Paracetamol Does Not Compromise Early Wound Repair in the Intestine or Abdominal Wall in the Rat

Rozemarijn J. van der Vijver, MD, Cees J. H. M. van Laarhoven, MD, PhD, Roger M. L. M. Lomme, and Thijs Hendriks, PhD

BACKGROUND: Paracetamol is a cornerstone for perioperative pain relief. Its mechanism of action may include a local antiinflammatory effect with inhibition of cyclooxygenase isoenzymes. The scarce literature available on its effects on wound healing consists of preclinical studies into the effect of paracetamol on healing of the musculoskeletal system. Although the drug is used abundantly for pain relief after surgery of the gastrointestinal tract, there are no published data on the influence of paracetamol on anastomotic and abdominal healing. This also holds for the crucial, early inflammatory phase of repair. The recovery of wound strength could therefore conceivably be affected by paracetamol.

METHODS: In 78 male Wistar rats, we constructed an anastomosis in colon and ileum. The rats received either low- or high-dose (50 or 200 mg/kg/d, divided over 2 doses) paracetamol or vehicle (controls) until they were killed on day 3 or 7 after surgery (n = 13 each). In anastomoses, the main outcome variables were 2 independent measures for wound strength, bursting pressure, and breaking strength, the latter being the primary outcome variable. In addition, collagen levels were measured and histology was performed. In fascia, breaking strength was analyzed.

RESULTS: No significant differences were found between control and paracetamol-treated groups at any time point for any of the variables. Wound strength increased significantly from day 3 to day 7 in all groups. In the colon anastomosis, the breaking strength increased from 130 ± 9 g (mean ± SEM) at day 3 to 232 ± 17 g at day 7 in the control group, from 144 ± 10 to 224 ± 9 g in the low-dose group, and from 130 ± 12 to 263 ± 29 g in the high-dose group. The lower limit for the 95% confidence interval was −11 for the difference between control and low-dose groups at day 3 and −25 for the difference between control and high-dose groups. No differences in collagen levels were found between the high-dose and control groups. Histology did not indicate the presence of gross differences between groups.

CONCLUSIONS: Perioperative use of paracetamol in a rat model of intestinal surgery does not significantly impede wound repair in the early postoperative period. (Anesth Analg 2012; X:●●●●●)

Optimal management of postoperative pain remains a topic of ongoing research because it directly relates to postoperative patient well-being and clinical outcome.1,2 Paracetamol, acetaminophen, is considered an effective analgesic and antipyretic drug that is a cornerstone in perioperative pain relief, and in patients who have intestinal surgery. Paracetamol decreases morphine consumption and therefore morphine-related adverse effects.3 With the exception of a few studies on the musculoskeletal system, surprisingly little has been reported on the potential effects of paracetamol on wound healing.

It is not quite clear which analgesic pathway is affected by the administration of this non-opioid drug, but multisite activity in the central nervous system has been suggested. Potential mechanisms include interaction with both the serotonergic and cannabinoid pathways. However, an antiinflammatory effect has also been reported through inhibition of cyclooxygenase (COX) isoenzymes.4–7 The inflammatory reaction constitutes the first phase of wound healing and is an essential link within the wound repair cascade. Inhibitors of the COX enzymes might interfere with inflammation and wound healing. Indeed, negative effects of nonsteroidal antiinflammatory drugs (COX inhibitors) on anastomotic healing have been described.8–12

Although the drug is used abundantly for pain relief after surgery of the gastrointestinal tract, there are no published data on the influence of paracetamol on anastomotic and abdominal healing. This also holds for the crucial, early inflammatory phase of repair. If paracetamol affects inflammation, it conceivably could interfere with the recovery of wound strength. Therefore, we examined the effects of paracetamol on early healing in ileum and colon anastomoses and the abdominal wall of the rat.

METHODS

Study Design

Seventy-eight male Wistar rats (Harlan BV, Horst, The Netherlands) were housed 2 per cage and accustomed to laboratory conditions for 5 days before the start of the experiment. They were randomly divided over 3 groups of 26 animals each, 1 control group and 2 experimental groups. At day 0, all rats underwent intestinal resection, and anastomoses were constructed in both colon and ileum. The 2 experimental groups received different doses (low
and high) of paracetamol daily from operation onward. Rats were observed closely, weighed daily, and had free access to water and standard rodent chow (Hope Farms, Woerden, The Netherlands) throughout the entire experimental period. Half of the rats within each group were killed at day 3 and day 7 after operation (Table 1). The Animal Ethics Review Committee of the Radboud University Nijmegen approved the study. The study was conducted in a manner that does not inflict unnecessary pain or discomfort upon the animal, as outlined by the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals (1996), prepared by the National Academy of Sciences’ Institute for Laboratory Animal Research.

