Effect of prenatal ethanol administration on microvillus membrane glycosylation in developing rat intestine

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Received 11 July 2003; revised 8 May 2004

Effect of prenatal ethanol exposure has been studied on microvillus membrane glycosylation in developing rat intestine. In utero ethanol administration did not affect the gestation period but reduced litter size in ethanol-exposed group. Body weight, intestinal length and weight of pups born to ethanol-exposed rats during gestation, aged 4 to 30 days were significantly low compared to the respective controls. Total hexoses (p<0.05) and sialic acid (p<0.05, p<0.01) contents of purified brush border membranes increased in pups exposed to ethanol prenatally, while the fucose content showed significant (p<0.05, p<0.01) decrease. The fucose-sialic acid molar ratio was also low in all age-groups of prenatally ethanol-exposed pups, compared to respective controls. The results indicate that ethanol ingestion during gestation alters the glycosylation pattern of intestine in rats, which may impair its functions during postnatal development.

Keywords: Glycosylation, prenatal ethanol exposure, microvillus membrane, brush border membrane, total hexoses, fucose, sialic acid.

Surface oligosaccharides play a vital role in various cell functions, such as cell-cell interactions, cell differentiation and membrane transport. Luminal surface of intestine contains considerable amounts of carbohydrates as a constituent of membrane glycoproteins and glycolipids. The glycosylation of microvillus membrane undergoes striking changes during postnatal development in rat intestine, which is rich in sialic acid and contains low amounts of fucose during prenatal period. This pattern of membrane sialylation and fucosylation is reversed upon weaning, primarily due to modifications in glycoproteins, rather than glycolipids. This process is associated with high sialyl-transferase and low fucosyl-transferase activities in intestine of suckling rats.

Administration of hormones to suckling animals also induces precocious maturational changes in intestinal glycosylation. Administration of ethanol to developing foetus leads to foetal alcohol syndrome (FAS), which is associated with multiple birth defects and delayed growth during postnatal development. In utero exposure to ethanol is reported to cause morphological injuries and a decrease in brush border enzyme activities in intestine during postnatal development. A marked decrease in absorption of IgG from intestine of pups exposed to ethanol during gestation has also been reported. In the present study, we have investigated effects of in utero ethanol exposure on microvillus membrane glycosylation, during postnatal development in rats.

Materials and Methods

Wistar strain albino rats, each weighing 100-120 g were maintained on commercial rat pellet diet (Hindustan Lever, India) ad libitum with free access to water. The first day of gestation (day 1) was checked by examining vaginal smears under light microscope as described. The pregnant rats were administered 1ml of 30% ethanol by Ryle’s tube daily @ 2 g/kg body wt/day from day 1 of gestation until delivery. Two control groups, one on isocaloric amounts of glucose (glucose control) and another on saline (saline control) were maintained up to certain stage of the experiment and later continued with the glucose control alone. The neonates were kept with their natural mothers and were sacrificed by decapitation between 9-10 A.M. at day 4, 8, 14, 20 and 30 of postnatal age. Intestinal tissue starting from the ligament of Treitz to caecum was removed, washed thoroughly with ice-cold normal saline and stored at –20°C, if not used immediately. Intestinal length and weight were recorded. Each group, control and ethanol-administered had 3-4 mother rats with a litter size of 5-8 pups.

Brush border membranes (BBMs) were isolated and purified from pooled intestinal tissues, using calcium chloride precipitation method. All procedures were carried out at 0 to 4°C, except otherwise mentioned. Total hexoses were estimated...
using anthrone\textsuperscript{17}, and sialic acid\textsuperscript{18} and fucose\textsuperscript{19} were estimated as described earlier. Protein was estimated by Lowry \textit{et al}\textsuperscript{20} with bovine serum albumin as standard. Statistical analysis of data was carried out using Student’s \textit{t}-test.

**Results**

Basal body parameters, viz., body wt, intestinal length and intestinal wt of developing pups from glucose control as well as prenatally ethanol-exposed groups are shown in Table 1. Pups from saline control group did not show significant difference from that of glucose control group on these parameters (data not shown). The gestation periods of saline control, isocaloric glucose-fed animals and mother rats fed ethanol during gestation were 21.8±0.5, 21.5±0.7 and 22.5±0.9 days, respectively. The litter size in saline control and isocaloric glucose-fed animals was 10.8 and 11, respectively, while it was 9.6±0.5 in group administered with ethanol prenatally.

The body wt of 4-days old glucose control pups was 9.0±0.7 g and gained 68.0±1.7 g in 30 days. The respective age-group pups in prenatally ethanol-exposed group weighed 5.3±0.3 g and 63.0±2.1 g. The intestinal wt of pups exposed to ethanol \textit{in utero} was significantly low, compared to respective control in all age groups (Table 1). A similar decrease \textit{(p<0.05)} was observed in intestinal length of suckling pups, except in 30-days old rats. The weight to length (W/L) ratio of intestine was unaffected in pups from ethanol-exposed groups, compared to control (Table 1).

