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The role of somatomedin in mediating ethanol induced growth retardation with potential impact on nursing practice related to fetal alcohol syndrome

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The Role of Somatomedin in Mediating Ethanol Induced Growth Retardation With the Potential Impact on Nursing Practice Related to Fetal Alcohol Syndrome

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Abstract

The effect of ethanol administration on plasma levels of growth hormone (GH) and somatomedin C (IGF-I) was investigated on 26 day old male and female rats. Ethanol (3gm/kg) was administered IP to 5 male and 5 female rats at ages 24 and 25 days. Saline (10 ml/kg) was administered to 5 male and 5 female control rats on the same days. At age 26 days blood was collected by cardiac puncture at sacrifice. IGF-I was extracted using C-18 cartridges, and plasma concentrations of GH and IGF-I were measured using specific radioimmunoassay. No significant difference was found statistically but trends showed 11-15% decrease of GH and IGF-I levels in ethanol groups compared to the control groups. Lowered plasma levels of IGF-I may be a result of variables in the extraction methods. It is possible that ethanol reduces plasma levels of GH and somatomedins which may have a role in mediating growth retardation associated with ethanol exposure conditions such as fetal alcohol syndrome.

The role of Somatomedin in Mediating Ethanol Induced Growth Retardation With Potential Impact on Nursing Practice Related to Fetal Alcohol Syndrome

Introduction

Throughout history there has been an established relationship between teratogenicity and maternal alcohol consumption. Prohibition of consumption of alcohol by the nuptual couple on their wedding night as a prevention of birth defects are found in ancient laws. The manifestation of fetal abnormality after exposure to alcohol, is varied and may be only a slight growth lag and irritability of the neonate or it may be severe retardation, extremely low birth weight, with several or many congenital anomalies. A specific pattern of these congenital malformations have been documented and identified as a condition known as fetal alcohol syndrome (FAS) (Behrman, & Vaugham, 1987). Identification of features from each of three categories, with a positive history of maternal alcohol abuse, establish the criteria for diagnosis of FAS. These categories are growth retardation, neurological impairment, and distinct abnormal features of the face (Anderson & Anderson, 1986). The craniofacial anomalies are most unique and are specifically linked to FAS. The effected infant may have one or more of the following traits: Narrow forehead, flat nasal bridge, short palpebral fissures (eye slits), small midface, indistinct philtrum, thin upper lip, and small chin (Whitney and Cataldo, 1983; Emhart, et al., 1987). Other defects associated with FAS are cardiac defects, minor joint, limb and genital anomalies (Anderson et al., 1986). Infants exposed to ethanol (ETOH) with less than three of these features are classified as having fetal alcohol effects (FAE) (Halmesmaki, 1988). Researchers estimate that 1-3 out of every 1000 live births will have either FAS or FAE (Abel, 1985), and the incidence of congenital defects in infants born to heavy drinkers to be 32% (Behrman et al., 1987). The effects of fetal alcohol exposure are considered to be permanent and sometimes fatal (Whaley & Wong, 1987).

Abel (1985) suggested that prenatal growth retardation is the most common fetal alcohol effect and is an accurate predictor of neonatal mortality and morbidity. Specifically, infants with a birth weight less than 1500g have a neonatal mortality rate of 45%. In a retrospective study of greater than 300 FAS infants, the average birth weight was 2000g (Abel, 1985). This suggests that infants with FAS, especially those with low birth weight, to be at risk for infant mortality. Therefore, deficient prenatal growth related to maternal ethanol consumption is a significant topic for study.

