

## Inhibition of Peritonitis by Amide Local Anesthetics

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Peritonitis was induced in rats by exposing the peritoneal surface to 0.1 M hydrochloric acid (HCl). Peritonitis was quantified by extraction of Evans blue-bound albumin from the tissue exposed to HCl and analyzed by a spectrophotometric technique. In the first set of experiments, one group of rats had the peritoneal surface exposed to HCl following local pretreatment with isotonic saline; a second group of rats had the peritoneum exposed to HCl after topical pretreatment with an equal volume of lidocaine 1%, whereas in a third group the peritoneal surface was exposed only to saline without HCl. The experimental design in the second set of experiments was similar to that of the first set except that bupivacaine 0.5% was used instead of lidocaine in the second group. Results show a significant inhibition of peritonitis in the groups pretreated with lidocaine ( $P < 0.01$ ) and bupivacaine ( $P < 0.05$ ) compared with rats in the saline pretreated group. In the lidocaine-pretreated group Evans blue-albumin extravasation did not differ significantly from the rats not receiving HCl, whereas the bupivacaine-pretreated group showed a slightly but significantly ( $P < 0.05$ ) more pronounced peritonitis than control rats not exposed to HCl. In the third set of experiments the peritoneum was topically treated with either lidocaine 1%, bupivacaine 0.5%, or isotonic saline after first having exposed the peritoneal surface to HCl. A significant inhibition of albumin extravasation was seen following lidocaine ( $P < 0.001$ ) or bupivacaine ( $P < 0.01$ ) treatment compared with treatment with isotonic saline. The penetration of local anesthetics into the tissue was visualized by autoradiography and, like the inflammatory reaction, was limited to the outer surface of the intestinal wall. Amide local anesthetics can prevent as well as modify the development of chemical peritonitis. (Key words: Anesthetics, local: bupivacaine; lidocaine. Measurement techniques: autoradiography, spectrophotometry. Microscopy, fluorescence. Peritonitis.)

INDEPENDENT of the primary cause, chemical peritonitis is characterized by an inflammatory reaction in the peritoneum leading to extravasation of fluid, electrolytes, and albumin into the abdominal cavity,<sup>1</sup> impaired intestinal fluid absorption,<sup>2</sup> and ileus<sup>3</sup> and intraperitoneal adhesions.<sup>4</sup> Irrigation of the peritoneal cavity using isotonic solutions is currently the most established treatment of peritonitis. The addition of anti-inflammatory agents to

these solutions may further potentiate their beneficial effects on chemical peritonitis.

Because amide local anesthetics are known to inhibit several of the steps involved in the pathophysiology of inflammation,<sup>5-9</sup> we evaluated the effects of the cationic amide anesthetics lidocaine and bupivacaine on peritonitis in the rat induced by hydrochloric acid and measured spectrophotometrically by quantification of extravasated Evans blue-albumin.

### Materials and Methods

Experiments were performed on Sprague-Dawley rats weighing 250-300 g. The experimental protocol was approved by the Animal Use Committee. Anesthesia was induced with pentobarbital (50 mg/kg) intraperitoneally and maintained by continuous iv infusion of chloralose ( $1.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). A tracheostomy was performed. Blood pressure was monitored using a pressure transducer (Statham P 23 AC) connected to a cannula into a femoral artery. Intravenous drugs were administered into a femoral vein.

Three sets of experiments were performed in which the abdomen was opened by a midline incision and the peritoneum covering the colon was visualized. In the first set of experiments three groups of rats were included. In one group of rats peritonitis was elicited by placing a piece of gauze ( $1 \times 1 \text{ cm}$ ) containing 0.5 ml of 0.1 M hydrochloric acid (HCl) on the peritoneal surface for five minutes after first having exposed the peritoneum to isotonic saline ( $pH 7.0$ ;  $n = 8$ ) (0.5 ml on gauze  $1.5 \times 1.5 \text{ cm}$ ) for five minutes. In a second group the peritoneum was pretreated for five minutes with lidocaine hydrochloride 1% ( $pH 6.9$ ;  $n = 8$ ) (0.5 ml on gauze  $1.5 \times 1.5 \text{ cm}$ ) before exposing the peritoneal surface to HCl for five minutes. In a third group ( $n = 8$ ) the peritoneum was exposed to isotonic saline ( $pH 7.0$ ) for five minutes but was otherwise not exposed to any additional chemicals or drugs.

