Am J Physiol Gastrointest Liver Physiol 327: G16–G24, 2024.
First published April 23, 2024; doi:10.1152/ajpgi.00083.2024

american physiological society

AMERICAN JOURNAL OF PHYSIOLOGY
GASTROINTESTINAL AND LIVER PHYSIOLOGY

RESEARCH ARTICLE

Cell and Animal Models of Gastrointestinal Disease

Buprenorphine affects the initiation and severity of interleukin-induced acute pancreatitis in mice

Sarah Jahangir,[1] Biswajit Khatua,[1] Nabil Smichi,[1] Prasad Rajalingamgari,[1] Anoop Narayana Pillai,[1] Megan J. Summers,[1] Bryce McFayden,[1] Sergiy Kostenko,[1] Naomi M. Gades,[2] and Vijay P. Singh[1]

[1]*Department of Medicine, Mayo Clinic Arizona, Scottsdale, Arizona, United States and* [2]*Department of Comparative Medicine, Mayo Clinic Arizona, Scottsdale, Arizona, United States*

Abstract

Acute pancreatitis (AP) is a common disease with no targeted therapy and has varied outcomes ranging from spontaneous resolution to being lethal. Although typically painful, AP can also be painless. Various agents, including opioids, are used for pain control in AP; the risks and benefits of which are often debated. As experimental AP in mice is used to study the efficacy of potential therapies, we studied the effect of a commonly used opioid, buprenorphine, on the initiation and progression of AP. For this, we administered extended-release buprenorphine subcutaneously before inducing the previously established severe AP model that uses interleukins 12 and 18 (IL12,18) in genetically obese (ob/ob) mice and compared this to mice with AP but without the drug. Mice were monitored over 3 days, and parameters of AP induction and progression were compared. Buprenorphine significantly reduced serum amylase, lipase, pancreatic necrosis, and AP-associated fat necrosis, which is ubiquitous in obese mice and humans. Buprenorphine delayed the AP-associated reduction of carotid artery pulse distention and the development of hypothermia, hastened renal injury, and muted the early increase in respiratory rate versus IL12,18 alone. The site of buprenorphine injection appeared erythematous, inflamed, and microscopically showed thinning, loss of epidermal layers that had increased apoptosis. In summary, subcutaneous extended-release buprenorphine interfered with the induction of AP by reducing serum amylase, lipase, pancreatic and fat necrosis, the worsening of AP by delaying hypotension, hypothermia, while hastening renal injury, respiratory depression, and causing cutaneous injury at the site of injection.

NEW & NOTEWORTHY Extended-release buprenorphine interferes with the initiation and progression of acute pancreatitis at multiple levels.

adipose; hypotension; lipase; renal failure; opioid

INTRODUCTION

Acute pancreatitis (AP) affects 270,000 patients/yr in the United States (1). The outcomes of AP can vary from being a mild self-limited illness in most cases to being severe and causing life-threatening multisystem organ failure and sometimes death (2). Abdominal pain during AP can vary from being severe to mild or painless (2–4). There are two main classes of analgesics used in AP, i.e., opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) (5, 6). The impact of pain medications in clinical pancreatitis is controversial, with some studies showing improved control with opiates like buprenorphine compared with NSAIDs (7), and others showing opioids beyond the first day of AP can worsen AP severity (8), even though worse pain may be associated with AP severity.

AP has no targeted therapy and is therefore commonly studied experimentally to evaluate the efficacy of drugs in preventing AP or for their therapeutic role in AP, with a goal to avert organ failure or reduce pancreatic necrosis. Different models induce AP by distinct mechanisms (9). These include giving supraphysiological doses of the cholecystokinin analog caerulein to rodents, or coadministering interleukins 12 and 18 (IL12,18) (10, 11), both of which are increased in human AP. Recent studies show that obese rodents are more likely to develop severe AP, including lung injury, renal injury, and hypotension or shock (12–14). Therefore, obese mice are commonly used for studies that focus on AP severity. Obese animals develop severe AP due to pancreatic injury spreading into the visceral fat surrounding the pancreas and causing visceral fat necrosis (12–14). Such visceral fat necrosis (15) is universal in human AP (14, 16–19) and multiple AP models that study the progression of AP to a severe disease (12–14, 20, 21).