The fact that alcohol impairs growth is well documented. Wright (1986a ;1986b) suggested a direct relationship between maternal alcohol consumption of greater than 100g per week (10 large wine glasses) and lowered birth weight and head circumference. Fetal growth retardation can be detected ultrasonically after 27 weeks gestation but there is no evidence that this growth lag can be modified if the women reduced their intake or abstained from drinking (Halmesmaki, 1988). The growth deficit seems to be permanent. Eight children with the diagnosis of FAS at birth, examined at 4 years of age, still had a weight and head circumference less that the expected mean for a 4 year old (Graham, Hanson, Darby, Barr, & Streissguth, 1988). The physiological mechanism whereby ethanol impairs growth is still unclear. Researchers seeking to find the answer have presented several hypothesis. Halmesmaki and Ylekorkala's (1986) study of fetal cardiotocography (CTG) of four deeply intoxicated women (blood ethanol levels 27.6mmol/l- 63.16 mmol/l) revealed no or poor variability and no reactivity to external stimuli or fetal movement. The CTG's returned to normal when the mothers became sober. It was hypothesized that the effects of Ethanol resembled the effect of cerebral depressants manifested in general suppression of fetal function which, if chronically repeated, could contribute to FAS

features. Ernhart et. al. (1987), suggested a clear dose-response relationship of anatomic anomalies (particularly of the craniofacial area) to prenatal ethanol exposure. A theory of alcohol related malnutrition as the immediate antecedents of the anomalies was offered. Others suggest the deprivation of glucose and B vitamins to the nervous system as the cause of most of the FAS symptoms (Whitney et al., 1983). Abel (1985) suggested several different hypothesis regarding the causal factors for growth deficiency. Among them was that an alteration in placental function, due to restricted blood flow in the presence of ethanol results in fetal hypoxia. Hypoxia reduces ATP synthesis and impairs the active transport of amino acids across the placenta limiting protein synthesis. Another possibility was that high gamma glutamyl transpeptidase, associated with ethanol consumption, may be related to increased anomalies (Wright, 1986b).

The possibility that the endocrine system, which regulates growth, is altered by ethanol exposure has been examined. The effect of ethanol on hormones is variable. Alcohol does not influence circulating prolactin levels, however thyroid stimulating hormone (TSH) levels rise under similar conditions (Mannisto et al., 1987). Both testosterone and estradiol increased the liver's alcohol metabolizing activity (Teschke, Wannagat, Lowendorf & Strohmeyer, 1986).

Of particular interest is the effect of ethanol on the growth hormone (GH) system because of its essential role in skeletal growth. Associated with GH are a group of circulating endogenous growth factors, somatomedins (Sms), which are dependent on and mediate the effects of GH. In an early GH study of the role of GH in ethanol induced growth retardation, five children with FAS were found to have normal to slightly elevated levels of GH (Tze, Friesen & Macleod, 1976). The age of the children were from 6 months to 7 years and showed severe growth failure (<3%/ht/wt/age). They hypothesized peripheral tissue insensitivity to GH to be the reason for poor growth. Blood levels of Sms were at normal levels. Animal studies suggest the same

result, that GH levels were not significantly different in the chronic ethanol group or the light drinking group (Mannisto et al., 1987). In one of the few strict studies of the effect of ethanol on Sms, Kennedy, Sheppard, Bhaumick, and Laverty, (1986), found that serum Sms (IGF-I) levels in mice were not effected by alcohol. However, there was a reduction of the binding of Sms to the placenta possibly initiating premature aging of the placenta. A recent study suggested a significantly lower peak of GH in the ethanol experimental group after stimulation with clonidine, than the control group (Mauceri & Conway, 1989).

It seems likely that GH and its dependent somatomedins have a role in mediating the effect of reduced growth in the presence of ethanol, based on its essential role in promoting skeletal growth, cell proliferation and differentiation. Somatomedins are endogenous GH-dependent hormones which are synthesized in the liver but are found in greatest concentration in the plasma bound to its carrier protein (Hadley, 1984). Sms are low molecular weight peptides (7500 MW) which are also called IGF-I and IGF-II so named for their insulin-like properties (Nissley, & Rechler, 1984). IGF-I corresponds to rat somatomedin C (SmC), and is a basic polypeptide with 70 amino acids (Hadley, 1984). IGF-II is neutral and corresponds with the rat Sms MSA which is only found in rats less than 25 days old (Van Wyk, 1984).

