A Method to Quantitatively Compare *In Vivo* the Effects of Gallstone Solvents on Intestinal Mucosal Function: A Controlled Study Comparing Mono-Octanoin with Methyl *tert*-Butyl Ether in the Rat (43895)

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Abstract. **During contact dissolution of gallstones, solvents may escape from the gallbladder and damage the intestinal mucosa. In order to compare the extent of this potential injury, we developed a method to objectively quantify the effects of two commonly used cholesterol solvents, methyl *tert*-butyl ether and mono-octanoin, on mucosal transport function in the rat intestine.**

Two intestinal segments in each of 184 anesthetized rats were cannulated. Three milliliters of either solvent were instilled in one segment and left for varying periods of time, while saline was instilled in the other as control. The segments were then washed and perfused for 45 min with an isotonic solution containing [^{3}H]polyethylene glycol 4000 (a nonabsorbable reference marker) and either [^{14}C]α-aminoisobutyric acid (a marker for active absorption) or [^{14}C]mannitol (a marker for passive permeability).

Methyl *tert*-butyl ether caused more inhibition of α-aminoisobutyric acid absorption (64%) than mono-octanoin (48%) and a greater reduction of dry weight per centimeter of the perfused segment (22%) compared with mono-octanoin (10%). Such effects appeared after only 1 min of solvent exposure and did not appreciably increase with longer exposures. Permeation of mannitol increased by 26% after 1 min of exposure to mono-octanoin and by 54% after a similar period of exposure to methyl *tert*-butyl ether. Longer exposures to both solvents did not seem to cause progressive increases in mannitol permeation.

The results indicate that brief exposure of the rat jejunum to either of the two solvents causes a reduction in active transport ([^{14}C]α-aminoisobutyric acid absorption), an increase in passive permeability (mannitol permeation), and a loss of mucosal constituents. We conclude that the intestinal mucosa is susceptible to solvent damage and may be used as a selectively sensitive model that can characterize the biological injury of gallstone solvents. The study also suggests that escape of the currently available solvents into the small intestine in patients undergoing contact dissolution of gallbladder stones may cause injury to the small intestine. [P.S.E.B.M. 1995, Vol 209]

Even though it was first described in 1891 (1), the procedure of chemical contact dissolution of gallbladder stones did not stimulate much enthusiasm until the discovery that the lipid solvent methyl *tert*-butyl ether (MTBE) can rapidly dissolve cholesterol gallstones *in situ* without injuring the gallbladder mucosa (2–4). In all reported studies, MTBE was manually syringed while limiting the infusion volume to an amount less than the capacity of the gall-

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bladder (overflow volume) in an attempt to prevent overflow of MTBE into the duodenum (3–5). However, unpredictable gallbladder contractions frequently occur resulting in solvent overflow into the intestine leading to gastrointestinal symptoms such as nausea, vomiting, abdominal pain, and the intense odor of MTBE appearing on the patient's breath (3, 5). Escape of MTBE into the duodenum has also been observed by endoscopy to cause mucosal erythema (4).

Although the gallbladder mucosa, for reasons that are unclear, appears to be remarkably resistant to injury by MTBE (2), the effects of this solvent on the small intestinal mucosa have not been well documented. Classically, the effects of various agents on the intestine are evaluated and compared using histological studies where responses are graded according to an individually defined method of scoring. Such studies are highly subjective, do not provide an accurate quantitative measure, and in many cases lack sensitivity. In this study, we quantified these subtle effects on the absorptive functions of the small intestinal mucosa which may otherwise not have been evident when using basic morphological or histological evaluations. Our aim was to develop a quantitative measure with which to compare prospective gallstone solvents in an animal model and not to study their clinical effects *per se*.

Materials and Methods

The effects of MTBE and mono-octanoin (MOC) (a solvent currently approved for dissolution of gallstones retained in the common bile duct) (6) on intact segments of the rat's intestine were studied and compared to saline which serves as control. The studies were approved by the institutional animal care committee. MTBE was obtained from EM Science (Gibbstown, NJ); MOC from Ethitek Pharmaceuticals Co. (Skokie, IL); α-aminoisobutyric acid (AIB) from Sigma Chemical Co. (St. Louis, MO); [^{14}C]AIB, [^{3}H]polyethylene glycol ([^{3}H]PEG) 4000, and [^{14}C]mannitol were obtained from NEN, Research Products-Dupont (Wilmington, DE).