The second set of experiments included three groups of rats. In one group of rats the peritoneal surface was pretreated for five minutes with isotonic saline (0.5 ml on gauze  $1.5 \times 1.5 \text{ cm}$ ) ( $pH 7.0$ ;  $n = 8$ ) followed by topical application of 0.1 M HCl (0.5 ml on gauze  $1 \times 1 \text{ cm}$ ) for five minutes. In a second group the peritoneum was pretreated in a similar way for five minutes with bupivacaine hydrochloride 0.5% ( $pH 7.1$ ;  $n = 8$ ) before exposing the peritoneal surface to HCl for five minutes. A third group was only exposed locally on the peritoneum to isotonic saline ( $pH 7.0$ ;  $n = 8$ ) for five minutes.

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FIG. 1. Evans blue-albumin extravasation from the colonic peritoneum exposed to 0.1 M HCl on the left and right side. The left side was locally pretreated with bupivacaine 0.5%, whereas the right side was pretreated with saline. Prior to taking the photograph the vascular bed was rinsed with 37° C isotonic saline through the superior mesenteric artery.

Fifteen minutes after these procedures Evans blue (20 mg/kg) was dissolved in saline and injected into a femoral vein. Five minutes later the superior mesenteric artery was cannulated, the superior mesenteric vein was cut, and the colonic vascular bed rinsed with 20 ml of 37° C saline to eliminate nontissue bound Evans blue. The experimental area was dissected, dried on filter paper to remove excess fluid, and immediately weighed. The samples were then placed in 4 ml formamide (HCONH<sub>2</sub>) and incubated for 24 h in a water bath at 50° C as described by Gamse *et al.*<sup>10</sup> Colorimetric measurements were performed in a Stasar (Gilford) spectrophotometer at the absorption peak of 612 nm. Three measurements were performed on each sample and the mean value was used for calculations. Calculations were based on external standards in formamide. All specimen analysis was performed by an assistant blinded to the experimental protocol.

In a third set of experiments the peritoneal surface of all groups was exposed to HCl 0.1 M as described above. After five minutes the peritoneum of one group (n = 7) was treated for 60 min with isotonic saline (0.5 ml; pH

7.0), in a second group (n = 8) with lidocaine 1% (0.5 ml; pH 6.9), and in a third group (n = 8) with bupivacaine 0.5% (0.5 ml; pH 7.1). Following this treatment colonic vasculature was rinsed with saline, the exposed tissue dissected, and albumin extravasation was quantified as described above.

The original pH of the solutions was 5.87 for isotonic saline, 6.01 for bupivacaine, and 6.67 for lidocaine. To avoid differences in pH among the treatment solutions, we titrated all the solutions to pH 7.0 ( $\pm 0.02$ ) and, taking into account the individual pKa-values of lidocaine and bupivacaine (7.9 and 8.1, respectively), the pH of the local anesthetic solutions was titrated to achieve similar degree of dissociation (approximately 10% nonionized agent). One hundred milliliters of each solution was continuously stirred (IKA-combimag Ret) and titrated with 0.5–1.0 mM NaOH by a micropipette (Transferpette, Brand). The pH of the solutions was continuously monitored by a pH-meter (Metrohm 1632) and correction of pH was achieved by 0.1–0.5 mM HCl until reaching predetermined pH level. The osmolarity of the solutions was controlled be-

fore and after titration by an osmometer (Advanced osmometer model 3W, Advanced Instruments), and dilution was found to be negligible (<0.5%).