**Surgery and Analgesics**

Procedures were performed under semisterile conditions using a Zeiss operation microscope (Carl Zeiss AG, Oberkochen, Germany). Animals were anesthetized by use of a mixture of isoflurane, oxygen, and nitrogen, while breathing spontaneously through a mask. A midline laparotomy was performed and in each rat a 1-cm segment was resected from the descending colon 3 cm proximal to the peritoneal reflection. Colonic continuity was restored by constructing an end-to-end anastomosis with 8 single-layer, inverting, interrupted sutures (Ethilon 8-0; Ethicon, Norderstedt, Germany). A similar procedure was performed in the distal ileum, 15 cm proximal to the cecum. The abdominal wall was closed with a running suture (Vicryl 3-0; Ethicon). The skin was closed with staples. During operations, body temperature was kept at 38°C using a heating pad and a lamp.

Intestines were covered with gauze pads soaked with 0.9% NaCl to minimize desiccation. To prevent dehydration, 10 mL of 0.9% NaCl was administered subcutaneously after the operation.

All rats were administered buprenorphine (Temgesic; Schering-Plough, Houten, The Netherlands), 0.02 mg/kg subcutaneously, every 12 hours (5 times in total) for 48 hours, the first dose just before surgery. The first experimental group received acetaminophen (Paracetamol; Sigma-Aldrich Chemistry, Steinheim, Germany) in a dose of 50 mg/kg/d (low dose) by oral gavage. The daily dose was divided into 2 parts, which were given at least 8 hours apart during the experimental period and starting just before surgery. The second experimental group (high dose) was given 200 mg/kg paracetamol per day. Paracetamol was dissolved in 0.5 mL 0.9% NaCl and 0.1% polysorbate 20 (TWEEN 20; Fluka Chemika, Buchs, Switzerland); the control group received 0.5 mL of this solution (vehicle) per gavage twice a day.

**Necropsy and Analysis of Wound Strength**

Ten rats from each group were killed by CO/CO2 asphyxiation on postoperative day 3 (Table 1). At day 7, another 10 rats were killed for wound strength analysis by cardiac puncture and cervical dislocation, to allow blood sampling (see below). Adhesions were dissected carefully without manipulation of the anastomosis. Segments of 2 cm length, containing the anastomoses in the middle, were resected with sutures left in place. The segments were placed over a plastic tube and secured with a vessel loop on one end and closed with a clamp on the other side. To measure bursting pressure, the segments were infused (2 mL/min) with 0.9% NaCl containing methylene blue. The maximum pressure (mm Hg) recorded immediately before sudden loss of pressure was taken as the bursting pressure. The site of rupture (within or outside the anastomotic line) was noted. Subsequently, the same segments were placed in a tensiometer, and the breaking strength (g) was measured.

A rectangle (approximately 1 × 2 cm) containing the laparotomy wound in the middle was carefully cut out from the abdominal wall. After removing the running suture, the segment was cut in half to create 2 samples with the midline laparotomy wound in the middle. The length of the laparotomy wound in each sample was noted before it was placed in the tensiometer. Subsequently, the breaking strength was measured and expressed in grams/millimeter tissue by using the mean value obtained from both measurements.

The anastomotic segments and abdominal wall were carefully cleaned from any adhering tissue and 5-mm samples, containing the suture line in the middle, were frozen in liquid nitrogen and stored at −80°C until further processing.

**Biochemical Analysis**

After weighing, tissue samples were frozen, lyophilized, and pulverized. The hydroxyproline content, as a measure of the collagen content, was measured in the control and high-dose groups by high-performance liquid chromatography after hydrolysis with 6 N hydrochloric acid and coupling to dabsyl chloride. The detection limit of the assay is 0.25 μg, and intraassay and interassay coefficients of variation (at 6-μg levels) are 2.3% and 10.3%, respectively.

In animals killed at day 7, blood was collected 1 hour after the last dose of paracetamol (or vehicle) and immediately before killing the animals. Paracetamol levels were measured in heparin plasma by the Cobas Integra® (Roche, Lima, Peru) system. Detection limit is 0.2 mg/L.