Total hexoses content of intestinal BBM increased from day 4 to day 30 of postnatal development suggesting a gradual rise in membrane hexoses with age (Table 2). A similar increase in the membrane hexoses was also apparent in rats exposed to ethanol prenatally. \textit{In utero} ethanol treatment led to a significant increase \textit{(p<0.05)} in total hexoses content of microvillus membranes in 20 and 30 days old rats, compared to respective controls. Sialic acid content of intestinal BBM decreased from day 4 to day 30 of postnatal development in control, as well as ethanol-exposed rats (Table 2), the decline was 72% and 61% respectively. However, sialic acid content from prenatally ethanol-exposed rats was significantly high at all ages of postnatal development, compared to respective controls. In contrast, fucose content of BBM increased gradually from day 4 to day 30 in both the groups (Table 2). Prenatal exposure to ethanol significantly reduced fucose levels on day 8 \textit{(p<0.05)} to day 30 \textit{(p<0.01)} of postnatal development compared to the respective controls. Fucose to sialic acid molar ratio in microvillus membrane showed significant increase during postnatal development in

**Table 1—Effect of prenatal ethanol exposure on intestinal parameters of pups during postnatal development**

<table>
<thead>
<tr>
<th>Group</th>
<th>Postnatal age (days)</th>
<th>Body wt (g)</th>
<th>Intestinal wt (W) (g)</th>
<th>Intestinal length (L) (cm)</th>
<th>W/L ratio (g/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>9.0±0.7</td>
<td>0.46±0.03</td>
<td>27.91±1.52</td>
<td>0.016</td>
</tr>
<tr>
<td>Ethanol-exposed</td>
<td></td>
<td>5.3±0.3**</td>
<td>0.41±0.01**</td>
<td>25.80±0.88**</td>
<td>0.015</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>11.0±0.4</td>
<td>0.61±0.01</td>
<td>33.12±0.87</td>
<td>0.018</td>
</tr>
<tr>
<td>Ethanol-exposed</td>
<td></td>
<td>7.8±0.2**</td>
<td>0.59±0.06**</td>
<td>32.07±0.42*</td>
<td>0.018</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>18.0±0.8</td>
<td>1.02±0.05</td>
<td>41.36±0.68</td>
<td>0.024</td>
</tr>
<tr>
<td>Ethanol-exposed</td>
<td></td>
<td>11.6±0.6*</td>
<td>0.97±0.03*</td>
<td>39.97±0.95*</td>
<td>0.023</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>36.0±1.4</td>
<td>2.28±0.23</td>
<td>56.40±0.58</td>
<td>0.040</td>
</tr>
<tr>
<td>Ethanol-exposed</td>
<td></td>
<td>29.5±1.8*</td>
<td>1.92±0.18*</td>
<td>55.10±0.78*</td>
<td>0.034</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>68.0±1.7</td>
<td>3.40±0.31</td>
<td>80.73±0.68</td>
<td>0.042</td>
</tr>
<tr>
<td>Ethanol-exposed</td>
<td></td>
<td>63.0±2.1*</td>
<td>3.02±0.17*</td>
<td>78.98±0.97</td>
<td>0.038</td>
</tr>
</tbody>
</table>

\( **p<0.01, *p<0.05 \) compared to the control.
both the groups (Table 2). However, the ratio was always low in prenatally ethanol-exposed pups, compared to controls at all ages of development.

**Discussion**

The model of chronic ethanol consumption used in this study was designed to roughly parallel the long-term ethanol intake among alcoholics. Female rats were administered ethanol orally at a dose equivalent to approx. 200 ml of whiskey consumed by a 70 kg person. Control animals received isocaloric amounts of glucose to minimize the nutritional imbalance that may arise due to the high-energy content of ethanol. As there was no change in the gestation period and litter size of saline or isocaloric glucose-fed groups, thus glucose-fed group was treated as control to study the effect of ethanol feeding on intestinal glycosylation during postnatal development.

No marked difference was observed in the gestation period of ethanol-fed female rats, compared to controls. These findings are similar to those reported earlier, where no delay in delivery period was observed when pregnant rats were administered alcohol at a dose of 1 or 2 g/kg body wt/day. However, other workers have reported that ethanol ingestion increased the gestation period by a day. The discrepancy in the present study may be due to the differences in timing and amount of alcohol exposure employed in these experiments. It is possible that the duration and amount of alcohol exposure may influence the gestation period. The reduced litter size in ethanol-treated dams is in agreement with earlier studies. The decrease in body wt of pups exposed to ethanol prenatally is in agreement with previous studies. Pups from alcohol-administrated mother rats exhibited low birth wt, despite an additional day in utero, which continued during lactation. Observations on intestinal length and weight suggest a delayed intestinal maturation in developing rats exposed to ethanol prenatally, compared to age matched controls and are consistent with the earlier studies. The low body weight of offsprings in ethanol-exposed group is due to the deleterious effect of ethanol on developing foetus during gestation. Maternal undernutrition is ruled out as the mother rats in control and experimental groups weighed identical during gestation and at the time of parturition.