The degree and localization of Evans blue-albumin extravasation in control tissue and following application to the peritoneum with HCl was visualized by fluorescence microscopy as described previously.<sup>11</sup> Following injection of Evans blue, irrigation of colonic vasculature, and dissection of the experimental colonic area, the preparation was fixed in 4% buffered formaline for 24 h. Following this procedure the segments were frozen in liquid nitrogen, sectioned into 10 μm slices in a cryostat, mounted on glass slides, and dried by heating to 30° C for one minute. The sections were examined in a fluorescence microscope (Leitz dialux) using epi-illumination technique (Ploek pam system) consisting of a UV-lamp, an excitation filter (450–490 nm), a beam-splitting mirror (510 nm), and a suppression filter (515 nm).

In a separate group of animals (n = 5) the peritoneum was exposed to <sup>3</sup>H-bupivacaine 0.5% (470 mCi/mmol; 0.5 ml; pH 7.0) for five minutes. The exposed segment of the colon was extirpated and immediately frozen in isopentane cooled by liquid nitrogen to -150° C. The preparates were cut into 20 μm thick sections in a cryostat, transferred to a glass slide, and heated to 70° C during 30 s. A dental x-ray film (Kodak DF-57) was placed over the sections and exposed at room temperature for varying periods of time (4–8 days). The localization of <sup>3</sup>H-bupivacaine in the tissue was indicated by the density of silver grains on the radiographs and compared with the corresponding histologic section.

Statistical analysis was performed using the Wilcoxon rank-sum test. Differences resulting in P values less than 0.05 were considered statistically significant. Data are expressed as mean ± SEM.

### Results

Exposure of the peritoneum to HCl induced a pronounced inflammatory reaction with extravasation of albumin (figs. 1 and 2). In the first set of experiments pretreatment of the peritoneum with lidocaine 1% significantly reduced ( $P < 0.01$ ) albumin extravasation following administration of HCl compared to saline pretreatment (fig. 2). No significant difference ( $P > 0.05$ ) was found between the lidocaine-pretreated group and the group exposed to isotonic saline only (fig. 2). In the second set of experiments pretreatment of the peritoneal surface with bupivacaine 0.5% significantly reduced peritonitis in response to HCl as compared to saline pretreatment ( $P < 0.05$ ) (figs. 1 and 2). Peritoneal inflammation in the bupivacaine-pretreated group was slightly but significantly ( $P < 0.05$ ) more pronounced than that in the saline-pretreated control group not exposed to HCl (fig. 2). Com-