**Histology**

The remaining 14 animals (Table 1) were killed as described above and used for descriptive histology. Intestinal

---

<table>
<thead>
<tr>
<th>Table 1. Experimental Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operated at day 0</td>
</tr>
<tr>
<td>Premature death (day)</td>
</tr>
<tr>
<td>Terminated at day 3 for biomechanical analysis</td>
</tr>
<tr>
<td>Terminated at day 3 for histological analysis</td>
</tr>
<tr>
<td>Terminated at day 7 for biomechanical analysis</td>
</tr>
<tr>
<td>Terminated at day 7 for histological analysis</td>
</tr>
<tr>
<td>Terminated at day 7 for histological analysis</td>
</tr>
</tbody>
</table>

Number of animals operated on day 0 and premature deaths. At days 3 and 7, 10 animals in each group were analyzed for wound strength and the remaining animals for histology.
samples of approximately 1 cm containing the entire anastomosis in the middle were carefully collected en bloc, opened at the mesenteric side, washed gently in 0.9% NaCl solution, and spread out in a cassette for paraffin embedding. From paraffin-embedded tissues, 4-μm sections were prepared and stained with hematoxylin and eosin. Sections were analyzed using a binocular light microscope.

**Statistics**

Historical data from our own group show the breaking strength of colonic anastomoses at day 3 typically to be 117 ± 27 (SD) g. Sample size was determined as sufficient to detect a loss of wound strength of 30 g (25%). Using an α of 0.05, a power of 0.8, and a 1-tail test, the group size was calculated (G*Power 3.1.2) to be 10 (actual power 0.84).

All data passed the normality test (Kolmogorov-Smirnov, P > 0.10). Comparison between control and both experimental groups was performed using a 1-way analysis of variance (ANOVA). Comparisons between 2 groups (hydroxyproline content) or, within 1 group, between values at 2 different days were performed with a 1-tailed unpaired t test. The lower limit of the (1-sided) 95% confidence interval (CI) for the difference between each of the 2 paracetamol groups and the control group was determined using a t test, with the Fisher least significant difference procedure (see Table 1 in Haworth et al.19). Results were considered statistically significant at P < 0.05.

**RESULTS**

**General Observations and Paracetamol Plasma Concentrations**

Four animals died prematurely. One animal from the low-dose group did not survive the surgery. Two animals (1 from the control group and 1 from the high-dose group) were removed from the experiment because of extreme weight loss and poor clinical condition. Both showed signs of ileus at dissection, but the anastomoses were all intact and conductant. One animal from the low-dose group died from self-mutilation on day 2 (Table 1).

At operation, the rats weighed between 250 and 295 g (average 286 g) without differences between groups. All animals experienced transient postoperative weight loss, which was maximal at day 3 to 4 and averaged approximately 9% in all groups. At day 7, the mean (± SEM) relative body weight (compared with the preoperative weight) was 98% ± 1.2%, 95% ± 2.1%, and 97% ± 0.9% in controls, low-dose group, and high-dose group, respectively. There were no signs of wound dehiscence of the laparotomy wound. No macroscopic signs of anastomotic leakage were found. Paracetamol levels were determined in plasma collected on day 7 one hour after drug administration. Average values (n = 4, ± SEM) were 0 ± 0 mg/L, 0.4 ± 0.4 mg/L, and 22.7 ± 6.5 mg/L in the controls and low- and high-dose groups, respectively.

**Wound Strength**

In the control and both paracetamol groups, the anastomotic bursting pressure remained low after 3 days and increased sharply (P < 0.0001, both in ileum and in colon) thereafter (Fig. 1). At day 7, in all groups, the bursting site had shifted away from the suture line in most animals (Fig. 1). At day 3, only in the ileum did the average values in the paracetamol-treated animals (44 ± 6 mm Hg [mean ± SEM] and 51 ± 7 mm Hg) for the low- and high-dose groups remain below those in the controls (73 ± 11 mm Hg), but this effect was nonsignificant (P = 0.06, ANOVA). While measuring the breaking strength, tearing always occurred within the suture line. The increase in anastomotic breaking strength from day 3 to day 7 was also clear and significant (P < 0.05 in ileum; P < 0.0001 in colon) in all 3 groups (Fig. 2, A and B). On day 3, the strength of the abdominal fascia was only just measurable and varied widely among groups, e.g., 3.2 ± 0.7 g/mm in controls and 1.9 ± 0.5 and 4.8 ± 2.4 g/mm in the low-dose and high-dose groups, respectively. In all groups, it increased many-fold (P < 0.0001) until day 7 when control values averaged 87 ± 10 g/mm (Fig. 2C). Paracetamol treatment did not affect wound breaking strength in any consistent or dose-dependent way: no significant differences (ANOVA) were found between control, low- and high-dose groups on either day. Table 2 shows average values together with the lower limit of the 95% CI for the difference between controls and paracetamol-treated groups, demonstrating that the probability for true loss of strength is quite limited.

