41

STRIP AGA TEST: A RAPID AND ACCURATE METHOD IN SCREENING FOR COELIAC DISEASE.

T. Not, A. Ventura, M. Bittolo*, G. Torre and M. Andolina

Clinica Pediatrica and Dipart.di Biologia, Univers. Trieste, ITALY

Antialfaggladin antibodies (AGA) are a useful tool in screening for Coeliac Disease (CD). Anyway, this test requires a rather specialized laboratory, a venous blood sample and about one week in order to obtain the result for the family paediatrician. We developed and evaluated a dot immunobinding assay to detect alfa-glutadin IgG and IgA antibodies in a single drop of whole blood. The method is based on the absorption of alfa-glutadin as a spot on to nitro-cellulose sheets, who are immobilized on plastic strips. The strips were incubated 30' with patient sample and 30' with alkaline phosphatase conjugate antihuman (IgA and IgG) antibodies. After short incubation (15') with the substrate solution, the strip was dried and finally examined for the results, which are expressed by a colorimetric reaction. Twenty patients with CD (10 with active disease, 10 during gluten free diet (GFD)), 11 children with other gastrointestinal diseases (8 IBD, 1 autoimmune chronic diarrhoea, 1 congenital microvillous atrophy, 1 unexplained chronic diarrhoea) and 35 healthy controls were examined with both strip AGA test and the classic ELISA test for AGA. The results are summarized as follows:

PATIENTS	n°	DOT IgA	IgG	ELISA IgA	IgG
CD untreated	10	+	+	+	+
CD on GFD	5	+	+	+	+
GASTROINT. CONTR.	5	-	-	-	-
HEALTHY CONTROLS	35	-	-	-	-

The method showed good correspondence with ELISA AGA test: the procedure is quick and simple and does not require any costly equipment. We think that this "Strip AGA test" could be very convenient both for screening and for the follow-up of CD. This test could also be useful in population and/or family screening for C.D.

42

HEPATOBIILIARY SCANNING IN PATIENTS WITH CYSTIC FIBROSIS AND LIVER DISEASE BEFORE AND AFTER URSODEOXYCHOLIC ACID (UDCA) THERAPY.

C. Colombo, M.R. Castellani*, E. Sereni*, J.G. Apostolo, S. Foran, A. Giunta

Dept. Pediatrics, University of Milan, *Nuclear Medicine Div. National Cancer Inst. ITALY

It has recently been indicated that 96% of patients with liver disease secondary to Cystic Fibrosis (CF) showed evidence of common bile duct stenosis on hepatobiliary scanning (Gaskin et Al: N.Eng. J. Med. 318:340, 1988). We performed IDA scan pre and 10 - 12 months post administration of the hydrophilic and choleretic UDCA (15mg/Kg/day) in 8 children with CF related liver disease. Morphological pattern as well as functional parameters (T½ of hepatic wash-out, time of visualization of the intestine) were evaluated. At baseline, evidence of severe biliary obstruction was found in 2 cases, striking visualization of secondary and tertiary bile ducts in 5 and dilatation of common bile duct in 4; an enlarged gallbladder with delayed emptying was present in 2 patients and in 3 the gallbladder could not be visualized. After UDCA therapy, morphologic appearance at IDA scan improved markedly in all patients: none showed evidence of biliary obstruction, dilatation of common bile duct and intrahepatic ducts was substantially reduced and in all cases the gallbladder was visualized and appeared to empty normally. Mean T½ of hepatic wash-out decreased from 50 ± 21 min. at baseline to 36.0 ± 18 min. after therapy and mean time of visualization of the intestine decreased from 46.8 ± 40.8 min. to 17.5 ± 9.3 min. after UDCA. In all patients on UDCA liver function tests improved significantly and enrichment of the biliary bile acid pool with UDCA (from 4.8 ± 2.2 % to 28.5 ± 9.6 %) occurred.

The modifications observed after UDCA therapy suggest that inspissated secretions in CF patients may be responsible for the typical appearance at hepatobiliary scanning and that the improvement during UDCA may be related to its choleretic effect.

43

DO TRANS FATTY ACIDS (TFA) IMPAIR BIOSYNTHESIS OF LONG-CHAIN POLYUNSATURATES (LCP) IN MAN?