Because of the essential role of GH and Sms in growth it is of interest to the examine the effect of ethanol on this system. Important questions to address are: Does ethanol have an impact on plasma levels of GH and Sms? Do lower plasma levels of GH and Sms mediate ethanol induced growth retardation?

The purpose of this pilot study is to determine the effect of ethanol on plasma levels of GH and IGF-I. The Hypothesis of this study is that plasma levels of Somatomedins and Growth hormone will be lower in animals treated with Ethanol than animals not treated with Ethanol.

Materials and Methods

Animals

Male and female Sprague-Dawley rats were bred and housed in the animal facility at Northern Illinois University. The environment was light (12 hours of light, 12 hours of dark) and temperature (22±2 C) controlled. Animals were 24 days of age, weaned to standard laboratory chow and water, ad libitum, and weighed 30-63 gm.

Methods

Ethanol treatment A group of 5 male and 5 female rats were injected IP with 25% Ethanol solution in 0.9% NaCl (3g/kg) at age 24 and 25 days. Control animals (5 males and 5 females) were administered 0.9% NaCl (10ml/kg) IP at the same ages.

Plasma collection At age 26 days the animals were sacrificed by cervical dislocation and blood immediately collected by cardiac puncture. The blood samples were centrifuged at 1800 rpm, 4 C for 20 minutes. The plasma was stored at -20 C until assayed.

IGF preparation Rat plasma 100ul was acidified with 100ul .5N HCL and incubated for 1-2 hours. During this time the C-18 Sep-Pak cartridges (Waters,Assoc., Milform Ma.) were solvated/primed using 5 ml isopropanol, 5 ml 100% methanol, and 10 ml 7% acetic acid(pH 2.5). The C-18 cartridge (column) is of octadecyl silane bonded to silica substrate. The sample was applied to the column and incubated for 3 minutes. The column was washed with 10ml 7% acetic acid. After the acetic acid was completely removed from the column, .5 ml of methanol was applied to the column and incubated for 2 minutes, followed by 1.5 ml of methanol to elute the isolate. The 2 ml of methanol with isolate(Sms) were evaporated using the Savant Speed-Vac concentrator for 3 hours. The isolate was reconstituted in 1 ml of .03M phosphate buffer, pH 7.5.(IGF-I assay buffer).

RIA

Sms assay The reconstituted isolate was assayed for IGF-I using IMCERA IGF-I radioiodination protocol. A double antibody, disequilibrium assay was used.

Assay protocol Day 1: 50ul of Rabbit anti-rat IGF-I 1:400 dil. (1S.D. antibody) was added to polypropylene tubes containing 50ul of reconstituted sample and 300ul of 0.03M phosphate buffer. The tubes were vortexed and incubated at 4C for 3 days.

Day 4: IGF-I was iodinated with 125I by chloramine-T oxidation reaction and passed over a Bio-Gel G-50 column in 0.03M phosphate buffer for elution. An initial collection of 20 drops was followed by fractions of 2 ml collected in tubes containing 100ul of 0.03 phosphate buffer. 10ul was removed from each tube and counted. The tube with the highest count, (tube 12), contained the largest amount of iodinated IGFI and was diluted in buffer to produce an activity 15000 cpm/100ul. 100ul of iodinated IGF-I was added to the incubated sample tubes. The tubes were vortexed and stored at 4C.

Day 5: 200ul of sheep anti-rabbit serum diluted 1:36 (2nd antibody) was added to each tube. The tubes were vortexed and 100ul of normal rabbit serum (NRS) in buffer was added. The tubes were vortexed again and refrigerated at 4C.

Day 6: 1ml of 1% egg white PBS (phosphobuffered saline), pH 5.0 and 1ml of PBS pH 5.0 were added to each tube. The tubes were centrifuged at 4C, 2000rpm, for 60 minutes. The supernatant was aspirated, the remaining pellet was counted and the IGF-I content was calculated by a LKB Wallac 1282 Compugamma Gamma Counter (Turku, Finland).Assay sensitivity was .2ng/tube to 100ng per tube.

