Does maternal drug ingestion cause Megacystis Microcolon Intestinal Hypoperistalsis Syndrome? *III. ethanol trial*

E. Balkan[1], S. Noyan[2], Ş. Sırmalı[2], H. Doğruyol[1]

Departments of Paediatric Surgery[1], and Histology and Embryology[2], The Medical Faculty of Uludağ University, Bursa (Turkey)

Summary

Purpose: Megacystis Microcolon Intestinal Hypoperistalsis Syndrome (MMIHS) is a congenital disease, and the etiology of the disease is unclear. It is speculated that maternal ingestion of some drugs during pregnancy may be an etiologic factor. In this study we aimed to investigate the effect of maternal ingestion of ethanol on the fetal bladder and colon in pregnant rats.

Methods: We separated animals into an ethanol group including 30 rats and a control group with 14 rats. Nothing was given to the control group during pregnancy. Orogastrically 2 cc/day 30% ethanol was given to the study group from the 6th to 12th day of pregnancy. All of them were sacrified on the 20th day of pregnancy. Histopathological examination of the fetal colon and bladder was performed.

Results: In the ethanol group a significant decrease in the colon and bladder diameter, a significant decrease in the thickness of the colon and bladder wall, an increase in vacuolar degeneration in the muscles of the bladder wall, an increase in connective tissue proliferation among the muscles of the bladder, a significant decrease in the number of ganglion cells in the myenteric plexus of the colon and a significant decrease in the thickness of the bladder tunica muscularis were determined.

Conclusion: In our rat model we found histological structural changes in the rats' colon and bladder wall similar to a pathological finding found in some of the MMIHS patients' bowel and bladder as a result of using ethanol on the 6th-12th days of pregnancy.

Key words: Ethanol; Megacystis Microcolon Intestinal Hypoperistalsis Syndrome; Maternal Drug Ingestion.

Introduction

The etiology of Megacystic Microcolon Intestinal Hypoperistalsis Syndrome (MMIHS) is still unknown but some authors have suggested various findings associated with it [1]. In 1983, Puri *et al.* demonstrated vacuolar degenerative changes in the smooth muscle cells of the bowel and bladder in MMIHS patients and alleged that this syndrome may depend on degenerative disease in smooth muscle cells [2]. It was considered that this syndrome has an autosomal recessive pattern of inheritance since it is also seen in sibblings [1, 3], whereas Penman and Linford have proposed that it is the result of an autosomal recessive end-organ receptor defect confined to the smooth muscle of the urinary and gastrointestinal tracts [4]. The cause of hypoperistalsis in MMIHS has been attributed to visceral myopathy [2], imbalance in gut peptides [5], defective autonomic inhibitory neuroeffector activity [6] and neuroaxonal dystrophy [7]. Srikanth *et al.* have speculated that the initial event in the pathogenesis of MMIHS is an intramural inflamatory process that affects the gastrointestinal and urinary tracts leading to extensive fibrosis that destroys the intestinal neural network [8].

In his two cases, reported in 1985 and 1989, Doğruyol determined that their mothers had used drugs such as clomiphene, scopolamin, trimethoprim-sulfadiazine, dypirone and bromide during the first weeks of gestation [9, 10]. The question of whether one of these substances has a teratogenic effect [11] similar to MMIHS pathology was worth being investigated and an experimental study was planned.

In this report we examined the effects of ethanol on the fetal colon and bladder when given to pregnant rats.