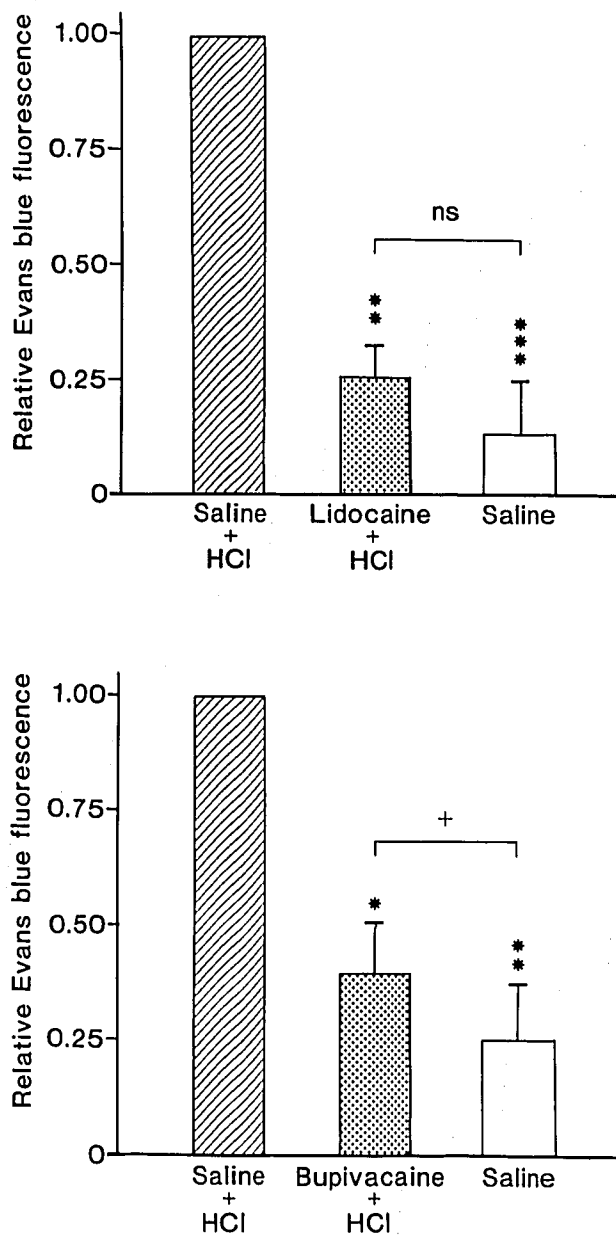


FIG. 2. Relative extravasation of Evans blue-albumin in the peritoneum topically pretreated with isotonic saline and subsequently exposed to 0.1 M HCl (shaded bar) and in HCl-exposed peritoneum locally pretreated with lidocaine 1% (dotted bar; upper panel) or bupivacaine 0.5% (dotted bar; lower panel). Open bars indicate albumin extravasation in peritoneum exposed to saline only. Tissue concentration of Evans blue was quantified by spectrophotometric technique. [ns = nonsignificant; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs. saline-pretreated HCl-exposed peritoneum (shaded bars). + $P < 0.05$  bupivacaine vs. control group only exposed to saline.] Data are given as mean ± SEM.

parison of albumin extravasation in lidocaine-pretreated peritoneum exposed to HCl (first set) with bupivacaine pretreatment (second set) revealed a significantly more pronounced inhibition of peritonitis by lidocaine ( $P$

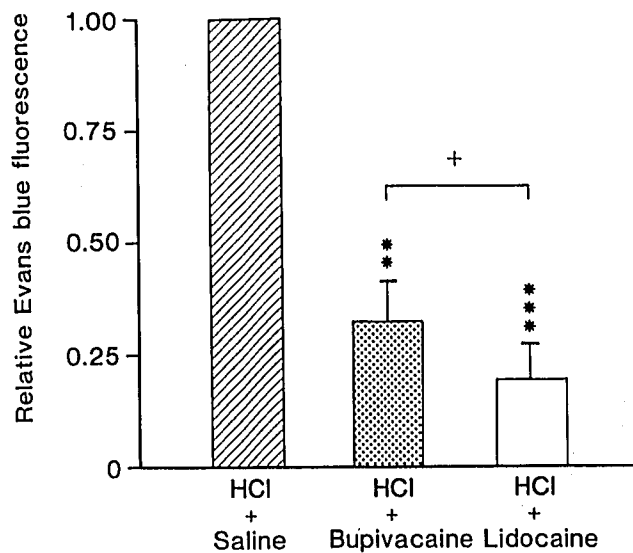


FIG. 3. Relative Evans blue-albumin extravasation in manifest peritonitis following exposure to HCl followed by topical treatment of the peritoneum for 60 min with saline (shaded bar), bupivacaine 0.5% (dotted bar), or lidocaine 1% (open bar). [\*\*\* $P < 0.01$ , \*\*\*\* $P < 0.001$  vs. HCl-exposed peritoneum treated with saline (shaded bar). \* $P < 0.05$  lidocaine vs. bupivacaine.] Data are mean  $\pm$  SEM.

$< 0.05$ ). No significant differences regarding albumin extravasation were found between the first and second set of experiments as for the saline-pretreated HCl-exposed groups (shaded bars) ( $P > 0.05$ ) or between the control groups exposed only to saline (open bars) ( $P > 0.05$ ; fig. 2).

