Does maternal drug ingestion cause megacystis microcolon intestinal hypoperistalsis syndrome? *II. bromide trial*

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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 and degeneration of smooth muscle cells in the fetus may be an etiologic factor. In this study we aimed to investigate the effect of maternal ingestion of bromide on the fetal bladder and colon in pregnant rats.

Method: We separated animals into a bromide group including 30 rats and a control group with 14 rats. Nothing was given to the control group during pregnancy. Intraperitoneally 8 mg/kg/day bromide was given to the study group from the 6th to 12th day of pregnancy. All of the rats were sacrified on the 20th day of pregnancy. Histopathological examination of fetal colons and bladders was performed.

Results: In the bromide group, an increase in the colon and bladder diameter, an increase in muscle atrophy in the colon and bladder wall, an increase in vacuolar degeneration in the muscles of the bladder and colon wall, and a significant decrease in ganglion cell numbers in the myenteric plexus of the colon and bladder were determined.

Conclusion: In our rat model, we found histological structural changes in the rats' colon and bladder walls as a result of using bromide on the 6-12th days of pregnancy similar to pathological findings found in some of MMIHS patients' bowels and bladders.

Key words: Bromide; Megacystis Microcolon Intestinal Hypoperistalsis Syndrome; Maternal drug ingestion; Colon and bladder.

Introduction

The etiology of Megacystis Microcolon Intestinal Hypoperistalsis Syndrome (MMIHS) is still unknown but some authors have suggesteed 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 has been 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 of 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 the 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 investigating and an experimental study was planned.

In this report we examined the effects of bromide 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 accepted 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 these were divided into two groups. Fourteen of these rats were given no drugs (group 1) and only normal nourishment. The second group, composed of 30 rats, was given a single dosage of intraperitoneal 8 mg/kg bromide between the sixth and twelfth days of pregnancy. All rats were fed with rat food and tap water. The rats were sacrificed on the twentieth 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 the laparatomy was carried out. After observation, the abdominal organs, the bladder and 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

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measurements (Leits Wetzlor, Periplan 6.3 X μ) were used in the histometric evaluation. Photographs were taken by a Nikon HFX-DX photo-microscope.

In the histopathologic examination organ diameter, wall thickness, atrophy and vacuolar degeneration in muscles, connective tissue proliferation among muscles and decreases in the number of ganglion cells in the myenteric plexus were investigated both in the colon and bladder. Apart from all of the above-mentioned parameters, epithelial atrophy and thickness of the tunica muscularis were studied in fetal bladders (Figures 1-4). 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 335 fetuses from the second were obtained. The mean fetus number obtained from pregnant rats was 10.4 and 11.4, respectively. There was no statistically significant difference between the two groups (Table 1). We determined that five fetuses from the first group and three fetuses from the second group had a placenta but no obvious fetal development. The mean fetal weight was 3.604 gr in the first group and 3.868 gr in the second. No statistically significant difference was determined in the evaluation of fetal weights (Table 2).

There was no obvious pathology upon examination of the abdominal organ in the abdominal exploration of fetuses. In evaluating the colon diameter, the mean value was found to be 356.888 μm and 384.129 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 154.129 μm in the bromide group and no statistically significant difference was determined (Table 4).

In evaluating the atrophy of the muscles in the colon wall, an increase was determined in the bromide group ($p<0.001$). Decrease in ganglion cell numbers in the myenteric plexus and vacuolar degeneration in the colon muscles were observed in the bromide group ($p<0.001$). Connective tissue proliferation among muscles in the colon wall increased but there was no statistically significant difference between the two groups.

The mean bladder diameter was found to be 1265.915 μm in the control group and 1333.098 μm in the bromide group and there was a statistical difference ($p<0.05$, Table 5). The mean values of the wall thickness of the bladder were found to be 362.168 μm in the control group and 368.957 μm in the bromide group with no statistically significant difference between the groups (Table 6). There were no statistically significant differences in the epithelial atrophy of the bladder. Regarding atrophy of muscles, an important increase was determined in the bromide group ($p<0.01$). When vacuolar degeneration in the bladder muscles was evaluated, there was a statistically significant increase in the bromide group ($p<0.001$). Connective tissue proliferation among the muscles of the bladder wall, showed no statistically significant difference between groups. There was a statistically significant decrease in ganglion cell numbers in the bladder myenteric plexus in the bromide group ($p<0.01$). The mean thickness of the tunica muscularis was found to be 187.673 μm in the control group and 195.169 μm in the bromide group and there was no statistically significant difference between them (Table 7).