All experiments were performed on male Sprague-Dawley rats of the Charles River strain weighing 250–300 g. The rats were housed in an animal housing facility under controlled conditions (24°C; 12:12-hr light: dark schedule) for at least 7 days before being used in the experiments that were done between 9:00–12:00 AM. The rats were fed rodent chow and water *ad libitum* until the day of experimentation when they were anesthetized with an intraperitoneal injection of Na-pentobarbital (45 mg/kg). During the experiment, the rats were placed on a heating pad that maintained their rectal temperature at 37°C.

Each of 184 rats' abdominal cavities were opened by a longitudinal mid-line incision and two 10-cm long jejunal segments were cannulated with inlet and outlet cannulae. Two to three milliliters of either undiluted MTBE or MOC were placed in one segment, care being taken to fill but not distend the segment, for periods of 1, 2, 3, 6, 9, 12, or 45 min. Three milliliters of isotonic saline were instilled in the second segment for identical periods of time. The segments were then washed off and perfused using a previously described recirculation technique (7), during which each of the cannulated segments was attached to tubes leading to and from a common reservoir which contained 13 ml of a perfusion solution. The solution consisted of either isotonic saline containing 2 m*M* of [^{14}C]AIB (an actively absorbed nonmetabolizable amino acid) and [^{3}H]PEG 4000 (a nonabsorbable reference marker), or 5 m*M* of [^{14}C]mannitol (a passively absorbed hexose) and [^{3}H]PEG. In order to attain a steady state, each segment was washed for 10 min by perfusing one of these solutions using a peristaltic pump. The wash solution was then replaced with fresh perfusate. This was recirculated for 45 min at a rate of 3 ml/min.

The perfused segments were resected at the end of the experiment, and their length, wet weight, and dry weight were determined. Samples of the perfusate were taken from the reservoirs at the beginning and end of the 45 min of perfusion and their [^{14}C] and [^{3}H] contents were determined by double isotope counting in a liquid scintillator.

Net absorption of AIB or mannitol was calculated as previously described (8) and expressed as μmole/45 min/cm of intestinal segment length. The segment's dry weight was expressed as mg/cm of intestinal length. Individual values for each time of exposure are presented as mean ± SEM (the *n* for each experiment is different and is outlined on the appropriate figure). The Student's *t* test for independent observations was used to test for statistical significance of the observed differences between test and control segments.

Results

Preliminary experiments were conducted to validate the methods that were used in this investigation. It was initially necessary to determine whether the nonabsorbable marker PEG 4000 is not itself absorbed after treatment of the intestine with MTBE or MOC. Absorption of this marker could have influenced the measured absorptive rates of AIB and mannitol leading to an underestimation of such rates. To avert this potential problem, we measured the recovery of [^{3}H]PEG in intestinal segments in which MTBE, MOC, or saline (control) had been instilled for 45 min. We used a method described previously (8) based primarily on recovery of the radioactivity (scintillation counts) in perfusates and wash solutions of a segment that had been perfused with a known initial total ra-

dioactivity counts of isotope. Recovery of the isotope ranged between 97%–98% and was not significantly different from control in either the MTBE or MOC treated tissues (Table I). This suggested that PEG is not appreciably absorbed and it serves, therefore, as a good marker of volume exchange in the present experiments.

The methodology was further validated in another group of experiments which tested whether the presence of MTBE or MOC in one segment of small bowel affects absorption in the adjoining segment. In several experiments, the rate of absorption of AIB or mannitol was measured in a 10-cm segment of jejunum that was not exposed to any solvent or saline and that was in proximity to another segment that contained either normal saline (0.9% NaCl) or one of the two undiluted solvents for 45 min. AIB or mannitol absorption in the virgin segment of the rats studied did not differ significantly for the three conditions tested and it was not influenced by the presence of MTBE or MOC in adjacent sister segments (Fig. 1).

Figure 2A shows the results for AIB absorption. The absorption rate of this amino acid was inhibited more by MTBE (64%) than by MOC (48%). Such inhibition appeared after 1 min of solvent exposure and increased only slightly with longer exposures. In all studies, water absorption was modest and varied directly with AIB absorption. The net absorption of water was, furthermore, influenced in a similar manner by the presence of MTBE and MOC.