Materials and Methods

Female Wistar Albino rats, weighing 250-300 gr, were kept with male rats in separate cages two times a day (morning and/or evening). Couples in which mating was observed were kept in a separate cage overnight. On the following day, the female rat was separated from the male and this was considered as the first day of pregnancy. Rats in which no pregnancy was determined were excluded from the study. During the study, 44 pregnant rats were used and divided into two groups. Fourteen of these rats were given no drugs and only normal nourishment (Group 1). The second group, comprised of 30 rats, was given a single dosage orogastrically of 2cc 30% ethanol between the sixth and twelfth days of pregnancy. All rats were fed with rat food and tap water. The rats were sacrificed on the 20th day. The fetuses were removed, counted and numbered separately, and put into 10% formaldehyde. After 24 hours, fetuses were weighed and examined for gross pathology and then laparatomy was carried out. After observing the abdominal organs and the bladder, one centimeter distal colon segments were removed for histopathologic examination. Five μm thick transversal cross-sections were taken from organs and formed into paraffin blocks. Preparations dyed with HSE (hematoxylin-eosin) were examined under light microscope. Micrometric measurements (Leits Wetzlor, Periplan 6.3 X μ) were used in the histometric evaluation. Photographs were taken by Nikon HFX-DX photomicroscope.

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In the histopathologic examination of the colon, organ diameter, wall thickness, atrophy and vacuolar degeneration in muscles, connective tissue proliferation among muscles and decrease in the number of ganglion cells in myenteric plexus were separately examined (Figures 1-2). In the histopathologic evaluation of the bladder, organ diameter, wall thickness, epithelial atrophy, atrophy and vacuolar degeneration in muscles, connective tissue proliferation among muscles, decrease in the number of ganglion cells in the myenteric plexus and thickness of tunica muscularis were separately examined (Figures 3-4). For the parameters in which no objective measurement could be done, we used the term "increase or decrease".

Statistical analysis was performed using analysis of variance and the Tukey test. A $p<0.05$ value was considered as significant.

Results

One hundred and forty-seven fetuses from the first group and 486 fetuses from the second group were obtained. The mean fetus number obtained from pregnant rats was 10.4 and 11.0, respectively. There was no statistically significant difference between the two groups (Table 1).

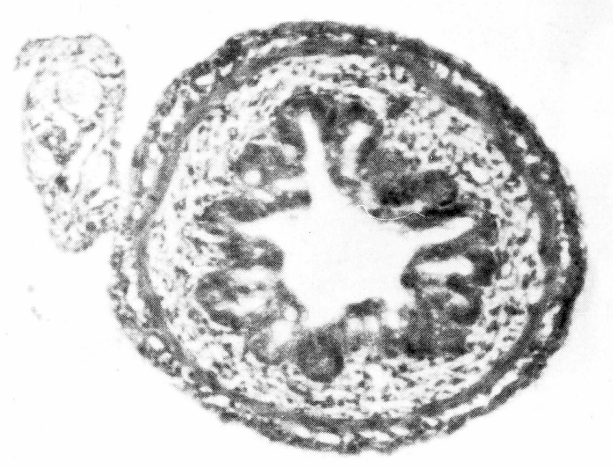

Figure 1. — Histologic appearance of colon belonging to control group. H-E, X800.

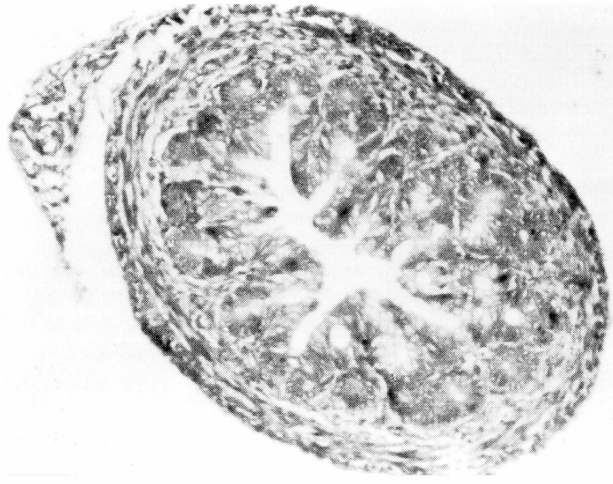

Figure 2. — Histologic appearance of colon belonging to ethanol group. H-E, X800.