Correspondence: V. P. Singh (singh.vijay@mayo.edu).
Submitted 21 March 2024 / Revised 19 April 2024 / Accepted 19 April 2024

Our understanding on the impact of analgesics on the initiation of AP and worsening of its severity in different models is evolving. For example, it was shown in the commonly used caerulein AP model that intraperitoneal injections were painful, and that this pain was not relieved by buprenorphine or metamizole (22). Interestingly, metamizole did not affect serum amylase, lipase, or pancreatic edema after caerulein-induced acute pancreatitis (23) or the development of pancreatic atrophy in chronic pancreatitis due to caerulein (24). Analgesics may also affect the course and interpretation of AP models. This can range from opioids interfering with the induction of caerulein AP as shown by Ogden et al. (25), and also worsening AP parameters and interfering with bacterial translocation and AP resolution, as shown by Barlass et al. (26). Similarly, NSAIDs, which are cyclooxygenase inhibitors, can inhibit initiation of caerulein-induced AP at various levels (27, 28) and are clinically known to decrease the risk of post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis (PEP) in clinical practice. However, NSAIDs do not affect the severity of AP that ensues among the patients who develop PEP despite NSAID prophylaxis (29).

The relevance of analgesics lies in the humane treatment of experimental animals, which is of crucial importance. The Institutional Animal Care and Use Committee (IACUC) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines state that pain medication should be a standard modality for any procedure that causes more than slight or momentary pain or distress (30, 31). Therefore, noting the complex impact of analgesics on the course of AP, we undertook a study to understand the impact of the common opioid buprenorphine on the initiation and worsening of an established severe AP model using IL12,18 in obese mice.

MATERIALS AND METHODS

Mice

The experimental protocol was approved by Institutional Animal Use Committee (IACUC) of the Mayo Clinic. Animal care and handling were done according to established guidelines (30, 31). The mice used in the experiments were genetically obese B6.Cg-Lepob/J, homozygous ob/ob mice, strain no.:000632, RRID:IMSR_JAX:000632 (commonly referred to as ob/ob), as used in previous studies (12, 14, 33). All mice were housed on a 12-h light/dark cycle at temperatures from 21°C to 25°C, fed standard laboratory chow, and allowed to drink ad libitum. The mice were 12–15 wk of age at the time of study.

AP Model

Experimental AP was induced using IL12 (150 ng/30 g; PeproTech) and IL18 (750 ng/30 g; R&D Systems) by injecting IL12,18. Each agent was given as two doses 24 h apart, intraperitoneally, as previously (10, 11, 14, 33–35). Each dose was freshly prepared with 150 μL of IL12 (2 μg/mL) and 150 μL of IL18 (10 μg/mL) dissolved in 300-μL phosphate-buffered saline (PBS) to a final volume of 600 μL.

Experimental Groups

The study was conducted on two groups of mice with baseline characteristics as described in Table 1. The first group (IL group) consisted of six mice, including four males and two females, who were injected IL12,18 intraperitoneally at 0 h and 24 h. The second group (IL + B group) consisted of six mice, including five males and one female, who were administered an extended-release formulation of buprenorphine (36) (dose: 3.25 mg/kg, concentration: 1.3 mg/mL, Ethiqa-XR) subcutaneously (SC) 30 min before the first dose of IL12,18. The second dose of IL12,18 was given 24 h later. The experimental setup and timeline have been depicted in Fig. 1A.

Table 1. *Baseline characteristics and treatment of mice*

	IL Group ($n = 6$)	IL + B Group ($n = 6$)	*P* Value
Sex (Male, Female)	4 M, 2 F	5 M, 1 F	1.00*
Body weight, g	53.8 ± 4.0	55.7 ± 15.3	0.97**
Body fat, g	23.4 ± 1.1	25.1 ± 9.3	1.00**
Age, wk	12–14	12–14	
Treatment	IL 12,18; IP, 2 doses	XR-Buprenorphine, SC, 30 min before administering first dose of IL12,18 IL 12,18; IP, 2 doses	

Values are means ± SD. IP, intraperitoneal; SC, subcutaneous; XR, extended-release. Comparisons were made by *Fisher's exact test and **Mann–Whitney test.

Monitoring, Observations, and Assays

Carotid pulse distension was measured using a neck collar sensor (MouseOx Oximeter, STARR Life Sciences, Pittsburgh, PA) as a noninvasive measure of blood pressure, and rectal temperatures were measured using digital clinical thermometer, as done previously (33, 34, 37). Vitals were monitored at baseline and 24-h intervals. Tail vein blood samples were collected at baseline and 24 h for biochemical analysis.

Euthanasia Criteria

Mice were euthanized between 54 h and 58 h when they fulfilled euthanasia criteria, such as inability to flip back from supine position with a carotid pulse distention drop >50% from baseline, or appearing moribund (37). All mice in the IL12,18-alone group met these criteria. Two of six mice with pancreatitis that also received buprenorphine met these criteria. The remaining four mice with pancreatitis receiving buprenorphine were electively euthanized at this time to compare the extent of pancreatic injury. The study was not designed to compare survival, and the results did not support a significant difference in survival between the two groups ($P = 0.06$).