Permeation of mannitol increased by 26% after a 1-min exposure to MOC and by 54% after exposure to MTBE (Fig. 2B). Longer exposure to solvent did not seem to cause progressive increase in mannitol permeation compared with control.

The possibility existed that the segments might have decreased in length due to shrinkage or contraction after exposure to the solvents but not to saline.

Table I. Recovery of [^{3}H]Polyethylene Glycol After Recirculation-Perfusion of Intestinal Segments That Have Been Exposed to MTBE, Mono-Octanoin, or Saline for 45 min

Solvent	Perfused DPMs[a]	Recovered DPMs[a,b]	% Recovery[c]
MTBE	1.43 ± 0.1	1.39 ± 0.09	97.3 ± 0.33
Mono-octanoin	1.77 ± 0.08	1.73 ± 0.08	98.0 ± 0.0
Saline (control)	1.47 ± 0.19	1.45 ± 0.19	98.7 ± 0.33

[a] Values are expressed as mean × 10^5 ± SE of three rats per group.
[b] DPM (disintegrations per minute) recovery was calculated from the radioisotopic counts in DPM of the final effluent solution plus all the counts recovered in three saline wash solutions of the perfused segments. Values were quench corrected.
[c] Mean ± SE of three rats per group. The differences are not statistically significant.

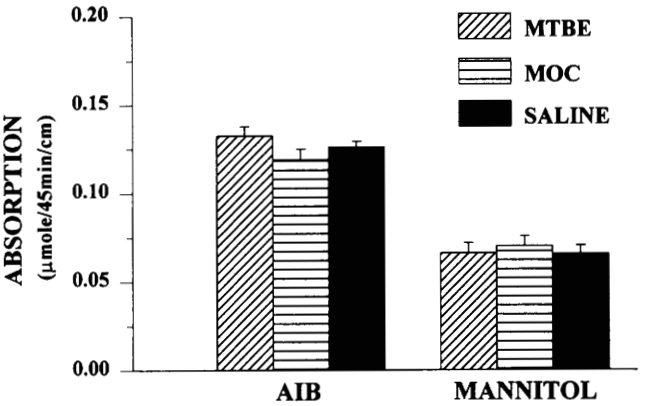

Figure 1. Absorption of AIB and mannitol in virgin jejunal segments that were adjacent to other test-segments that were exposed for 45 min to either MTBE, MOC, or normal saline as control. Values are expressed as mean ± SE of five experiments. The differences are not statistically significant.

This would have resulted in an over estimate of AIB absorption or mannitol permeation, since they are expressed per centimeter of intestinal length. However, when the results were expressed per gram dry weight the findings were consistently similar (Fig. 3).

Dry weight per centimeter of the perfused segment was compared in the three segments tested and is shown in Figure 2C. The dry weight per unit length decreased by 10% after exposure to MOC and by 22% after exposure to MTBE. Most of the weight loss occurred, again, after 1 min of solvent exposure. This indicates loss of tissue mass (i.e., cells or cell constituents). In addition, by calculating the wet/dry weight ratio of the segments at each time interval, the possibility of tissue dehydration due to treatment with these solvents was examined. The ratio for any of the solvents was not significantly different from that of control (Table II) indicating that the solvents caused no tissue dehydration.

Discussion

This report outlines a useful method and algorithm that can be followed in evaluating the relative toxic effects of potential gallstone solvents. The methods used are not intended to closely mimic the clinical condition *in vivo* or to establish the clinical effects of the solvents, but merely to compare the biological effects of such solvents with each other and with a saline control. Because of this, the solvents were compared in their undiluted form.

The results of this study show that a brief exposure of the rat jejunum to MTBE or MOC causes reduction in active transport (AIB absorption), increased passive permeability (mannitol permeation), and loss of mucosal weight. The changes were more pronounced with MTBE than after exposure to mono-octanoin. It is unclear at present how these changes that occur in the rat's intestine compare with what