GH assay The plasma samples were assayed for GH using the NIADDK's Rat Growth Hormone Radioimmunoassay (rGH RIA) kit. Following is a brief description of the protocol.

Day 1: Rat GH was iodinated with 125I by chloramine-T oxidation reaction and passed over a Bio Gel P-60 column using barbital buffer for elution. An initial collection of 20 drops was followed by fractions of 8 drops collected in tubes containing 100ul 1%BSA (buffer). 10ul was removed from each tube and counted for radioactivity. The tubes with the highest count (usually tubes 6-8) contained the largest amount of iodinated rGH and diluted in buffer to produce an activity of 15000cpm/100ul.

Each sample assay tube contained 50ul of plasma sample and 150 ul of 1% BSA phosphosaline buffer (pH 7.6). 100ul of monkey anti-rat GH antibody (1st antibody), diluted 1:5000 with buffer, was added to each tube. After they were vortexed, 100ul of the iodinated rGH was added to the tubes, which were vortexed again and refrigerated at 4 C.

Day 3: 100 ul of goat anti-monkey gamma globulin (2nd antibody), diluted 1:20 in buffer, was added to each tube. The tubes were vortexed and 100ul of 1% normal monkey Serum (NMS) in buffer was added to each tube, which were again vortexed. The tubes were refrigerated at 4C.

Day 5: 1 ml 1% egg white PBS pH 7.0 and 1 ml PBS pH 7.0 were added to each tube. Cold centrifugation of the tubes at 1800rpm for 35 minutes at 4 C were run and the supernatant was aspirated from the tubes. The pellet that remained was counted and the GH content was calculated by a LKB Wallac 1282 Compugamma Gamma Counter.

A log:logit standard curve was used. Reference and standard tubes followed the protocol for this assay. Sensitivity was .095ng/tube to 25ng/tube .

Statistics

The data was analyzed using the students t. P levels of .05 or less indicated a significant difference. Means, standard deviation and standard error were determined.

Results

This preliminary study was undertaken to examine the effects of acute ethanol on IGF-I and GH plasma levels. The administration of 3gm/kg of ethanol IP, was associated with reduced plasma levels of GH in both male and female rats (table 1). Males treated with ETOH had GH levels 11% lower than the male control rats treated with saline (10 ml/kg,IP). Female rats treated with ETOH had GH levels 13% lower than the female control rats. The female GH levels in both ETOH and saline groups were higher than male GH levels of the same groups (figure 1).

IGF-I plasma levels reflected trends similar to the GH levels (table 2). Male and female rats treated with ETOH (3gm/kg, IP) had lower plasma IGF-I levels than male and female rats treated with saline (10 ml/kg,IP). Male rats treated with ETOH had IGF-I plasma levels that were 15% lower than the saline treated males. The female rats in the ETOH treated group had plasma IGF-I levels 11% lower than the female rats in the saline treated control group. The male IGF-I level was slightly higher than the female level (802.64 ±116.36 to 768.87 ±172.55 ng/ml, respectively) in the saline treated group. The ETOH treated males and females had IGF-I plasma levels essentially the same (684.73 ±76.25 to 684.38 ±107.80 ng/ml, respectively), (figure 2).

Due to the small sample size (tables 1&2), of the experimental and control groups, differences in the data were not statistically significant.

