Int. J. Dev. Biol. 45 (S1): S151-S152 (2001)

Short Report

Prenatal exposure to alcohol affects the cytoskeleton in the developing rat liver

INMACULADA AZORIN[1], FRANCISCO LAZARO[1], JUAN MANUEL MARTINEZ[1], MANUEL PORTOLES[1], CONSUELO GUERRI[2] and JAIME RENAU-PIQUERAS[1]*

[1]Sección Biología y Patología Celular, Ctr- Investigación, Hospital La Fe, Valencia and [2]Instituto Investigaciones Citológicas, Valencia, Spain

ABSTRACT Prenatal alcohol exposure alters the liver and induces pathological changes in the morphology and function of the Golgi apparatus (GA), including an accumulation or reduced transport of newly synthesized glycoconjugates which suggests that ethanol could affect the cytoskeleton (CSK) in hepatocytes. To test this possibility we have analysed, using various techniques, the effect of ethanol "in utero" on several CSK proteins including actin, α- tubulin, cytokeratin, vimentin and the motor associated proteins dynein and kinesin. Our results show that this treatment increases the amount of both α-tubulin and cytokeratin and alters its distribution.

Introduction

Maternal consumption of ethanol severely affects the developing fetus inducing growth deficiency, behavioural abnormalities, microcephaly and central nervous system damage (fetal alcohol syndrome) (1). In addition, cardiovascular abnormalities, hepatic dysfunction and histopathological alterations have been found in children with this syndrome. Similar alterations have been found in experimental animals where prenatal exposure to ethanol affects the morphological, structural and functional features of the Golgi apparatus (GA). Moreover, ethanol "in utero" decreases, in fetal hepatocytes, the protein synthesis, the activity of galactosyltransferase, alters the amount and distribution of several carbohydrate residues in the GA and induces an accumulation (or reduced transport) of newly synthesized glycoproteins in this cell compartment (2). Since there are not any data demonstrating that an inhibition of glycosylation impedes protein trafficking, it is possible that the accumulation of glycoproteins in the GA could be the result of an alteration in transport due to an effect of ethanol on cytoskeleton (CSK) elements, mainly microtubules (MT). However, information on the effects of prenatal alcohol exposure on hepatocyte CSK is sparse. Therefore, the goal of this study is to test this possibility using immunoblotting, immunofluorescence and immunogold techniques.

Materials and Methods

Animals

Females Wistar rats were fed the Lieber-DeCarli liquid diet (3) either containing 5% (wt/vol) ethanol or isocalorically balanced for pair-fed controls. Female rats received the liquid diet for a minimum the 40 days before exposure to males rats. After mating, the rats were placed in separate cages with the same liquid diet. Twenty-one-day old fetuses were decapited and the liver processed for the study of CSK.

Antibodies

The following antibodies were used: Monoclonal anti-α-tubulin (B-7, Sta Cruz Biotechnology), monoclonal anti-vimentin (V9, Sigma), monoclonal anti-pancytokeratin (Sigma), monoclonal anti-dynein (intermediate chain, Sigma) and monoclonal anti-kinesin (light chain, Chemicon).

Western blot analysis

Tissues were homogenised 1: 10 (wt/vol) in Tris buffer 6 mM, pH 7.6 supplemented with EDTA 10 mM and SDS 2%. One dimensional SDS polyacrylamide gels (10%) were run, and proteins were electrophoretically transferred to nitrocellulose paper. Membranes were blocked with 1% BSA in TBS 0.01% and then incubated with the same buffer containing the appropriated dilution of the antibody. Finally, gels were quantified using the Scion Image program.

Fluorescence studies

Light and immunofluorescence microscopy was carried out on frozen sections of about 3 μm. Actin filaments were stained with fluorescent phalloidin whereas the remainder CSK were demonstrated using indirect immunofluorescence procedures.

Electron microscopy

Immunocytochemistry of CSK in hepatocytes was carried out on extracted tissue (4). After extraction, tissues were incubated with the first antibody (anti-α-tubulin, anti-vimentin and anti-cytokeratin), rinsed with PBS and incubated with the second antibody conjugated with colloidal gold and processed for embeddeding in Epon. Ultrathin sections were examined in a Philips CM100 EM. The number and distribution of gold particles per length unit (μm) of MT or intermediate filament was analysed.

Results and Conclusions

Immunoblotting analysis of CSK liver proteins in control and prenatal exposed to alcohol animals reveals that alcohol increases the amount of α tubulin and cytokeratin (Fig. 1) whereas no significant variations in the levels of vimentin, kinesin and dynein

***Address correspondence to:** Jaime Renau-Piqueras. Sección Biología y Patología Celular, Ctr- Investigación, Hospital La Fe, Avda Campanar 21, 46009 Valencia, Spain. e-mail: renau_jai@gva.es

