0.0006$). Increased dietary levels of calcium diminish both adaptive and neoplastic growth in the colon, and calcium also blunts the co-carcinogenic stimulus of massive enterectomy.

ASSESSMENT OF RAT COLONIC TUMOURS BY FLOW CYTOMETRY: METASTASES ARE COMMONLY DNA ANEUPLOID

G.V.N.Appleton, P.Quirke, M.F.Dixon, C.C.Bird and R.C.N.Williamson

University Department of Surgery, Bristol Royal Infirmary, Bristol, BS2 8HW, U.K.

The study of azoxymethane-induced colonic carcinogenesis in rats helps in understanding human colorectal cancer. We have used flow cytometry to investigate the presence of DNA aneuploidy in rat intestinal tumours, and to evaluate the rat model as an experimental system. Fifty male Sprague-Dawley rats weighing 185.6+9.2 g were given azoxymethane 15 mg/kg/week s/c for 6 weeks and then underwent 80% small bowel resection (n=25) or jejunal transection (n=25). Half the animals in each group had calcium lactate 24g/1 added to the drinking water. Ten further non-operated rats (NOP) received azoxymethane 10 days later than the others. Forty-three rats survived 26 weeks and yielded 149 colonic and duodenal tumours of which 140 were measurable by flow cytometry. The incidence of DNA aneuploidy was 43% in NOP which was higher than in rats with resection (9%; $p<0.0005$) or transection (24%; $p<0.0005$). There was no significant difference in the prevalence of DNA aneuploidy between adenomas (32%) and carcinomas (17%) or between calcium treated (11%) and non-calcium groups (12%). However metastases were more commonly DNA aneuploid than the primary tumours (62% vs 20%; $p<0.005$). DNA aneuploidy is present in rat intestinal tumours and levels can vary widely with manipulation of the model. Metastases are associated with a high incidence of DNA aneuploidy.

ASSESSMENT OF COLONIC ADAPTATION BY CRYPT CELL PRODUCTION RATES IN ORGAN CULTURE: AN

EARLY SEVERE SHOCK!

G.V.N.Appleton, E.E.Wheeler, J.B.Bristol, D.N.Challacombe and R.C.N.Williamson

University Department of Surgery, Bristol Royal Infirmary, Bristol BS2 8HW, U.K.

The stathmokinetic method of measuring crypt cell production rate (CCPR) acurately assesses cell turnover in intestinal epithelium but ethical constraints limit its application in man. Organ culture might overcome this problem, although the exact cytokinetics of colorectal mucosa within this system have yet to be determined. We therefore studied 2 x 2 mm mucosal explants from the lower descending colon of male Sprague-Dawley rats (n=35), which were placed on still grids in culture dishes containing supportive medium and gently rocked in an atmosphere of 95% O_2, 5% CO_2. After 18 hr, vincristine (0.5 µg/ml) was added to the medium, and 6 sequential specimens were removed during the next 3 hr. A linear increase in metaphase arrests was observed with a CCPR of 4.78±0.41 cells/crypt/hr (means±S.E.M.). By contrast, in further experiments vincristine was added either ab initio or 3, 6 or 9 hr after the commencement of culture. During the first 5 hr of organ culture there was almost no increase in arrested metaphase figures per crypt (CCPR=0.03; $p<0.0001$). However, if 6 or 9 hr culture preceded addition of vincristine, CCPR was 4.01 and 4.06 respectively (p=n.s. vs 18 hr). Colorectal mucosa undergoes severe shock during the initial 5 hr of organ culture. A 6 to 9 hr period of culture yields satisfactory data on CCPR and could reflect original proliferative rates more closely than an 18 hr culture.

TOXICITY OF COMPOUNDS RELATED TO DENTAL MATERIALS IN CULTURED HUMAN BUCCAL CELLS

K.Arvidson, B.Silva, Y.Liu, K.Sundqvist and R.C.Gräfström

Karolinska Institutet, Stockholm, Sweden.

Clinical reports have clearly associated different dental materials with pathological effects in buccal mucosa. Therefore in vitro models using cultured normal human buccal epithelial cells and fibroblasts have been developed. Adult human buccal mucosa, obtained from surgery, was either maintained in a serum-free growth medium to derive epithelial cells, or in a low-serum (0.6%) culture medium to derive fibroblasts.

The effects of several metal-ions corresponding to metals commonly used in dental materials were investigated. The doses required to decrease the colony forming efficiency (CFE) of fibroblasts to 50% after 1 hr exposure were: Hg(II), 1 µM; Ni)II), 1 µM; Cr(VI), 1µM; Cd(II), 3 µM; Cr(III), 100 µM; and Co(II), 300 µM. Formaldehyde, a reactive compound known to be released from denture base polymers, was also found to decrease CFE of fibroblasts; a 50% inhibition was found at 30 µM. Preliminary experiments indicate that individually these agents were equally toxic to buccal epithelial cells grown at clonal density. The results show that different cultured human buccal cell types can be grown and used for studying pathobiological effects of dental materials.

HUMORAL ENHANCEMENT OF METASTASIS : IgG BINDING BY TUMOUR-BEARER T LYMPHOCYTES AND CONCURRENT CHANGES IN HELPER/SUPPRESSOR RATIOS

C.A.Aslakson and J.R.Starkey

Department of Microbiology, Montana State University, Bozeman, U.S.A.

Using the RT7-4b hepatocarcinoma of the inbred BD-IV rat, we have previously shown that metastasis can be enhanced using the IgG2b fraction of tumour-bearer serum. Flow cytometric analyses of lymphocytes from tumour bearing, serum enhanced tumour-bearing, and naive rats revealed that IgG from tumour-bearer serum bound to a subset of T lymphocytes. PBLs from serum enhanced tumour-bearing rats bound predominantly the IgG2b isotype (74% T cells exhibiting this isotype specific fluorescence) and this binding was saturated in vivo. Tumour-bearer T splenocytes, sorted on their IgG binding parameters, enhanced metastasis (approximately 2-fold) in the lung colony assay. As well as the additional macrometastases, numerous micro-metastasis were seen in the enhanced rats. During the development of metastasis, helper:suppressor T cell ratios fell progressively, being most rapid for PBLs and serum enhanced animals. Suppressor cells appear to be involved in humoral enhancement of metastasis.

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THE EFFECT OF HYROXYUREA ON GENE AMPLIFICATION IN HUMAN NEUROBLASTOMA CHP-100 CELLS

G.F.Atkinson and S.M.Bougourd

Department of Biology, University of York, York YO1 5DD, U.K.

Evidence exists to suggest that