ALCOHOL CONSUMPTION DURING PREGNANCY ALTERS GLYCOCONJUGATES, GALACTOSYLTRANSFERASE ACTIVITY AND β-COP PROTEINS IN THE GOLGI APPARATUS OF HEPATOCYTES IN DEVELOPING LIVER.

José María SEGUÍ[1], Rosa GUASCH[2], Inmaculada AZORIN[1], Consuelo GUERRI[2] and Jaime RENAU-PIQUERAS[1]
[1]: *Cell Biology and Pathology, Centro de Investigación, Hospital "La Fe", Valencia, and*
[2]: *Instituto de Investigaciones Citologicas, Amadeo de Saboya 4, Valencia, Spain*

Alcohol exposure during development has been demonstrated to produce a wide spectrum of alterations in offspring, including growth deficiency, behavioral abnormalities, microcephaly and CNS damage (fetal alcohol syndrome). Hepatic disfunction and histopathological alterations have also been described in childrens with this syndrome. In previous studies we have demonstrated that prenatal alcohol exposure affects the morphological, structural and functional features of the Golgi apparatus (GA), thus altering the glycosylation process in developing hepatocytes. In order to elucidate the cellular mechanisms underlaying these alterations, we have analyzed the effect of alcohol exposure in utero on the activity of liver galactosyltransferase (GT), the distribution and amount of sugar residues and the Golgi β-COP protein in hepatocytes. For this, livers from 21-day-old fetuses were obtained from control and ethanol-fed rats. GT activity was determined in isolated GA cis and trans fractions. Colloidal gold labelled lectin cytochemistry was used to analyze sugar residues and, finally, the distribution of β-COP was analyzed by immuno-cytochemistry.

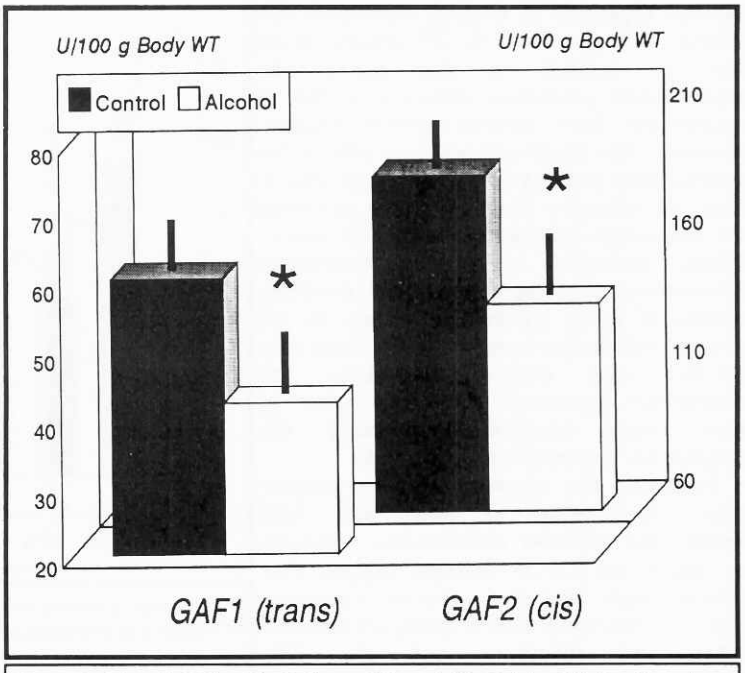

Fig 1: Activity of GT in GAF1 (trans GA and GAF2 (cis GA) fractions from control and prenatal alcohol-exposed hepatocytes. The activity is expressed as U/100 g. body weight. Units refer to nmol of galactose transferred per hr. Each point represent the average ± SD in 9-12 litters from control or alcohol-fed pregnant rats. *P < 0.05 from controls.

Prenatal alcohol exposure induces a significant increase in both liver weight and total protein in the trans GA. The analysis of enzyme marker distribution and electron microscopy indicated a good separation of the different GA fractions and agrees with previous studies in adult liver. Our results showed that alcohol exposure significantly reduced the GT activity in both trans (GAF1) and cis (GAF2) GA fractions (fig. 1).