**Collagen and Histology**

Hydroxyproline levels were measured in anastomotic samples from the control and high-dose groups. In both groups, the hydroxyproline content, expressed as μg/5 mm, increased significantly (P < 0.05) from day 3 to day 7 (Fig. 3). There was no significant difference between groups on day 3 or day 7, neither in the ileum nor in the colon. The same was true for the wound hydroxyproline concentrations.
Paracetamol and Wound Repair

Figure 2. Wound breaking strength. Individual values and means (horizontal bars) are given for ileal (A) and colonic (B) anastomoses in control (C) and low-dose (L) and high-dose (H) groups. Panel C shows data for abdominal wounds, day 3 left y-axis and day 7 right y-axis.

Table 2. Wound breaking strength

<table>
<thead>
<tr>
<th></th>
<th>Control mean</th>
<th>Low dose mean</th>
<th>95% CI</th>
<th>High dose mean</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>Day 3</td>
<td>130</td>
<td>144</td>
<td>–11</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
<td>232</td>
<td>224</td>
<td>–54</td>
<td>263</td>
</tr>
<tr>
<td>Ileum</td>
<td>Day 3</td>
<td>59</td>
<td>47</td>
<td>–31</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
<td>92</td>
<td>109</td>
<td>–8</td>
<td>98</td>
</tr>
<tr>
<td>Abdomen</td>
<td>Day 3</td>
<td>3.2</td>
<td>1.9</td>
<td>–4.9</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
<td>87</td>
<td>87</td>
<td>–28</td>
<td>91</td>
</tr>
</tbody>
</table>

Data represent mean values in the anastomoses (g) and abdominal wall (g/mm).

* Represents the lower limit of the (1-sided) 95% confidence interval (CI) for the difference between each of the paracetamol groups and the control group (t-test, Fisher LSD procedure).

Figure 3. Hydroxyproline content of anastomotic segments. Bars represent mean and SEM in ileal and colonic segments from the control groups (gray bars) and the groups that received a high dose of paracetamol (white bars) on days 3 and 7. Asterisk denotes significant (P < 0.05) difference between values obtained at day 3 and day 7 within the same group.

DISCUSSION

Paracetamol does not have a detrimental effect on the strength of the healing intestinal anastomosis in the ileum or colon in rats within the first 7 days after operation. Also, the strength of the abdominal fascia is not compromised by paracetamol.

The main outcome variables to assess anastomotic healing in the intestine relate to mechanical strength. Although the bursting pressure is a measure of the capacity to withstand intraluminal pressures, the breaking strength represents longitudinal strength. Both can be measured sequentially on the same segment and are thus essentially independent variables for wound strength. Because the breaking strength always represented the true strength of the suture line (while at day 7 the bursting site was often outside the wound area), it is considered to be the primary outcome variable.

In the first days after operation, wound strength remains low and chances for dehiscence are believed to be highest. From 3 days onward, collagen deposition increases and therefore wound strength increases. Thus, the first postoperative week is most crucial to undisturbed healing and therefore measuring points were set at day 3, when anastomotic strength is lowest, and day 7, when strength should be considerably increased.

Wound breaking strength, as the primary outcome variable, did not change in any consistent or dose-dependent manner by the administration of paracetamol. There were no significant differences among groups and analysis of the 95% CI for differences between control and paracetamol-treated groups (Table 2) demonstrated the probability for considerable, and possibly clinically relevant, loss of strength to be very low. Although at day 3 the expressed as μg/mg dry weight (data not shown). Although the number of animals available for histological analysis was limited, no gross differences between paracetamol-treated rats and control rats were observed in the architecture of either the anastomosis in ileum and colon or the abdominal wound (not shown). Figure 4 illustrates normal healing and closing of the mucosal gap in the ileum between days 3 and 7 after surgery in the control and high-dosed animals.
average bursting pressure in the ileum was below that of the controls in both paracetamol-treated groups, this effect remained nonsignificant. Moreover, the bursting pressure increased sharply thereafter. Altogether, analysis of the variables for wound strength and collagen levels in anastomoses and abdominal wounds did not reveal any differences, even when paracetamol was given at a high dose.