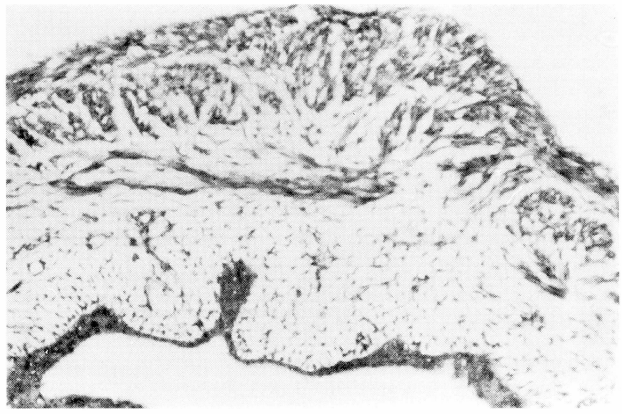

Figure 3. — Histologic appearance of bladder belonging to control group. H-E, X800.

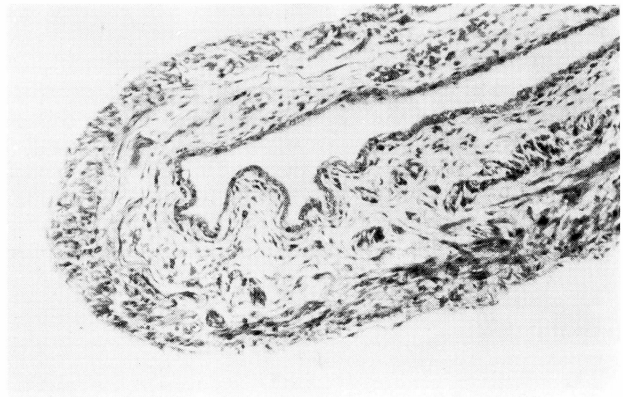

Figure 4. — Histologic appearance of bladder belonging to ethanol group. H-E, X800.

We found five fetuses from both the first and second group with placentas but no obvious fetal development. The mean fetal weight was 3.604 gr in the first group and 3.471 gr in the second. No statistically significant difference was determined in the evaluation of fetal weights (Table 2).

There was no obvious pathology on examination of the abdominal organs in the abdominal exploration of the fetuses. In evaluating colon diameter, the mean value was found to be 356.888 μm and 326.356 μm, respectively, and there was a statistically significant difference between the two groups ($p<0.001$, Table 3). The mean wall thickness of the colon was found to be 148.351 μm in the control group and 105 μm in the second one and a statistically significant difference was determined ($p<0.001$, Table 4). In evaluating the atrophy of muscles in the colon wall and vacuolar degeneration in colon muscles, there was no statistically significant between the groups. In evaluating the decrease in ganglion cell numbers in the myenteric plexus, there was a statistically significant difference between the two groups ($p<0.01$). Increases in connective tissue proliferation among muscles in the colon wall were not statistically significantly different between the two groups.

The mean bladder diameter was found to be 1265.915

Table 1. — *Fetus numbers obtained in pregnancy according to groups*

	Control	Ethanol
Minimum	4	6
Maximum	16	15
Mean	10.428	11.033
Standard deviation	3.480	2.2521

Table 2. — *Fetal weight according to groups (gr)*

	Control	Ethanol
Minimum	1.651	1.440
Maximum	5.064	6.000
Mean	3.604	3.471
Standard deviation	1.054	1.174

Table 3. — *Measurement values of colon diameter according to groups (μm)*

	Control	Ethanol
Minimum	245.000	227.500
Maximum	507.500	455.000
Mean	356.888	326.356
Standard deviation	47.057	47.349
Standard error	4.854	3.283

Table 4. — *Measurement values of the thickness of the colon wall according to groups (μm)*

	Control	Ethanol
Minimum	105.000	87.500
Maximum	210.000	192.500
Mean	148.351	105.000
Standard deviation	20.660	20.506
Standard error	2.131	1.422