Electron microscopy showed, as reported earlier, that prenatal exposure to ethanol induces, in about 30% of hepatocytes, a disorganization of the GA. The appearance of this cell component in the remaining treated hepatocytes was similar to that of control cells. Immunolabeling of β-COP protein was seen over GA cisternae and vesicles in both control and alcohol exposed hepatocytes. However,this treatment induced a 75% reduction, from the control values, in the number of anti-β-COP binding sites (fig. 2). On the other hand, the labelling pattern for each lectin over GA was similar to that reported for other cell types, indicating that lectins interact with different sugars that are added stepwise by a series of glycosyltransferases distinctively located among the GA cisternae. Our results indicated that alcohol increases α-L-Fuc residues (UEA I), while it decreases α-Man (Con A), GlcNAc-(β 1,4,GlcNAc)$_{1,2}$ (WGA), GalNAc α 1,3GalNAc (HPA), GalNAc (MPA) and α-Gal (BS-I) residues.

We show for the first time that prenatal ethanol exposure induces, as in the adult liver, an increase in liver weight and enhancement of the total liver protein. These results are consistent with the enlargement of hepatocytes and with the liver glycoprotein accumulation observed in previous studies after this ethanol treatment. Impairment of glycosylation and secretion of glycoproteins as well as water retention, due to the corresponding osmotic effect of protein accumulation, have been suggested to participate in hepatocyte swelling after acute and chronic ethanol treatments. Therefore, it is possible that a similar mechanism might be involved in the enlargement of hepatocytes observed after prenatal alcohol exposure.

We also confirm that prenatal alcohol exposure induces morphological changes in about 30% of GA. In addition, we demonstrate that this treatment causes a significant decrease in the labeling of β-COP protein. β-COP is a protein associated with the non-clathrin-coated vesicles which appears to be involved in intra-GA-transport, transport from the endoplasmic reticulum to GA and protein transit between the intermediate compartment and

cis-GA. Moreover, it appears that β-COP plays an important role in regulating structural aspects of GA, The effect of ethanol on this protein could explain the morphological changes observed in this organelle after alcohol exposure and may suggest an effect of ethanol on vesicular trafficking. In addition, a perturbation of the skeletal scaffold associated with GA could also been involved in these morphological changes. Our results show that prenatal alcohol exposure increases α-L-Fuc residues , while it decreases α-Man , GlcNAc-(β 1,4,GlcNAc)$_{1,2}$, GalNAc α 1,3GalNAc , GalNAc and α-Gal residues. Although, at present we do not know why alcohol affects differently the sugar residues, our results suggest that alcohol induces defects in glycosylation during one or several steps of this process in both cis and trans GA. However, the qualitative and quantitative extent of these modifications in the oligosccharide chain composition is an alcohol-induced alteration of glycosyl-transferases and / or glycosylases. This interpretation is supported by the finding that prenatal exposure to ethanol decreases GT activity. This decrease in GT activity could also be related to the glycoprotein accumulation previously observed in GA of hepatocytes from alcohol-exposed fetuses. Moreover, this treatment not only affects the glycosylation process in the liver, but also in other cell systems. Thus, we have observed that astrocytes exposed to alcohol "in utero", display alteration in plasma membrane glycoproteins and decreases in the secretory process of some glycoproteins such as the nerve growth factor receptor. It has been also reported that alcohol decreases the biosynthetic syalilation of gangliosides in chick brain neurons, depressing the ganglioside- dependent neurogenesis.