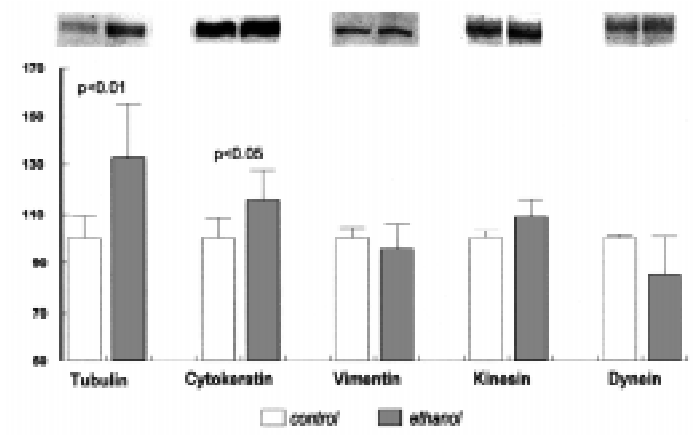

Fig. 1. Immunoblotting of CSK proteins in rat liver from control and experimental animals who had been prenatally exposed to ethanol. *As shown, ethanol increases the levels of tubulin and cytokeratin.*

were found. Immunofluorescernce staining of the liver slices reveals (Fig. 2) a staining pattern similar to that described by Ohta el al (1988)(4) for cytokeratin and actin. Both proteins were localised mainly at the cell border. In contrast, tubulin was localized in the cytoplasm. Finally, vimentin showed a weak staining pattern and was not clearly delineated. We have not found clear differences in

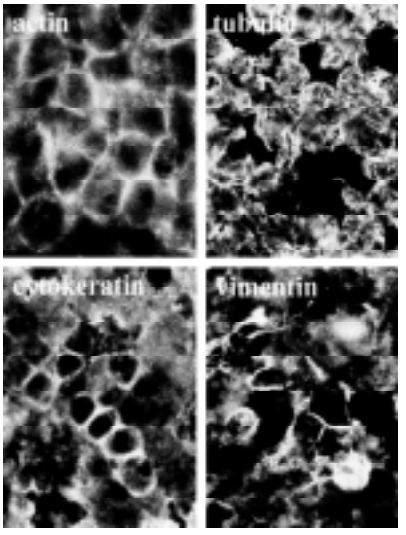

Fig. 2. Fluorescence of CSK proteins in rat liver slices from control animals. *Similar patterns were found in ethanol exposed rats.*

the immunostaining patterns between control and alcohol exposed livers. Electron microscopy showed the distribution of immunogold complexes on MT and intermediate filaments (Fig. 3). Preliminary data indicate differences between control and alcoholic animals in the number of distribution of gold particles corresponding to tubulin and cytokeratin. In both cases, an increase in the number of particles was found.

Taken together, our results indicate that prenatal exposure to alcohol alters CSK in neonatal rat hepatocytes which could consti-

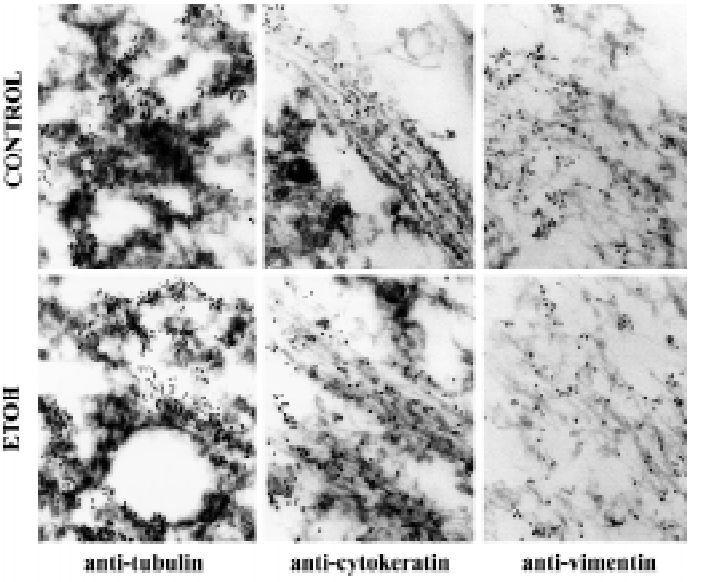

Fig. 3. Immunogold electron microscopy of CSK proteins in extracted livers from control and ethanol exposed animals.

tute an important mechanism involved in the alcohol-induced alterations of glycosylation and intracellular glycoconjugate trafficking.

This work was supported by FISS 00/0006-01

References

LIEBER C.S. and DECARLI L.M. (1976). Animal models of ethanol dependency and liver injury in rats and baboons. *Fed. Proc.* 35: 1232-1236.

OHTA M., MARCEAU N. and FRENCH S.W. (1988).Pathologic changes in the cytokeratin pericanalicular sheath in experimental cholestasis and alcoholic fatty liver. *Lab. Invest.* 59: 60-74.

RENAU-PIQUERAS J., SANCHO-TELLO M., BÁGUENA-CERVELLERA R. and GUERRI C. (1989). Prenatal exposure to alcohol alters the synthesis and glycosylation of proteins in fetal hepatocytes. *Alcohol. Clin. Exp. Res.* 13: 817-823.

RENAU-PIQUERAS J., GUASH R., AZORIN I., SEGUI J.M. and GUERRI C. (1997). Prenatal alcohol exposure affects galactosyltransferase activity and glycoconjugates in the Golgi apparatus of fetal rat hepatocytes. *Hepatology* 25: 343-350.

SPECIAL REPORT (10th) to the U.S. Congress on Alcohol and Heatlth, NIAAA, June 2000.