Tissue Collection and Processing

On necropsy, gross appearances of the intraperitoneal cavity including pancreas, abdominal fat pads (12), and the skin at buprenorphine injection site were noted. Terminal blood was collected for biochemical analysis. Serum lipase, amylase, and BUN assays were performed according to the manufacturer's instructions (Pointe Scientific, Canton, MI), as done in previous studies (33, 34). Creatinine was measured with the CHEM8+ cartridge using the i-STAT blood analyzer (Abbott Point of Care, Orlando, FL) (34), requiring 95 μL of blood. Control cardiac samples were used when tail vein blood samples were insufficient.

Histology and Staining

Pancreas, fat pad, and skin tissue were harvested and fixed in 10% neutral-buffered formalin and processed for embedding in paraffin. Sections were used for hematoxylin and eosin (H&E) staining. Digital images of sections were captured with a digital microscope (Olympus BX45) with a ×4, ×10, and ×40 objective. On H&E-stained pancreatic sections, pancreatic acinar necrosis was defined as diffuse pink areas with loss of cell boundaries, and also cytoplasmic, nuclear detail. Necrotic areas were quantified as a percentage of total pancreas tissue area, as described previously (14). Skin tissue was used for TUNEL immunostaining using the ApopTag Peroxidase In Situ Apoptosis Detection Kit from EMD Millipore (Cat. No. S7100) (12, 14, 33, 34, 38).

Statistical Analysis

Continuous data are represented as mean ± standard deviation. When comparing two groups, a *t* test or Mann–Whitney test was used, depending on the normality of distribution. Graphing was done using GraphPad Prism (v. 8.0.0 for Windows, GraphPad Software, San Diego, CA, www.graphpad.com).

RESULTS

Buprenorphine Interferes with the Initiation of AP

To determine the effect of buprenorphine on the initiation of AP, we compared serum markers and histological evidence of pancreatic injury in both groups at similar time points. For this, blood samples were collected at baseline, 24 h after the first IL12,18 injection, and at the time of euthanasia 54–58 h after the first IL12,18 injection. This is shown in the schematic in Fig. 1*A*.

Serum lipase was similar in the two groups, averaging at 98 ± 87 U/L at baseline. The increase was higher at 24 h in mice receiving IL12,18 alone (IL group) than in mice