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 female rats that mated without following the cycle, the first day of pregnancy can be determined [12, 13]. We followed the same protocol at the beginning of our 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.

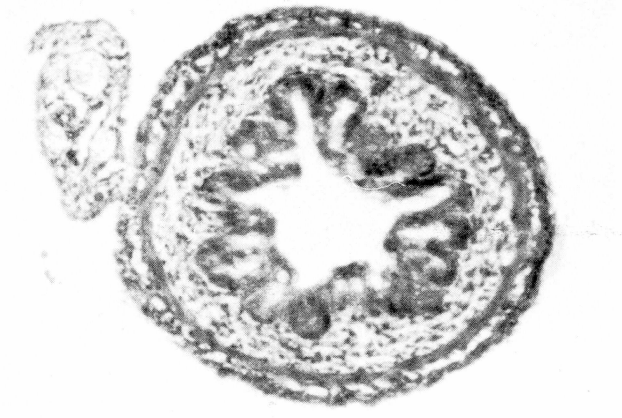

Figure 1. — Histologic appearance of colons belonging to the control group. H-E, X800.

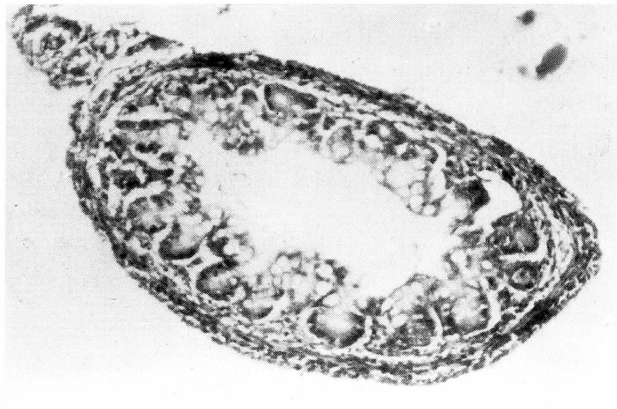

Figure 2. — Histologic appearance of colons belonging to the bromide group. H-E, X800.

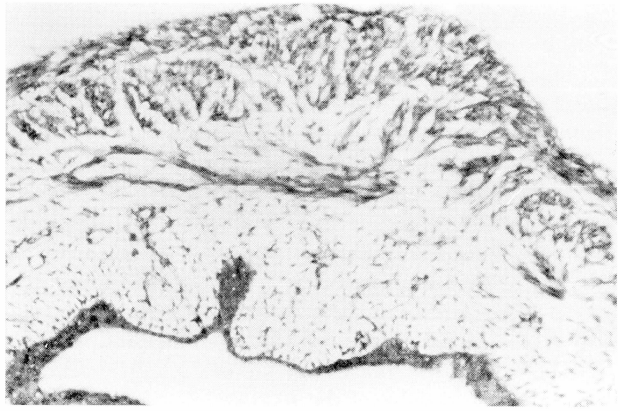

Figure 3. — Histologic appearance of bladders belonging to the control group. H-E, X800.

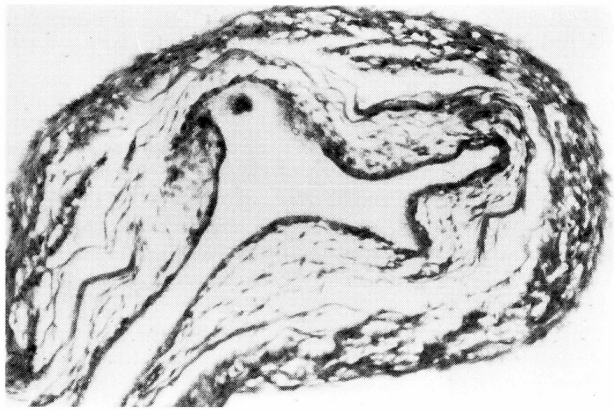

Figure 4. — Histologic appearance of bladders belonging to the bromide group. H-E, X800.