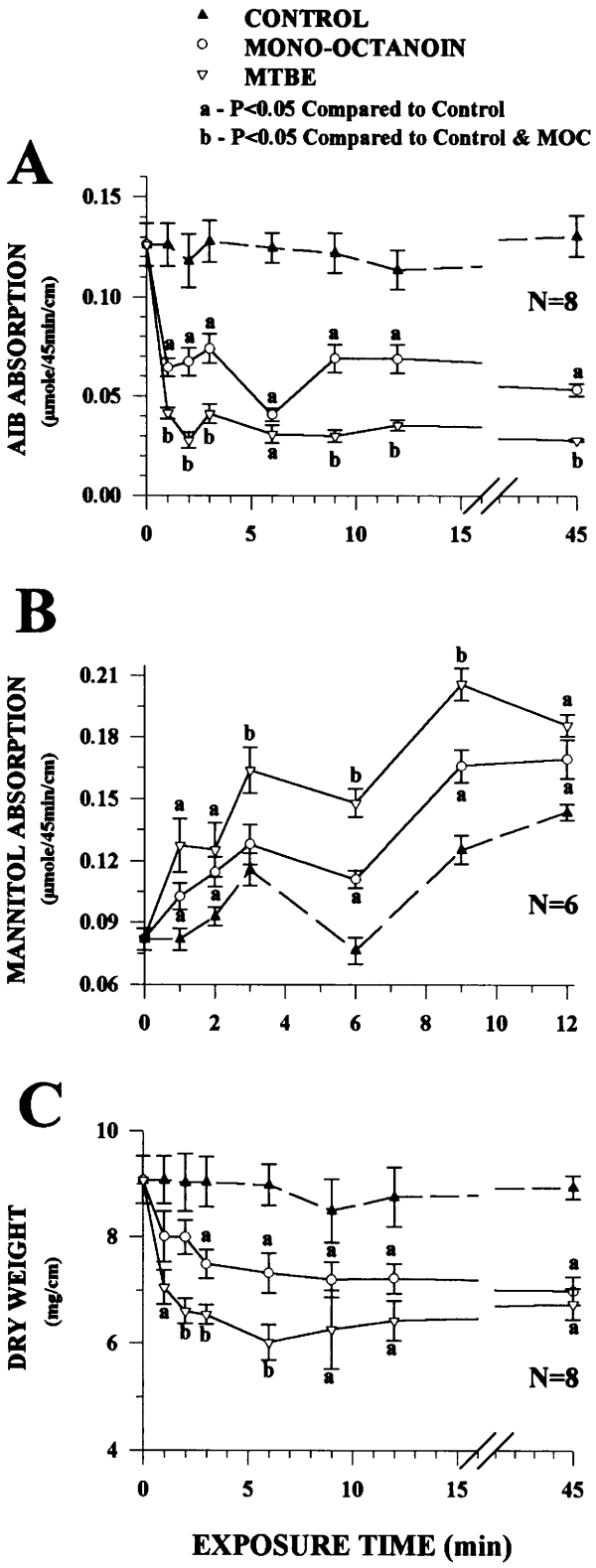

Figure 2. (A) AIB absorption, (B) permeation to mannitol, and (C) intestinal dry weight after increasing periods of exposure to MTBE, mono-octanoin, and physiological saline. The results are expressed in µmole/cm of intestinal length ± SE.