In animals with manifest peritonitis following exposure to HCl (third experimental set), a significant inhibition of Evans blue-albumin extravasation was seen following treatment of the inflammatory area during 60 min with bupivacaine 0.5% ( $P < 0.01$ ) or lidocaine 1% ( $P < 0.001$ ) compared to treatment with saline (fig. 3). The anti-inflammatory effect of lidocaine was significantly more pronounced than that of bupivacaine ( $P < 0.05$ ) (fig. 3). Autoradiographic examination of tissue penetration of  $^3\text{H}$ -bupivacaine 0.5% showed an accumulation of the drug in the external layers of the colonic wall (fig. 4). Under the fluorescence microscope a bright red fluorescence was seen where albumin-bound Evans blue had extravasated. The red fluorescence was patchy in appearance and was mainly confined to the peritoneal tissue and the external muscle layer, whereas the mucosa and submucosa were nonfluorescent. In control tissue not exposed to HCl, a weak Evans blue fluorescence was seen only in the walls of small blood vessels.

### Discussion

Damage to the peritoneum is followed by an inflammatory response with stimulation of granulocyte delivery

to the inflammatory site<sup>7,12,13</sup> and release of inflammatory agents such as histamine, serotonin, kinins, and prostaglandins<sup>14-16</sup> and of lysosomal enzymes<sup>9</sup> into the damaged tissue. These agents in turn cause hyperemia, increased vascular permeability,<sup>17,18</sup> and extravasation of plasma components,<sup>1</sup> leading to a deranged protein and fluid balance. Another complication of peritonitis is the formation of intraperitoneal adhesions,<sup>19,20</sup> which may result in life-threatening intestinal obstruction.<sup>21</sup> Administration of steroids intraperitoneally have been shown previously to reduce the inflammatory reaction in the peritoneum and the subsequent formation of peritoneal adhesions.<sup>22-24</sup> The use of steroids is however limited due to systemic side effects.

The results of the present study show that cationic local anesthetics can effectively reduce or modify the development of chemical peritonitis. To exclude that the anti-inflammatory effects of lidocaine and bupivacaine were simply due to dilution or titration of tissue pH to a value that is less irritating, control experiments were performed using isotonic saline having the same volume and pH as the anesthetic solutions. Our results suggest that the inhibition of peritonitis by amide anesthetics is due to properties of the anesthetics *per se* rather than due to dilution or titration of pH.

Chemical peritonitis in humans may be caused by various conditions such as intraabdominal bleeding, abdominal surgery, leakage of gastric or intestinal contents, or acute pancreatitis. In our study HCl was used to induce peritonitis because this experimental model proved easy to grade and reproduce. There are, however, no data to indicate that the pathophysiology of peritonitis varies for different etiologic factors, suggesting that our results may also apply to peritonitis caused by other substances.

Interestingly, the inhibition of peritonitis was consistently more pronounced with lidocaine than with bupivacaine. This is surprising in view of previous results showing a significantly greater inhibition of leukocyte activation by bupivacaine.<sup>25</sup> The differences between lidocaine and bupivacaine in the present investigation cannot be explained by different degrees of dissociation in the anesthetic solutions because, taking into consideration the pKa values of the agents, pH was titrated to give similar concentrations of nonionized agent in both solutions. A possible explanation could be different effects for the two anesthetic agents on inflammation, *i.e.*, lidocaine has more potent effects on changes in vascular permeability, whereas bupivacaine has greater inhibitory effects on leukocyte activation.

Our results are in agreement with previous results by McGregor *et al.*<sup>26</sup> who showed that iv infusion of lidocaine almost completely abolished the delivery of polymorphonuclear granulocytes (PMN) to the inflammatory site in aseptic peritonitis in the rabbit and that this effect was