Berthold Koletzko

Universitäts-Kinderklinik D-4000 Düsseldorf, FRG.

Humans consume large amounts of TFA due to the extensive use of hydrogenated fats in food production. TFA consumption is considered safe for man, but side effects including impaired synthesis of LCP (20 & 22 carbons) have been observed in animal studies. We have previously documented materno-fetal transfer of TFA in humans. Therefore, we looked for possible effects of TFA on LCP status during early life, when LCP modulate tissue growth and development. Methods: Blood plasma samples were obtained in 29 clinically well premature infants (gest. age 34.0 ± 1.8 wks., birthwt. 1694 ± 173 g, M ± SD) prior to feeding in the morning of day 3 post partum, when milk intake was still very low. No infant had received fat infusions. Fatty acids in lipid classes were determined by high-resolution gas-liquid-chromatography. Results: Both total TFA and elaidic acid (18:1t), the main dietary TFA, were inversely correlated to LCP in plasma lipids. In triglycerides, linear correlation coefficients (r) for 18:1t were significant (P < 0.05* & 0.01**) for LCP (n=6-LCP: -0.41*, n=3-LCP: -0.50**, total LCP: -0.55**) and for product substrate ratios of LCP biosynthesis (20:4/18:2n-6: -0.47**, 22:6/18:3n-3: -0.50**). Similar results were found in other lipid classes for 18:1t and total TFA. Conclusions: 1. TFA exposure may impair biosynthesis of n-6- and n-3-LCP in man. 2. Since the capacity for LCP biosynthesis is limited during early life and LCP accretion is essential for normal functional development of membrane rich tissues (e.g. brain), a high intrauterine TFA exposure may have serious risks for fetus and neonate. 3. This observation is the first indication of possible untoward effects of TFA in man.

44

LKM-1 ANTIBODY POSITIVE AUTOIMMUNE HEPATITIS IN IDENTICAL TWINS: IN VITRO INHIBITION OF THE TARGET ANTIGEN CYTOCHROME P450 db1 AND IN VIVO PHENOTYPE

S. Koletzko, H. Löhr, M. Eichelbaum, F. Borchard, C. Rittner, K.-H. Meyer zum Büschenfelde, M. Manns

Kinderklinik & Patholog. Institut, Univ. D-4000 Düsseldorf, and I. Medizinische Klinik & Rechtsmedizin, Univ. D-6500 Mainz, FRG.

A subgroup of autoimmune type chronic active hepatitis (AI-CAH) is associated with LKM-1 autoantibodies. They are directed against Cytochrome P450 db1 and inhibit its function in vitro. We observed LKM-1 positive AI-CAH and colitis in a 13 year old girl. Liver disease responded well to corticosteroids. Father, mother, brother, the patient and her identical twin sister were investigated for HLA class I-III phenotypes, auto-antibodies, in vitro inhibition of P450 db1, and in vivo phenotype for drug metabolism (sparteine) mediated by this enzyme. Both twins had the autoimmune HLA haplotype A1, B8, DR3, C4A-Q0. While the mother is a homozygous extensive metabolizer (EM) (metabolic ratio 0.33), the father is a homozygous poor metabolizer (MR 65.25) and all children are heterozygote EM (MR 1.01, 0.99 & 1.76). Only the index patient had signs of liver disease and was positive for LKM-1 antibodies, and only her serum inhibited P450 db1 catalysed oxidation of sparteine in vitro up to 90 %. Conclusion: We describe for the first time occurrence of LKM-1 positive AI-CAH in a pair of identical twins, who were discordant for the disease. Since both twins are of the EM metabolizer type, i.e. express functionally intact P450 db1, and share the autoimmune HLA haplotype, we conclude that environmental factors trigger the manifestation of this autoimmune liver disease.

45

PROSTAGLANDINS IN SMALL INTESTINAL MUCOSA OF CHILDREN WITH ACTIVE COELIAC DISEASE AND ON A GLUTEN FREE DIET.

M. Bonamico, P. Lionetti, P. Mariani*, D. D'Alessandro*, P. Triglione, P. Mariani, E. Ferrante and G. Ballati

I Pediatric Clinic, II Surgical Clinic, "La Sapienza" University, Rome, Italy.