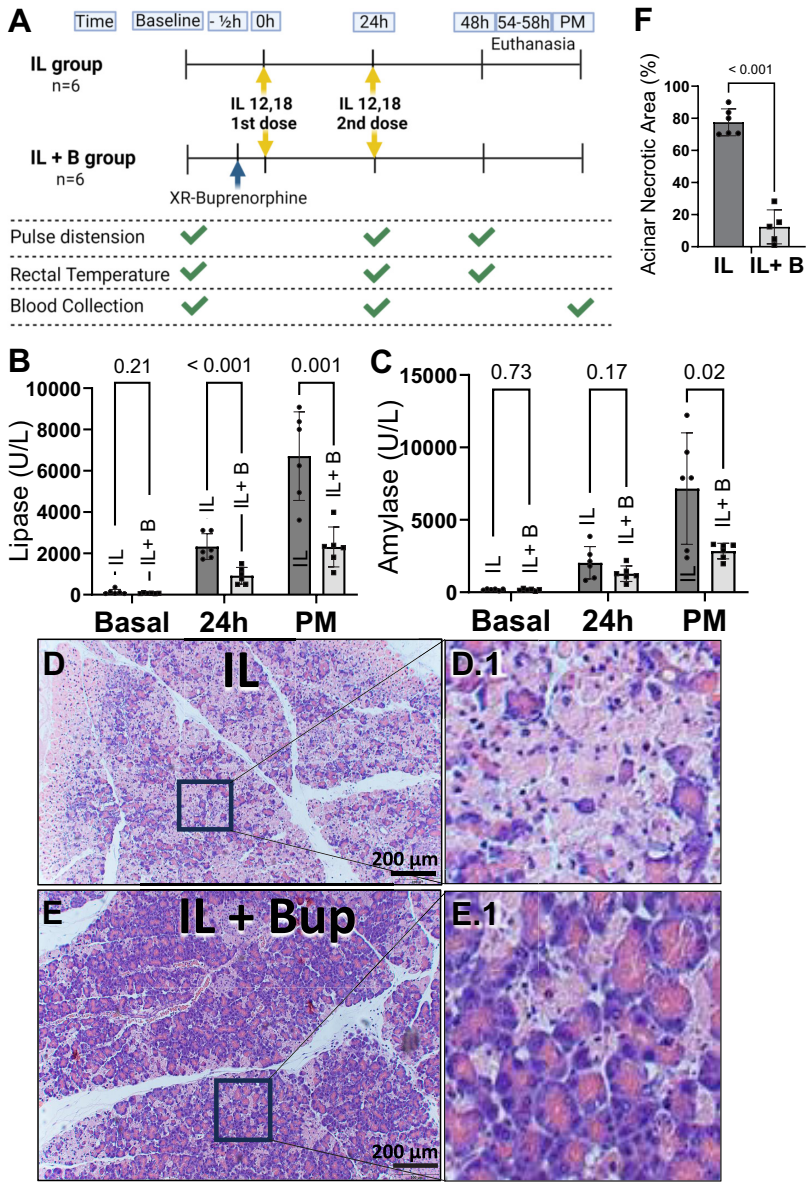

Figure 1. Time course of pancreatitis and effects of buprenorphine on its initiation. *A*: schematic illustrating experimental setup with time points for injections, monitoring, blood collection, and euthanasia. IL group: mice received two doses of IL12,18, intraperitoneally, at 0 h, and 24 h. IL + B group: mice were injected extended-release buprenorphine subcutaneously 30 min before administering the first dose of IL12,18 at 0 h and second dose at 24 h. Times of monitoring and blood collection are shown with green ticks (created with BioRender.com). Bar graphs showing levels of serum lipase (*B*) and amylase (*C*) in mice injected IL12,18 alone (IL, dark gray bar, $n = 6$) and IL1218 with buprenorphine (IL + B, light gray bar, $n = 6$) at baseline, 24 h after the first injection and postmortem. H&E-stained histological pancreatic sections at ×10 magnification in IL12,18 AP (*D*) and AP with buprenorphine (*E*). Magnified areas from box *D* and *E* highlighting the differences in diffuse pink necrotic areas with loss of cellular outline and nuclei in IL12,18 AP (*D.1*) compared with those receiving buprenorphine (*E.1*). *F*: bar graph comparing pancreatic acinar necrotic area in IL12,18 AP (IL, dark gray bar, $n = 6$) and those coadministered buprenorphine (IL + B, light gray bar, $n = 6$). Comparisons were made by *t* tests, *P* values are reported above the pairs of bars. AP, acute pancreatitis; IL, interleukin; PM, post-mortem; XR, extended release; *n*, number of mice in each group.

receiving IL12,18 with buprenorphine (IL + B group), 2,329 ± 627 U/L versus 918 ± 403 U/L ($P = 0.001$). This trend remained over the course of AP with postmortem serum lipases being, respectively, 6,710 ± 2,144 U/L versus 2,314 ± 965 U/L ($P < 0.001$) (Fig. 1*B*). Serum amylase showed a similar trend with baseline levels being 180 ± 55 U/L in the IL group and 168 ± 68 U/L ($P = 0.73$) in the IL + B group, and after 24 h being, respectively, 2,034 ± 1,119 U/L, 1,284 ± 541 U/L ($P = 0.17$) in the IL + B group. This difference was significant ($P = 0.039$) when statistically comparing matched mice that were studied simultaneously in the two groups. Postmortem amylase values were higher without buprenorphine at 7,153 ± 3,850 U/L versus 2,843 ± 543 U/L ($P = 0.02$) with buprenorphine (Fig. 1*C*). On histology, acinar necrosis was identified as diffuse pink areas with loss of cell outlines, cellular contents, and nuclear detail on H&E-stained sections. These areas were extensive in the IL group (Fig. 1, *D* and *D.1*), whereas in the IL + B group, these areas were sparse (Fig. 1, *E* and *E.1*). On quantification, the acinar necrotic area was reduced from 77.5 ± 8.3% in the IL group to 12.4 ± 10.6% in the IL + B group ($P < 0.001$) (Fig. 1*F*). Therefore, mice given buprenorphine with IL12,18 had reduced serum amylase, lipase, and necrosis compared with those given IL12,18 alone consistent with buprenorphine interfering with the initiation of AP.