The scarce literature available consists of studies describing the effect of paracetamol on healing of tissues such as alveolar bone,\textsuperscript{16} femur,\textsuperscript{17} ligament,\textsuperscript{18} or the patellar tendon.\textsuperscript{19} Here, paracetamol was given once a day or through the chow, while we divided the daily dose in 2 parts, which were given at least 8 hours apart. More importantly, the studies mentioned above examined repair at least 14 days after operation when the inflammatory phase of healing should long be over. For that reason, we studied variables for repair at day 3, characterized by low wound strength during the inflammatory phase, and at day 7, characterized by increasing strength during the early proliferative phase. Presumably, wounds are most sensitive to disruption as their mechanical strength is lowest.

Although the minimal plasma paracetamol level required for analgesia is believed to be 10 to 20 mg/L,\textsuperscript{30} the concentrations measured in humans 80 minutes after 1 g oral paracetamol were substantially lower and demonstrated to range between 0 and 13 (median 5) mg/L.\textsuperscript{20} The low-dosed rats were administered 50 mg/kg paracetamol a day, which compares on a per-weight basis with the maximum recommended human dose of 4 g. This dosage is based on studies examining the effect of paracetamol on wound healing and the dose-related effect on hyperalgesia in the rat.\textsuperscript{17–19,21} Some studies on other processes in rodents,\textsuperscript{22–25} although fairly dated, report a weak antiinflammatory effect with higher doses of paracetamol. The group receiving 200 mg/kg paracetamol a day was therefore included to ensure that any possible effect on wound healing would be noticed, even if it occurred after administration of supranormal dosages. Plasma concentrations in this group were comparable to those found after 2 g orally given to patients.\textsuperscript{20}

The mechanism behind the suggested antiinflammatory effect of paracetamol remains speculative. Paracetamol has periodically been proposed to inhibit one or more of the cyclooxygenase enzymes COX-1 and COX-2.\textsuperscript{2,22,26} COX enzymes catalyze conversion of arachidonic acid to prostanooids involved in inflammation. However, paracetamol generally lacks the other local effects of nonsteroidal anti-inflammatory drugs on platelet aggregation and gastric mucous production. This could be explained if paracetamol acts only centrally, and not peripherally, as a COX inhibitor.\textsuperscript{27} Perhaps this is why paracetamol does not influence the peripherally located processes of anastomotic and abdominal wall healing.

Anastomotic leakage remains a feared and potentially devastating complication after gastrointestinal surgery. A leaking anastomosis is associated with increased morbidity and considerable mortality.\textsuperscript{28,29} Many clinical studies have sought to identify risk factors for impaired anastomotic healing, but very little attention has been given to the use of analgesic drugs. The finding that anastomotic leakage takes place in the absence of any currently known risk factor illustrates that much remains unknown.\textsuperscript{30}

Because paracetamol is widely prescribed to patients who need construction of an intestinal anastomosis, there should be no doubt as to its safety regarding the development of anastomotic strength. The present report supplies the first preclinical data which demonstrate that the perioperative use of paracetamol does not affect the progression of wound repair in the intestine or the abdominal wall in its most crucial early postoperative period.

\textbf{Figure 4.} Anastomotic histology in the ileum. Each panel shows a tissue segment with the anastomosis in the middle and the mucosal layer at the bottom at a magnification of approximately ×40, representing typical examples obtained in the control group at day 3 (A) and day 7 (B), and in the high-dose group at day 3 (C) and day 7 (D). In each panel, an arrow identifies a typical finding. In A, the mucosal villi of the intestine is pointed out. In B, a suture is marked. In C, the inflammation, shaped as a triangle, is marked. In D, the submucosal muscular layer of the intestine is marked.
DISCLOSURES
Name: Rozemarijn J. van der Vijver, MD.
Contribution: This author helped design the study, conduct the study, analyze the data, and prepare the manuscript.
Name: Cees J. H. M. van Laarhoven, MD, PhD.
Contribution: This author helped design the study and prepare the manuscript.
Name: Roger M. L. M. Lomme.
Contribution: This author helped conduct the study and analyze the data.
Name: Thijs Hendriks, PhD.
Contribution: This author helped design the study, analyze the data, and prepare the manuscript.
This manuscript was handled by: Steven L. Shafer, MD.

REFERENCES
23. Flower RJ, Vane JR. Inhibition of prostaglandin synthetase in brain explains the anti-pyretic activity of paracetamol (4-acetamidophenol). Nature 1972;240:410–1