The age-wise increase in the total hexoses and fucose contents with a concomitant decrease in sialic acid levels of intestinal BBM in both control and experimental groups, is similar to earlier studies, indicating that during suckling period, the membrane is rich in sialic acid and has low fucose levels, which is reversed upon maturation. Prenatal exposure to ethanol led to a significant rise in the levels of membrane total hexoses and sialic acid, compared to control, but a decline in fucose content during the postnatal development, in consistent with the earlier study. It is reported that during pre-weaning period (1-2 weeks) sialyl transferase activity is high in enterocytes and then declines 5-fold to reach adult levels after weaning, however, fucosyl transferase

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>4</th>
<th>8</th>
<th>14</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total hexoses (μg/mg protein)</td>
<td>Control</td>
<td>214 ± 2</td>
<td>223 ± 5</td>
<td>239 ± 3</td>
<td>249 ± 4</td>
<td>273 ± 5</td>
</tr>
<tr>
<td></td>
<td>Ethanol-exposed</td>
<td>218 ± 3</td>
<td>234 ± 9</td>
<td>246 ± 4</td>
<td>261 ± 3*</td>
<td>291 ± 4*</td>
</tr>
<tr>
<td>Sialic acid (μg/mg protein)</td>
<td>Control</td>
<td>83.50 ± 4</td>
<td>65.00 ± 3</td>
<td>41.00 ± 4</td>
<td>25.30 ± 4</td>
<td>23.00 ± 4</td>
</tr>
<tr>
<td></td>
<td>Ethanol-exposed</td>
<td>97.50 ± 4**</td>
<td>82.40 ± 3**</td>
<td>63.80 ± 5*</td>
<td>40.00 ± 3*</td>
<td>38.00 ± 5*</td>
</tr>
<tr>
<td>Fucose (μg/mg protein)</td>
<td>Control</td>
<td>28.00 ± 3</td>
<td>45.00 ± 4</td>
<td>58.00 ± 4</td>
<td>76.00 ± 6</td>
<td>110.00 ± 3</td>
</tr>
<tr>
<td></td>
<td>Ethanol-exposed</td>
<td>20.00 ± 2</td>
<td>32.00 ± 3*</td>
<td>40.00 ± 5*</td>
<td>52.00 ± 3**</td>
<td>94.00 ± 3**</td>
</tr>
<tr>
<td>Fucose/sialic acid ratio (mole/mole)</td>
<td>Control</td>
<td>0.54 ± 0.01</td>
<td>1.08 ± 0.02</td>
<td>2.14 ± 0.09</td>
<td>5.70 ± 0.18</td>
<td>10.12 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>Ethanol-exposed</td>
<td>0.11 ± 0.03</td>
<td>0.53 ± 0.05</td>
<td>1.10 ± 0.04</td>
<td>2.10 ± 0.09</td>
<td>4.80 ± 0.27</td>
</tr>
</tbody>
</table>

The values of p vs. control *<0.05, **<0.01.
levels are increased under these conditions\(^7\). Also, the number of epithelial cells lining the intestinal lumen is decreased significantly in pups born to rats administered with ethanol during gestation\(^12\). Since majority of membrane enzymes lining microvillus membrane are glycoproteins, a decrease in these enzymes and the number of enterocytes in intestine could explain the observed changes in membrane sialylation and fucosylation in pups exposed to ethanol\(^13\).

A number of factors, such as the levels of glycosyl transferases, availability of sugar nucleotides, acceptor molecules and various cofactors influence membrane glycosylation. A high sialic acid and low fucose level during early suckling period is attributed to the sialyl-fucosyl transferase activities\(^7\). Thus, alterations in these factors could be implicated in observed changes in glycosylation in rat intestine. Sialic acid and fucose generally occupy the terminal non-reducing ends of membrane oligosaccharides and act as receptors for various toxins and adhesion of microorganisms\(^1,2\). The low fucose to sialic acid molar ratio in the intestinal BBM of ethanol-exposed pups suggests that prenatal exposure to ethanol in developing rats may influence the ecology of microflora in intestinal lumen.

Ethanol exposure is known to impair glycosyl transferase activity in hepatocytes\(^28\). It is likely that a similar phenomenon may occur in intestine, which may result in modifications in membrane glycosylation in response to prenatal ethanol exposure affecting various metabolic processes of enterocytes leading to aberrations of intestinal functions. The findings suggest that \textit{in utero} ethanol exposure influence microvillus membrane sialylation and fucosylation processes and may lead to aberrations in intestinal structure and functions during postnatal development in rats.

**Acknowledgement**

This study was supported by a grant from Indian Council of Medical Research, New Delhi.

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