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

	Control	Bromide
Minimum	4	4
Maximum	16	17
Mean	10.428	11.379
Standard Deviation	3.480	2.945

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

	Control	Bromide
Minimum	1.651	2.620
Maximum	5.064	6.946
Mean	3.604	3.868
Standard Deviation	1.054	0.722

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

	Control	Bromide
Minimum	245.000	167.500
Maximum	507.500	525.000
Mean	356.888	384.129
Standard Deviation	47.057	56.067
Standard Error	4.854	3.589

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

	Control	Bromide
Minimum	105.000	105.000
Maximum	210.000	192.500
Mean	148.351	154.129
Standard Deviation	20.660	17.890
Standard Error	2.131	1.145

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

	Control	Bromide
Minimum	7000.000	8400.000
Maximum	2240.000	2170.000
Mean	1265.915	1333.098
Standard Deviation	252.971	231.554
Standard Error	21.229	12.825

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

	Control	Bromide
Minimum	140.000	140.000
Maximum	700.000	490.000
Mean	362.168	368.957
Standard Deviation	96.189	76.766
Standard Error	8.044	4.252

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

	Control	Bromide
Minimum	52.500	52.500
Maximum	280.000	280.000
Mean	187.673	195.169
Standard Deviation	53.956	53.306
Standard Error	4.512	2.952

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

	Bladder	Colon
Organ Diameter	↑	↑↑↑
Wall Thickness	—	—
Epithelial Atrophy	—	
Atrophy in Muscles	↑↑	↑↑↑
Vacuolar Degeneration in Muscles	↑↑↑	↑↑↑
Connective Tissue Proliferation in Muscles	—	—
Decrease at Ganglion Cells Myenteric Plexus	↑↑	↑↑↑
Thickness of Tunica Muscularis	—	

Empty: Not Determined, —: $p<0.05$, ↑ and ↓: $p<0.05$, ↑↑ and ↓↓: $p<0.01$, ↑↑↑ and ↓↓↓: $p<0.001$

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.

We administered bromide at a high dosage [11] to our subjects between the sixth and twelfth days as it coincided with the organogenesis period of the urinary and gastrointestinal system of rat embryology [14].

Bromide has been used as a sedative, anticonvulsant and in intrauterine interventions with the aim of temporary paralyzis. It is also present in food. It has been determined that excess bromide intake might cause toxicity as its biologic half-life period is 12 days. Transplacental passage and neonatal bromide intoxication have been defined but the symptoms are nonspecific. Defects in the central nervous system, growth deficiency, cardiac, gastrointestinal and skeletonal system anomalies and MMIHS were determined in babies whose mothers had excessive bromide intake from drugs or food during pregnancy [10, 11, 15-19].

In clinical studies, although intrauterine development defects were determined in babies whose mothers had bromide intake during pregnancy no difference was seen in fetal weight in our study group [17, 19, 20].

Although no histological findings of the bowel and bladder wall were determined in most of the MMIHS studies published, 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 in the colon of 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, 21-25]. Plexus was 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 walls of fetuses was done according to the findings determined in MMIHS events.

In the bromide group an increase in colon and bladder diameter, an increase in muscle atrophy of the colon and bladder wall, an increase in vacuolar degeneration of the muscles of the bladder and colon wall, and a significant decrease in ganglion cell numbers in the myenteric plexus of the colon and bladder were determined (Table 8).

A Medline search using five different key phrases produced no previous experimental studies examining the effect of bromide on the colons and bladders of fetuses. In this study concerning the etiology of MMIHS, we followed methods similar to those seen in human cases and also in animal experiments.

Acccording to the results obtained, a similarity to the findings of MMIHS was determined. By looking at these findings it is impossible to prove the hypothesis that bromide causes MMIHS as an etiological agent. However, we found histological structural changes in the rats' colon and bladder walls as a result of using bromide on the 6th-12th days of pregnancy similar to the pathological findings found in some of the MMIHS patients' bowels and bladders.

Conclusion

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

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