LABORATORY INVESTIGATIONS

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The Effects of 10% Methohexital on the Rectal Mucosa in Mice

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The effects of 10% methohexital (pH 10 and osmolarity 820) on the mucosal lining of the mouse rectum was examined after transrectal instillation and application to a rectal mucosal pouch. In addition to 10% methohexital, control solutions of pH 4, 7, 12, and osmolalities of 823 mOsm/l, 1927 mOsm/l, and 1372 mOsm/l were also applied to the rectal pouch. Using a lesion index scoring system for grading macroscopic and microscopic changes in the rectal pouch 10% methohexital produced a superficial mucosal lesion of significantly higher severity score than that produced by control and solutions of varying pH or osmolalities. The methohexital-induced rectal mucosal lesion had its onset within minutes of drug application to the mucosal lining, appeared to mature by 60 min and left minimal effects 24 h after mucosal contact. The lesion appeared to be due to the methohexital itself and not secondary to the alkalinity or hyperosmolarity. In summary, the authors’ data in mice supports an extensive 20-yr clinical experience that 10% rectal methohexital, despite inducing a transient mucosal lesion, is free of long-term serious complication related to this lesion. (Key words: Anesthetics, rectal: methohexital. Anesthetic techniques: rectal. Complications: mucosal irritation. Gastrointestinal tract: rectum.)

The rectal administration of drugs is more acceptable to many children than is intravenous or intramuscular injection. In 1936, Bourne described the induction of anesthesia with rectal tribromoethanol in children; also in 1936, Weinstein reported on the efficacy of anesthetic induction in children with rectal thiopental. Intravenous methohexital was introduced as an anesthetic induction agent by Stoelting in 1957. Orallo and Eather in 1965 reported favorably on the use of this ultrashort-acting barbiturate as a rectal induction agent for children.

It has long been appreciated that the barbiturates are capable of producing tissue reactions. Stone and Donnelly clearly defined the chemical reaction of the arterial lining to thiopental in a greater than 2.5% concentration. Even when Taylor and Stoelting reported their experience with intravenous 1% methohexital in 3,340 adults, they noted a 60% incidence of venous pain on injection.

When rectal methohexital was introduced into clinical practice, it was as a 10% solution that is ten times the concentration recommended for intravenous use. Does the application of such a highly concentrated barbiturate solution onto the rectal mucosa cause any tissue reaction and, if so, what is its clinical importance?

Orallo and Eather reported a 2% incidence of rectal stinging and a 16% incidence of rectal soiling and/or urgency in children given rectal methohexital. Laishley et al. likewise described a 13% incidence of rectal soiling with methohexital administered rectally. Recently, Forbes and Vandewalker found a 20% incidence of rectal soiling after rectal methohexital with both 2% and 10% solutions. With such small injectate volumes, it would seem unlikely that distention alone accounts for these results. As interest in the transmucosal delivery of drugs increases, especially across the nasal mucosa and buccal mucosa, we were interested in defining the histopathologic effect of methohexital on the rectal mucosa. In this study, we have developed an animal model to define the effects of methohexital on the mucosal lining of the rectum.

Materials and Methods

This project was conducted in two phases. The first phase involved the transrectal instillation of methohexital in mice. The second phase concerns observations made using a rectal pouch model. Both phases were conducted in mice and were approved by the Institutional Animal Care and Use Committee of the Dartmouth Medical School.

Transrectal Instillation

Twenty-eight CD-1 mice weighing 25 g each were randomly assigned to one of four groups (n = 7 for each group). The four groups were as follows: group A1—control; group A2—rectal catheter without injection; group A3—rectal catheter with normal saline (10 μl) injectate; group A4—rectal catheter with 10% methohexital (10 μl) injectate (i.e., equivalent to a 30-mg/Kg dose).

The physicochemical characteristics of the solutions used were as follows: normal saline—pH 6.5–7.85 mOsm/l; 10% methohexital—pH 10.819 mOsm/l. A 2.5 cm 24-g plastic catheter was used. After prefilling the microliter syringes, each catheter was attached and its dead space filled with the solution to be injected. Each catheter
tip was inserted 1 cm proximal to the anal opening. A volume of 10 μl was injected in groups A3 and A4.