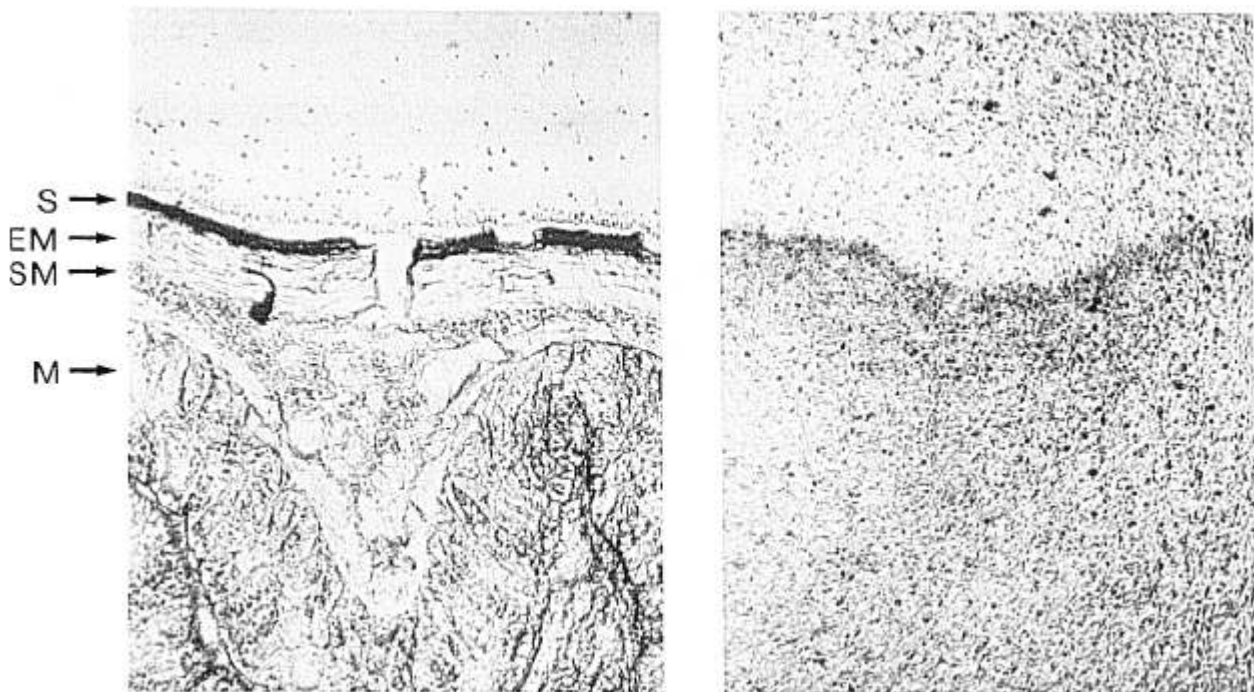


FIG. 4. Autoradiograph of the colon exposed to  $^3\text{H}$ -bupivacaine 0.5% during five minutes on the peritoneal surface (right panel). The corresponding histologic section is illustrated in the left panel. Exposure time seven days. S = serosa; EM = external muscle; SM = submucosa; M = mucosa. Penetration of bupivacaine is mainly confined to the external layers of the colonic wall.

more than tenfold greater than the effect of methylprednisolone. The mechanisms underlying this potent anti-inflammatory effect of amide local anesthetics could be several: antagonism of the action of prostaglandins in the inflammatory area,<sup>27</sup> inhibition of leukocyte migration and metabolism,<sup>28</sup> and inhibition of lysosomal enzyme release from PMN.<sup>9</sup>

Surprisingly, treatment of the peritoneum with local anesthetics resulted in a reduction of the amount of extravasated Evans blue-albumin from the area with manifest inflammation. These results suggest either a stimulated reuptake of albumin by direct effects on membrane properties<sup>29</sup> in capillary and lymphatic vessels or by inhibition of continued extravasation.

Examination of the histologic sections revealed an inflammatory reaction confined to the external layers of the gut wall and to the peritoneal surface. The penetration into the tissue of  $^3\text{H}$ -bupivacaine shown in the present study and of  $^{14}\text{C}$ -lidocaine shown in a previous study<sup>30</sup> suggest that the drugs cover well the area of inflammation and is in line with their potent inhibitory actions on albumin extravasation along the peritoneum.

In conclusion, the present results suggest that addition of cationic local anesthetics to the solutions used in the treatment of peritonitis, *e.g.*, following acute pancreatitis, may enhance their beneficial effects on the inflammatory reaction in the peritoneum.

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