Table 5. — *Measurement values of bladder diameter according to groups (μm)*

	Control	Ethanol
Minimum	700.000	700.000
Maximum	2240.000	2940.000
Mean	1265.915	1189.938
Standard deviation	252.971	260.046
Standard error	21.229	14.492

Table 6. — *Measurement values of the thickness of the bladder wall according to groups (μm)*

	Control	Ethanol
Minimum	140.000	35.000
Maximum	700.000	490.000
Mean	362.168	331.606
Standard deviation	96.189	79.708
Standard error	8.044	4.442

μm in the control group and 1189.938 μm in the ethanol group which was statistically significantly different ($p<0.05$, Table 5). The mean values of wall thickness of the bladder were found to be 362.168 μm in the control group and 331.606 μm in the ethanol group and the differences were statistically significant ($p<0.01$, Table 6). There was no statistically significant increase in bladder epithelial atrophy and atrophy in muscles in the ethanol group. When vacuolar degeneration in bladder muscles is evaluated, there was a statistically significant increase in the ethanol group ($p<0.01$). In evaluating the connective tissue proliferation among muscles in the bladder wall, there was a statistically significant difference between both groups ($p<0.05$). There was no statistically significant decrease in ganglion cell numbers in the bladder plexus or myenteric plexus in the ethanol group. The mean thickness of the tunica muscularis was found to be 187.673 μm in the control group and 171.995 μm in the ethanol group and the differences were statistically significant ($p<0.05$, Table 7).

Table 7. — *Measurement values of the thickness of the bladder tunica muscularis according to groups (μm)*

	Control	Ethanol
Minimum	52.500	52.500
Maximum	280.000	280.000
Mean	187.673	171.995
Standard deviation	53.956	46.869
Standard error	4.512	2.612

Table 8. — *Results of the histopathologic evaluation of the ethanol group compared to the control group*

	Bladder	colon
Organ diameter	↓	↓↓↓
Wall thickness	↓↓	↓↓↓
Epithelial atrophy	–	
Atrophy among muscles	–	–
Vacuolar degeneration among muscles	↑↑	–
Connective tissue proliferation among muscles	↑	–
Decrease of ganglion cells of the Myenteric Plexus	–	↓↓
Thickness of the tunica muscularis	↓	

Empty = not determined; – = $p>0.05$;
↑ and ↓ $p<0.05$
↑↑ and ↓↓ $p<0.01$
↑↑↑ and ↓↓↓ $p<0.001$

Discussion

In rat studies in which pregnancy is determined and the gestation date is required, the cycle of the female rat is generally followed. Female rats are left with males and then by observing the copulatory plug in the female rats that mated without following the cycle, the first day of pregnancy can be determined [12-14]. We followed the same protocol at the beginning of the study but had two problems. Some rats whose cycle was suitable did not let the male rat approach. This could be attributed to vaginal trauma which occurred while taking the vaginal smear.

The other problem was that we could not determine the copulatory plug in many rats which we observed during their mating. Thus, in our study the female and male rats were left together in the same cages and those that mated were left together overnight. The female was included in our study and the first day of pregnancy was considered as the morning after.

In experimental studies, hydronephrosis was observed when high doses of ethanol were given to rats on the 10th day or during the second trimester of pregnancy [14]. We administered a high treatment dosage of ethanol to our subjects between the sixth and twelfth days as it coincided with the organogenesis period of the urinary and gastrointestinal system when considering rat embryology [11, 13, 15].