Enhanced synthesis of Prostaglandins (PGs) has been reported in coeliac disease (CD) and a possible involvement of these substances in the pathogenesis of diarrhoea in coeliac patients has been supposed. The aim of our study was to evaluate Prostaglandin E2 (PGE2) and 6-ketoprostaglandin F1α (6-Keto-PGF1α) synthesis in small intestinal mucosa of three groups of patients: group A, consisting of 11 children with active CD and total or subtotal mucosal atrophy; group B consisting of 7 children on a gluten free diet for at least 1 year with mild villous atrophy or normal intestinal mucosa; group C (control) of 6 non-coeliac patients with normal intestinal mucosa. Only 6 children from group A had chronic diarrhoea while children from group B were asymptomatic. Children from group C suffered from failure to thrive; we excluded from this group children who showed diarrhoea. The amounts of PGs generated by intestinal mucosa were measured by a standard method, using a RIA system. In group A PGE2 generation was significantly higher (2652 ± 407, Mean ± SE) than in group C (603 ± 140) (p < 0.003). Children from group A with chronic diarrhoea and those without this symptom both showed significant higher PGE2 generation (2569 ± 687, 1431 ± 171, respectively) than group C (p < 0.02). In group B PGE2 generation was higher (1632 ± 567) but not significantly different from group C. 6-Keto-PGF1α generation although higher in group A and B than C did not show any statistically significant variation. Our results indicate that PGE2 generation in CD is not always related to the presence of diarrhoea. Elevated PGs levels in intestinal mucosa in CD may be due to both enhanced synthesis and decreased degradation. Alternatively, as hypothesized by Branski (J Pediatr Gastroenterol Nutr 3, 672, 1984), it could be the expression of an adaptive mechanism. This latter hypothesis could explain our results of high PGE2 levels in asymptomatic coeliac children on a gluten free diet. Maybe is necessary a long period of diet before to cease this adaptive mechanism.

46

SMALL INTESTINAL TRANSPLANTATION (SIT) IN CHILDREN.

Goulet O., Révillon Y., Jan D., Brousse N., Cerf-Bansussan N., Mougenot J.F., De Potter S., Ricour C., Necker Hospital Paris.

After experiments on piglets and rats SIT using Cyclosporine A (CsA) and after obtaining consent form from parents, we performed six SIT in children (6mths-9yrs) with short gut syndrome on home TPN for 0.5 to 6 years. All but one donor and recipients were Isoblood group ABO, with negative cross match reaction. Graft were harvested on brain dead neonates (n=2) or children 6-17 yrs. 110 ± 10 cm of jejunum ileum underwent both vascular and luminal washing using Collins (n=3) or UW (n=3). After aorta and inferior vena cava anastomosis total ischemic time ranged from 1 hr 20 to 6 hr 30. Graft was anastomosed on proximal end; both distal graft and own intestine were exteriorized as stomas. Initial immunosuppression included solumedrol 2 mg/Kg/d and Cyclosporine as a continuous infusion for RIA serum levels 200-300 ng/ml. Antilymphoglobuline (ALG) (5 mg/Kg/d) for 14 days were added in the last 4 cases. Systemic antibiotics and total decontamination of the graft are associated. The first graft was removed after 8 hours for ischemia; the child was discharged on home PN. Early (< 15 days) or delayed (2-6 mths) acute graft rejection (GR) occurred in all cases. Late clinical symptoms included increased ileostomy drainage while histologic pattern changed early including progressively: T cell infiltrates (CD4+, CD8+, CD25+), increased HLA DR expression by crypts enterocytes, villi oedema, destruction of crypt and surface epithelium, crypt abscess and finally mucosal sloughing. GR was treated with ALG or OKT3 but led to graft removal in 3 cases 3 wks to 6 mths after SIT. One graft was removed on the 17th mth because of chronic rejection. Four graft were progressively used from the 4th wk tolerating up to 90 % of total energy intakes, like in one patient still living with the graft for 10 mths. Functional assessment included baryum transit, enzyme activities, and absorption tests. Those results show that SIT is possible but depending on important immunosuppression. No GVH reaction was observed. GR can be limited by repeated immunohistochemical study and early treatment.