GROWTH HORMONE AND ETHANOL STUDIES			
GROUP	NO	TREATMENT	GH* (ng/ml)
Male	5	3 gm/kg ETOH	27.68 ± 1.56
Male	5	10 ml/kg Saline	30.96 ± 3.22
Female	5	3 gm/kg ETOH	31.87 ± 2.90
Female	5	10 ml/kg Saline	36.72 ± 8.57
			* MEAN ± SEM

TABLE 1

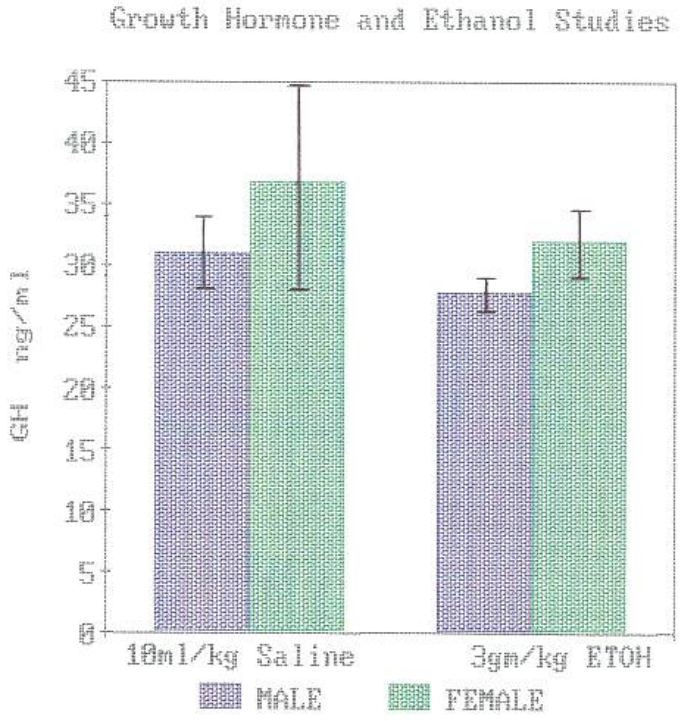

FIGURE 1

IGF-I AND ETHANOL STUDIES			
GROUP	NO	TREATMENT	IGF-I* (ng/ml)
Male	5	3 gm/kg ETOH	684.73 ± 76.25
Male	5	10 ml/kg Saline	802.64 ± 116.36
Female	5	3 gm/kg ETOH	684.38 ± 107.80
Female	5	10 ml/kg Saline	768.87 ± 172.55
			* MEAN ± SEM