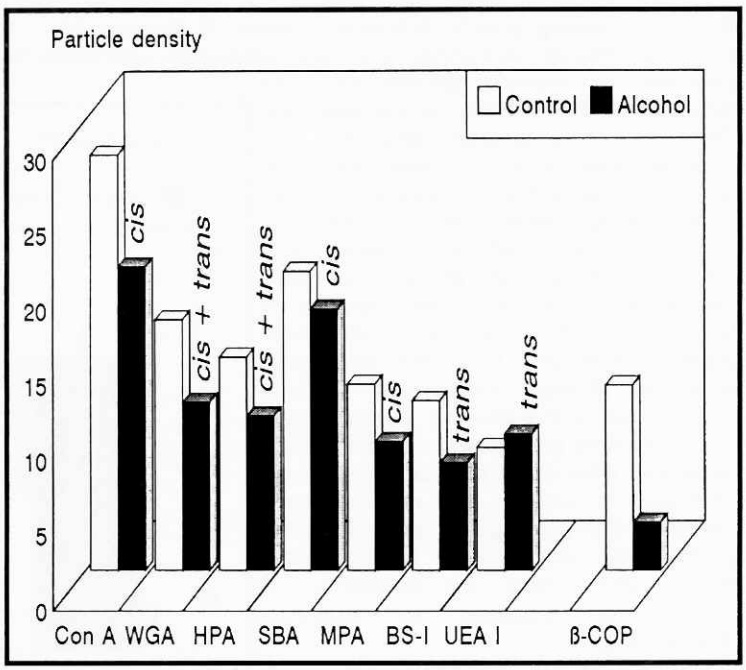

Fig.2: Labeling density in lectin cytochemistry and immunocitochemistry of GA of control and prenatally exposed to alcohol hepatocytes.

However, the question of why ethanol alters glycosilation in adult and fetal hepatocytes remains unanswered, although our results on β-COP strongly suggest that ethanol could alter the vesicular trafficking within the GA stack and in transport between endoplasmic reticulum and the GA. Neverthless, other possibilities could also been considered: a) acetaldehyde, the main metabolite of ethanol, could directly inhibit GT activity and, in fact, it has been reported that this substance produces a significant dose-dependent inhibition of glycosyltransferase in the serum of alcohol-abusing patients. It has also been demonstrated in cultured hepatocytes that treatment with acetaldehyde causes transferrin to shift to the more basic isoforms, thus indicating that glycosylation at the GA is inhibited. b) one of the biological consequences of ethanol in cells is reflected in its stimulatory and inhibitory effects on a variety of membrane-bound enzymes, receptors and ion-channels. These effects could be attributed to alcohol-induced alterations of the physical properties of the lipids surrounding the membrane tethered proteins, or to metabolic alterations of the fatty acids incorporated into phospholipids and in membrane biogenesis. Since GT is a membrane-bound protein whose activity is modulated by phospholipids, it could be suggested that the alcohol -induced alterations in GT activity could be due to modifications in those membrane lipids involved in the GT function.

In summary, our results demonstrate that prenatal exposure to ethanol induces alterations in several levels of the structural organization of the GA and in the glycosylation process. All of these changes may explain the accumulation of glycoproteins in the GA and the enhancement of hepatic proteins in fetal liver after alcohol exposure.

REFERENCES

Egea G.(1993) Lectin cytochemistry using colloidal gold methodology.In:Gabius HJ, Gabius S. eds. Berlin: Springer Verlag: 215-233.

Griffiths G,Pepperkok R, Locker JK and Kreis TE.(1995).Immunocytochemical localization of β-COP to the ER-Golgi boundary at the TGN.J.Cell Sci..108:2839-2856.

Guasch R, Renau-Piqueras J, Guerri C.(1992).Chronic ethanol consumption induces accumulation of proteins in the liver Golgi apparatus and decreases galactosyltransferase activity. Alcohol Clin.Exp.Res.16:942-8.

Renau-Piqueras J, Guerri C,Sancho-Tello M, Báguena-Cervellera R, Miragall F.(1989) Effects of prenatal exposure to ethanol on rat liver development. A review. Int.J.Dev.Biol.33:345-60

Renau-Piqueras J, Guasch R, Azorín I, Seguí JM,Guerri C.(1996).Prenatal alcohol exposure affects galactosyltransferase activity and glycoconjugates in the Golgi apparatus of fetal rat hepatocytes. Hepatology. (In Press).

Rothman JE, (1994).Mechanism of intracellular protein transport. Nature,376:55-63.

Streissguth AP, Landesman-Dywers S, Martin JC, Smith DW.(1980).Teratogenic effects of alcohol in human and laboratory animals. Science. 209: 253-61

Tuma DJ, Casey CA and Sorrell MF.(1991).Effects of ethanol on hepatic protein metabolism and trafficking.Alcohol and alcoholism, suppl 1:297-303.