Two animals were killed from each group at 2, 6, and 24 h following transrectal catheterization and/or injection. All animals were killed with an overdose of ether.

The distal rectum was excised and a ligature was placed at the distal anal opening. Each specimen was fixed in formalin and stained with hematoxylin-eosin. Serial histologic sections were cut proximal and distal to a 1-cm distance from the ligature at the predicted injection site.

Microscopic examination by a blinded observer involved classifying the presence of inflammation, ulceration, hyperemia/hemorrhage, and edema, then grading the changes on a 0–4 scale of severity with 4 indicating the most pathologic lesion.

RECTAL POUCH MODEL

Eighty-one CD-1 mice weighing 25 mg each were randomly assigned to nine experimental groups: (n = 9 for each group; table 1). All animals were anesthetized with an intramuscular pentobarbital (0.08 mg/g)/ketamine (0.003 mg/g) mixture. The distal rectum was isolated through a vertical abdominal incision. A linear vertical incision was made in the isolated rectal segment. A pouch was created by passing sutures through the upper and lower margins of the rectal incision. The rectal pouch model was similar to the gastric pouch model developed by Ritchie.11 Each animal had a 10 μl volume of the experimental solution applied to the pouch. All surgical procedures and observations were done under an operating microscope (×5 magnification).

After 60 min of solution residence time in the rectal pouch, the animals were killed and each pouch segment was excised and placed in 10% formalin for fixation. All rectal pouches were sectioned in their midregion and stained with hematoxylin-eosin.

A lesion index (LI) scale described by Ritchie11 was used to quantitate macro- and microscopic changes (table 2). All macroscopic changes were assessed at 60 min under the operating microscope. Microscopic histologic assess-

Table 1. Rectal Pouch Model Group

<table>
<thead>
<tr>
<th>Group*</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>Control</td>
</tr>
<tr>
<td>B2</td>
<td>0.9% NaCl</td>
</tr>
<tr>
<td>B3</td>
<td>Buffered solution—pH 4.0</td>
</tr>
<tr>
<td>B4</td>
<td>Buffered solution—pH 7.0</td>
</tr>
<tr>
<td>B5</td>
<td>Buffered solution—pH 12.45</td>
</tr>
<tr>
<td>B6</td>
<td>15% Mannitol—925 mOsm/L</td>
</tr>
<tr>
<td>B7</td>
<td>25% Mannitol—1372 mOsm/L</td>
</tr>
<tr>
<td>B8</td>
<td>3% NaCl—1027 mOsm/L</td>
</tr>
<tr>
<td>B9</td>
<td>10% methohexital—60 min postdrug</td>
</tr>
</tbody>
</table>

* n = 9 for each group.

ment was also based on examination of a view through the midregion of each pouch after 60 min of solution contact time by a blinded observer. Each feature in the LI scale was scored on a scale of 0–3, giving potential LI scores of 0–18, with 18 indicating the most pathologic lesion. Means and standard errors were calculated for each group. Level of significance was proven by the Wilcoxon signed-rank test.

Results

TRANSRECTAL INSTILLATION

Microscopic examination of sections of the rectum in groups A1, A2, and A3 showed no differences. The insertion of a transrectal catheter alone or followed by the injection of normal saline had no effect on the mucosal lining of the rectum. Group A4:10% methohexital, however, showed evidence of mucosal irritation manifested by inflammation, ulceration, and hyperemia/hemorrhage (table 3). Mucosal lesions were seen at 2 h after transrectal instillation of 10% methohexital (Fig. 1b). There was little residual mucosal irritation at 24 h.

As can be seen in figure 1b, when compared to the normal intact rectal mucosal lining (figure 1a), the methohexital resulted in a pathologic lesion in the mucosa. There is an inflammatory response manifested by an increase in inflammatory cells. In addition, there is ulceration of the epithelial lining giving the luminal surface a rough moth-eaten appearance when compared to normal controls. While some areas demonstrated involvement into the lamina propria, there was no evidence of any deeper effects on the muscularis mucosa or submucosa. Therefore, the methohexital lesion appears to be a fairly superficial mucosal lesion of the rectum.