Many studies have been carried out to explain the mechanism of the effect of ethanol on fetuses. Imai *et al.* [16] determined visceral immaturity and hemorrhage in the histopathologic examination of the brain, thymus, liver, lungs and kidneys of fetuses of pregnant rats given ethanol. Sharma *et al.* [17] showed that in rat fetuses ethanol caused the inhibition of protein, RNA and DNA metabolism in the fetal liver and brain. Okonmah *et al.* [18] determined that choline acetyltransferase was twice as high in female rat fetuses given ethanol and acetylcholinesterase activity was four times as high. They stated that the effect of ethanol on these enzymes, which are effective in acetylcholine metabolism, may show different developmental neurologic abnormalities. Inselman *et al.* [19] stated that the direct toxicity of ethanol or its metabolites impaired placental transport of nutrients or oxygen in fetuses. Fisher *et al.* [20] determined that ethanol decreased the placental transport of aminoacids and hypothesized that the aminoacids may worsen the direct fetotoxic effect of ethanol in fetal organogenesis. Brown *et al.* [21] found that structural defects may occur as cell proliferation decreases in the organogenesis phase and is directly related to the effect of ethanol. Vasilliauskas *et al.* [22] determined chronic intestinal pseudoobstruction in five children subjected to fetal alcohol. They stated that alcohol may act as a neurotoxin, altering neuronal migration or arresting the myenteric plexus. When 5.8 gr/kg 95% alcohol was intragastrically administrated to pregnant rats on the 10th day, hydronephrosis and hydroureter were determined in 40-50% of fetuses by Randall *et al.* and 24% by Boggan *et al.* [14, 23]. Yokoi *et al.* [24] determined that ethanol significantly impairs detrusor contractility in rats. Ohmura *et al.* [25] stated that responsiveness of rabbits' lower urinary tract was significantly reduced by exposure to ethanol. Malformations, intrauterine death, growth retardation, and central nervous system, urogenital and cardiovascular abnormalities and behavioral deficits have all been demonstrated in humans and laboratory animals exposed to alcohol in utero [11, 14, 20, 26].

In many studies, although the administration of ethanol during pregnancy causes fetal weight loss, no weight loss was seen in some studies [16, 26-28]. In our study, no weight lost was observed in the fetuses of rats having ethanol.

In many MMIHS studies published, although no histological findings of the bowel and bladder wall were determined, some authors found important anomalies. Thinning of colon longitudinal muscles in nine cases, vacuolar degeneration of the colon in six and in the bladder muscles of five, connective tissue proliferation of the colon in four and in the bladder of six, an increase in the thickness of the bladder wall in five and elastosis of the bladder in three cases were determined [1, 2, 8, 29-33]. Plexus were examined in 53 out of 75 cases and ganglion cell numbers and appearance were found to be normal in 42 [1]. The histopathologic examination of the colon and bladder wall of the fetus was done according to the findings determined in MMIHS studies.

In the ethanol group a significant decrease in the colon and bladder diameter, a significant decrease in the thickness of the colon and bladder wall, an increase in vacuolar degeneration of the muscles of the bladder wall, an increase in connective tissue proliferation among muscles of the bladder, a significant decrease in the number of ganglion cells in the myenteric plexus of the colon and a significant decrease in the thickness of the bladder tunica muscularis were determined (Table 8).

A Medline search using five different key phrases produced no previous experimental studies examining the effect of ethanol on the colon and bladder of fetuses. In this study concerning the etiology of MMIHS, we followed the methods similar to those seen in human studies and also in animal experiments. The results obtained were similar to the findings of MMIHS studies. By looking at these findings, it is impossible to prove the hypothesis that ethanol is an etiological factor in the cause of MMIHS. However, we found structural histological changes in the rats' colon and bladder walls similar to the pathological findings found in some of the MMIHS patients' bowels and bladders as a result of using ethanol on the 6th-12th days of pregnancy.

Conclusion

In our rat model we found histological structural changes in the rats' colons and bladder walls as a result of using ethanol on the 6th-12th days of pregnancy. This finding is similar to the pathological findings found in some MMIHS patients' bowels and bladders.

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Address reprint requests to:
HASAN DOĞRUYOL, M.D.
Department of Paediatric Surgery
The Medical Faculty of Uludağ University
Gorukle, 16059, Bursa (Turkey)