TABLE 2

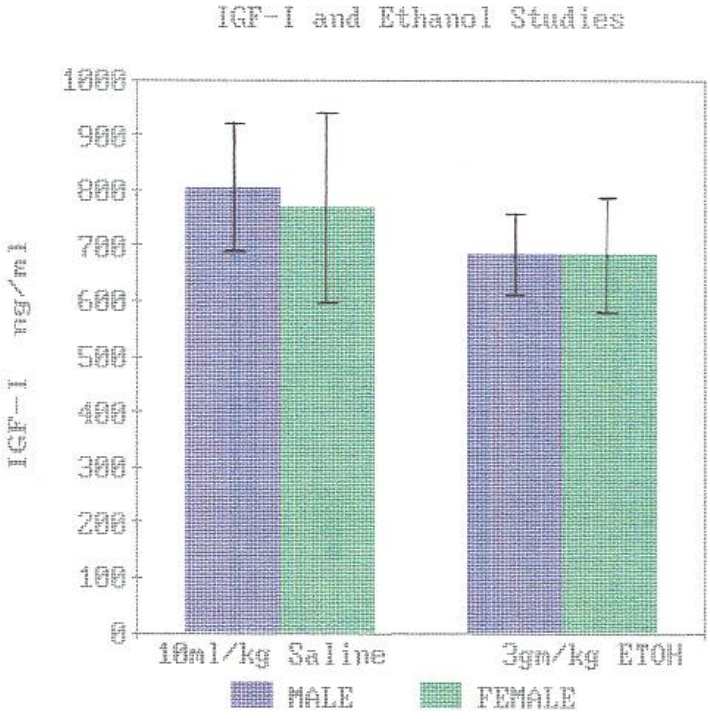

FIGURE 2

Discussion

The purpose of this study is to determine the effect of ethanol on plasma levels of GH and IGF-I and to examine the potential role of somatomedins in mediating ETOH induced growth retardation. The treatment used in this study, the administration of ethanol 3gm/kg mimics binge drinking in humans and maintains the rats blood ethanol level at approximately 200 mg/100ml dl. The data from this study show differences of GH and Sms levels between males and females as well as between treatment and control groups (figures 1&2). Male GH level is lower than females. This is possibly due to the different pattern of GH secretion in male and female rats. Male rats have a lower trough or baseline level but have high amplitude peaks at intervals of approximately 3-3.5 hours (Moherek, 1988). Females have a higher baseline, with more frequent, lower amplitude peaks of the hormone. The male releases more GH. The slightly higher Sms level in males, is possibly related to the presence of higher total amount of GH as SMS are GH dependent. Data from this study, though not statistically significant, also suggest a trend of lower levels of both GH and IGF-I in the ETOH treatment groups. Data from a previous study of Clonidine induced surges in the presence of ETOH (3gm/kg) also showed a trend of lowered GH levels in the ETOH treatment group which may suggest diminished synthesis (Mauceri et al., 1989). These rats had not been exposed to ETOH before day 24 and had not been able to build a tolerance for it. The site of synthesis for Sms, the liver, may have been occupied with the task of detoxifying the blood of alcohol and may have had reduced Sms synthesis efficiency. Another possible hypothesis to account for the reduction of somatomedin in the plasma is the possible pH change in the plasma related to the ETOH injection. A decrease from the optimal pH in the rat plasma may cause the binding protein to

release the Sms, thus shortening its life and presence in the plasma. A change of the pH could also reduce the retention capabilities on the C-18 cartridge. In the initial acidification of the sample to separate the sms from the binding protein, the plasma was incubated in HCL. Alcohol in the presence of strong acids act as bases thus raising the pH (Bailar et. al., 1984). The process of separating Sms from the binding protein would be hindered. The bound Sms would be washed with the other proteins and the C-18 cartridge would retain less Sms. This would be reflected in lower Sms levels in the ETOH group when in fact there may be levels greater or equal to the control. Another possibility for error is found in the technique of extraction. The C-18 non-polar cartridge could have less retention abilities for some sample than others. The apparatus for extraction can only extract six tubes at one time. The protocol for the assay necessitated the tubes be run in duplicate, therefore, only 3 samples could be eluted at a time. Often these samples were from the same group and were subjected to the same techniques. Other samples were run at different times and different days with widely varying vacuum pressures and flow rates. There also may have been occasions when the column became dried between the solvation and the elution step also effecting the retention potential toward the isolate. These variables need to be controlled for. Other possible effects on the Sms level may result from starvation and negative nitrogen balance. Van Wyk (1984) suggests that negative nitrogen balance and low caloric intake can reduce the Sms C level. Since we did not monitor the food/water intake we do not know if the ETOH had a deleterious effect on the animals appetite. A suggestion for future studies be that diet be closely monitored and any change in dietary intake be noted.

Although this study did not establish a significant difference between the control and the ETOH groups, a trend may be seen suggesting some diminishing effect of ETOH on the growth hormones, with potential depression of growth which may provide a basis for other studies. The literature has given much evidence for the potent teratogenicity of ETOH, with growth being a factor quite often effected. The mechanism underlying ethanol physiological impact on plasma GH and Sms remains nclear but future studies may elucidate the pathophysiology providing for the possibility of improved intervention and treatment for nursing practice. Because safe criteria for drinking during pregnancy has not been established, strict abstinence from alcohol while pregnant or trying to conceive is to be encouraged as the best intervention (Ernhart, et. al. 1987). While many of the effects of FAS seem to be permanent, some of them may be modified or reversed with early intervention strategies in the newborn (Barbour, 1989). A most important intervention strategy is prevention of FAS through assessment and identification of high risk women, early entrance into a treatment program with personal counselling, and continued monitoring of fetal progress in pregnant women (Bowen, & Sammons, 1988; Halmesmaki, 1988). Because nursing practice is based on scientific knowledge, more research is needed to build a body of science relating to FAS on which valid and successful interventions can be based and evaluated against.

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