Table 2. Lesion Index Scale

<table>
<thead>
<tr>
<th>Feature</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroscopic</td>
<td></td>
</tr>
<tr>
<td>Edema</td>
<td>0–3</td>
</tr>
<tr>
<td>Hyperemia</td>
<td>0–3</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>0–3</td>
</tr>
<tr>
<td>Microscopic</td>
<td></td>
</tr>
<tr>
<td>Pseudomembrane formation</td>
<td>0–3</td>
</tr>
<tr>
<td>Epithelial Slough/Ulceration</td>
<td>0–3</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0–3</td>
</tr>
</tbody>
</table>

Table 3. Transrectal Instillation Results at 2 h

<table>
<thead>
<tr>
<th>Group*</th>
<th>Inflammation</th>
<th>Ulceration</th>
<th>Hyperemia/Hemorrhage</th>
<th>Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>A3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>A4</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* n = 2 for each group at 2 h.
RECTAL POUCH MODEL

Mean LI scores and statistical data for groups B1–B9 are represented in table 4. Groups B2–B5 had mean LI scores of <3 and were not significantly different than the mean LI score for the control group (B1). Therefore, there did not appear to be an association between alkaline pH and mucosal irritation.

Groups B6–B8 had mean LI scores in the 4–5 range. The slightly higher LI scores in these hyperosmolar groups, however, were due to the presence of macroscopic features only. Both surface edema and hyperemia were judged to be more obvious in these groups. There were no microscopic lesions observed in these groups.

The significantly higher LI scores were observed in group B9 when 10% methohexital was administered into the rectal pouch. Macroscopically, using the operating microscope (3–5X) just after the application of the methohexital to the mucosal pouch lining mucosal surface,

swelling occurred. Along with the edema there was an increase in vascularity that increased over the first 30 min. In some animals extravasation of blood onto the mucosal surface was seen.

When the rectal pouch is submitted for microscopic examination, the mucosal lesion can be defined as in figure 2b. When compared to the microscopic cross section of a control rectal pouch (figure 2a) the degree of epithelial sloughing, hyperemia, and inflammation is seen.

Discussion

While rectal instillation of drugs is an old practice there is little information about what occurs at the mucosal-drug interface. In the present study, we have determined that a concentrated barbiturate solution placed in contact with a mucosal surface lining results in irritation. The mucosal lesion that we describe in mice after 10% methohexital is most likely of minor clinical significance. Whether the 2–20% incidence of rectal soiling described after recent methohexital in children is associated with a mucosal lesion is unclear. Furthermore, there seems to be no long-term consequences of this clinical practice. Interestingly, several investigators have reported that 2% and 5% rectal methohexital solutions have been just as effective as the 10% solution in achieving anesthetic induction. Nevertheless, we still think that the administration of 10% rectal methohexital is a safe clinical practice.

Caution should always be used in extending research findings from one species to another. The use of rodents for the study of gastrointestinal tract inflammation, however, has been accepted in previous studies. In this regard, we feel comfortable in assuming that the mucosal histopathology in our study serves as a model of what could be expected in the human.

Clearly, 10% methohexital causes mucosal changes that have been described with other rectally administered drugs. Meisel et al. demonstrated mucosal lesions similar
to the type we have described when humans were given Fleet’s enema and rectal bisacodyl. In addition, Leriche et al. 15 produced superficial mucosal lesions in humans after hypertonic enemas and the changes could be confused with quiescent ulcerative colitis on proctoscopic examination. On the other hand, Pedersen and Nissen 16 found no indication of mucosal irritation in humans after the administration of rectal aminophylline. Looked at in the context of these and other studies, the 10% methohexital-induced mucosal lesion would appear to be of minor importance. We would certainly be concerned, however, about inducing such a lesion in patients who are immunocompromised or who have neutropenia, and who are likely to be at greater risk for bacteremia from such a lesion.

The immunocompromised patient is constantly at risk for infection. Alterations in mucosal barriers in this group of patients invariably leads to local or systemic infections. The high incidence of perirectal cellulitis in these patients and subsequent bacteremia indicate some special concerns about administering rectal medications to them. 17, 18 Theoretically, an alteration in the protective rectal mucosal barrier, as we have described with 10% rectal methohexital, may be disadvantageous in immunocompromised patients.

In summary, we have defined a mucosal lesion of the rectum in mice that is induced 1 h after the administration of 10% methohexital. This superficial mucosal lesion would appear to have minimal consequences for children in clinical practice.

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References