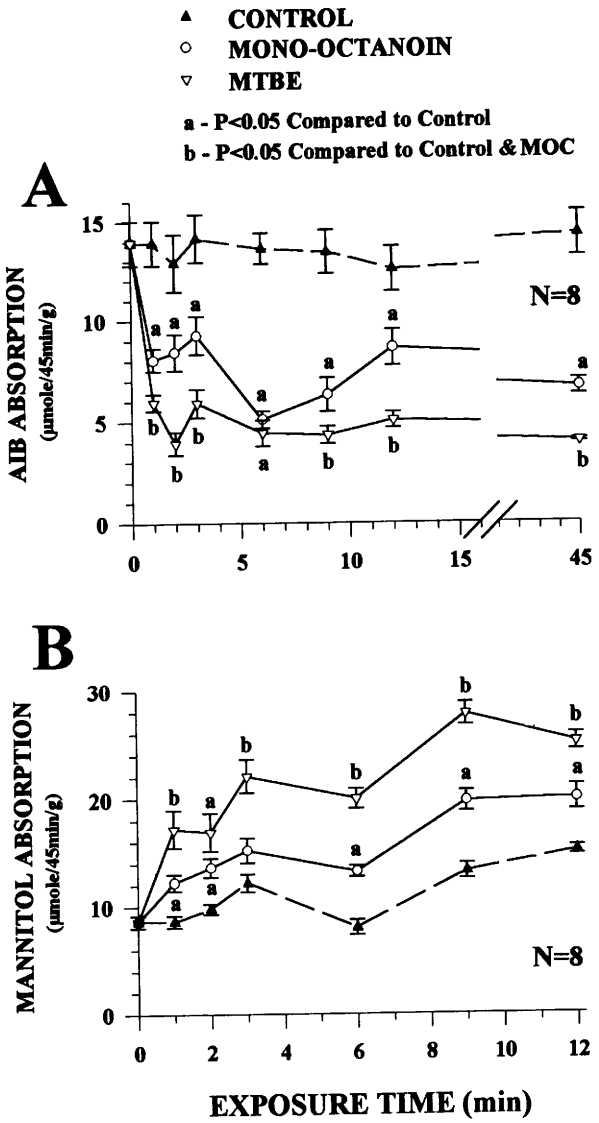

Figure 3. (A) AIB absorption and (B) permeation to mannitol after increasing periods of exposure to MTBE, mono-octanoin, and physiological saline. The results are expressed in µmole/g of dry weight ± SE.

may occur in humans during gallstone dissolution. The observed changes are nevertheless useful when comparing the effects of the two most commonly used solvents.

Changes in intestinal morphology in the form of erythema, punctate hemorrhages, and erosions have been observed endoscopically after inadvertent overflow of MTBE into the duodenum during the course of its percutaneous instillation into the gallbladder for contact dissolution of gallstones. These changes were reversible, since endoscopy several days later has shown complete disappearance of the abnormal findings (3). Further work is needed to determine whether changes in mucosal function, such as those observed in our study, are readily reversible as well. It is conceivable that a state of deranged intestinal

Table II. The Wet/Dry Weight Ratio of the Intestinal Segments After Exposure to MTBE, MOC, and Control

Solvent	Exposure time (min)						
	1	2	3	6	9	12	45
MTBE	3.65 ± 0.16	3.63 ± 0.16	3.59 ± 0.17	3.51 ± 0.24	3.54 ± 0.17	3.55 ± 0.19	3.53 ± 0.20
Mono-octanoin	3.63 ± 0.14	3.61 ± 0.14	3.78 ± 0.15	3.65 ± 0.17	3.63 ± 0.17	3.68 ± 0.15	3.60 ± 0.16
Saline (control)	3.74 ± 0.06	3.74 ± 0.06	3.74 ± 0.12	3.83 ± 0.08	3.59 ± 0.18	3.74 ± 0.15	3.63 ± 0.13

Note. Values are expressed as mean ± SE. The differences are not statistically significant.

absorptive function may persist for long enough to cause some degree of weight loss or nutrient deficiencies. Such clinical effects have not been described after treatment with mono-octanoin although it has been in use for many years. However, they may not have been looked for in a systemic fashion and they may be more pronounced, as our study indicates, after MTBE therapy.

To our knowledge, it is not known whether the absorptive functions of the gallbladder mucosa are affected in the same fashion after exposure to solvents such as MTBE or mono-octanoin. If the gallbladder mucosa was affected in this fashion, it may significantly contribute to early gallstone reformation after successful dissolution, because the gallbladder mucosa plays an important role in modifying bile while it is stored in the gallbladder. In bile, there is a fine balance between cholesterol, the phospholipid lecithin, and bile salts which is instrumental in solubilizing cholesterol in the aqueous medium of bile (9). If the proportion of the two cholesterol solubilizing agents lecithin and bile salts fall below a critical level cholesterol will precipitate from bile (10). One could speculate that if the gallbladder mucosa becomes leaky, bile acids could diffuse back into the circulation (following their concentration gradients) rendering bile to be supersaturated with cholesterol and hence lithogenic. This hypothesis may explain the incidence of early recurrence noted after percutaneous contact dissolution with MTBE both by Thistle *et al.* (3) and by ourselves (11). Although it is believed that early recurrence is usually due to insoluble debris left behind after dissolution of the cholesterol moiety (3, 11), the possibility of a mucosal functional defect should not be disregarded. For obvious reasons, the hypothesis deserves further study and testing.

In any event, the intestinal mucosa of the rat appears susceptible to solvent damage, suggesting that escape of solvent into the small intestine in patients undergoing contact dissolution of gallstones may be hazardous. Until a solvent safer than mono-octanoin or MTBE is developed, utmost care should be exercised and all possible means be employed to prevent any solvent overflow from the gallbladder into the intestine during the procedure of percutaneous contact dissolution of gallstones.

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