Buprenorphine Reduces the Development of Fat Necrosis

As obese mice develop fat necrosis during the evolution of mild AP to severe AP associated with organ failure (12–14), we looked for its presence macroscopically and microscopically in visceral fat. Mice in the IL group had numerous foci of chalky-white fat necrosis distributed throughout the visceral fat, including intrapancreatic, retroperitoneal, perinephric, and gonadal fat pads (Fig. 2, *A* and *A.1*). In contrast, mice in the IL + B group had remarkably less fat necrosis (Fig. 2, *B* and *B.1*). On histology, fat necrosis appeared as adipocytes with loss of peripheral nuclei and cytoplasm filled with pink and bluish amorphous material, which were abundant in the IL group (Figure 2*A.2*) but much less in the ones who got buprenorphine (Fig. 2*B.2*). The IL group also had grossly visible intrapancreatic fat necrosis (Fig. 2, *C* and *C.1*). Although peripancreatic fat necrosis on the interface of fat and pancreas was abundant in the IL group (Fig. 2, *E* and *E.1*), these areas were less extensive in the IL + B group (Fig. 2, *F* and *F.1*). Whether this is related to buprenorphine interfering with AP initiation, or if this is a mechanistically distinct phenomenon, is not known.

Buprenorphine Alters Physiological and Biochemical Parameters Related to AP Severity

To determine the effects of buprenorphine on the development of organ failure and severity of AP, we compared the relevant parameters for *1*) hypotension and shock, i.e., carotid artery pulse distention; *2*) renal failure, i.e., BUN and creatinine elevation; and *3*) hypothermia, i.e., rectal temperature drop.

At baseline, the carotid pulse distention was similar in both groups (429 ± 111 μm in the IL vs. 382 ± 60 μm in the IL + B group; $P = 0.39$). At 24 h, it was significantly reduced to 320 ± 86 μm in the IL group compared with baseline ($P < 0.05$), while remaining the same as baseline in the IL + B groups 407 ± 50 μm ($P = 0.66$; Fig. 3*A*). By 48 h, the IL + B did develop hypotension (carotid pulse distention 246 ± 21 μm) compared with baseline, but this remained higher than in the IL group (138 ± 36 μm; $P < 0.001$; Fig. 3*B*). Thus, buprenorphine interfered with the development of hypotension in this model. Interestingly, BUN elevation occurred earlier in the IL + B group (Fig. 3*C*) with significantly higher values compared with the IL group at 24 h (31.9 mg/dL vs. 15.8 mg/dL; $P = 0.03$). At the time of necropsy, both groups had a similar increase in BUN compared with baseline and creatinine compared with control mice (Fig. 3, *C* and *D*; $P < 0.005$ for both parameters). This indicated a similar development of renal injury despite the delay in hypotension and earlier renal injury in the IL + B group mentioned earlier. Buprenorphine also caused early respiratory depression as noted with a reduced respiratory rate (168 ± 20 vs. 222 ± 25 breaths/min, $P = 0.01$) compared with the IL group alone. This effect was later reversed, with the IL group having a lower respiratory rate at 48 h (Fig. 3*E*). At 48 h, buprenorphine also affected the development of hypothermia. This was noted as lower rectal temperatures in the IL group 28.4 ± 1.8°C versus 32.9 ± 2.2°C ($P = 0.003$) in the IL + B group (Fig. 3*F*). Therefore, while mice receiving IL12,18 alone developed more pronounced hypotension and hypothermia, the buprenorphine-treated group with AP developed earlier renal injury and respiratory depression, supporting interference at multiple levels.

Extended-Release Buprenorphine Causes Skin Injury at the Injection Site

We examined the skin of mice where buprenorphine extended-release formulation was injected at the time of necropsy. On gross appearance, the skin surrounding the buprenorphine depot was swollen and erythematous (Bup SC inj., Fig. 4*A.1*). Erythema and hemorrhage surrounding the injection site were also noted on the inner surface (Fig. 4*A.2*). On histological examination of H&E-stained sections of skin, the buprenorphine depot formed a cavity in the subcutaneous fat (note empty blue dashed outlined oval in subcutaneous adipose tissue on the left side of Fig. 4, *B* and *C*). The epidermis was thinner on top of the injection site, with compression and loss of epidermal layers (Fig. 4*B.1*) compared with the normal skin away from the injection site (Fig. 4, *B.2* and *B.3*) that was notable on high power magnification. To look for cell death, we did TUNEL staining. There were numerous TUNEL-positive brown nuclei signifying cell death in the epidermal cells on top of the injection site (Fig. 4, *C.1* and *C.2*), which progressively reduced and became TUNEL negative on moving away from the injection site (Fig. 4*C.3*). Therefore, extended-release buprenorphine caused injury and thinning of the epidermis at the injection site, along with causing surrounding erythema and hemorrhage.

DISCUSSION

In the current study, we induced a severe model of AP and noted buprenorphine affecting the clinical, biochemical, and histopathological course of disease. Interference with AP

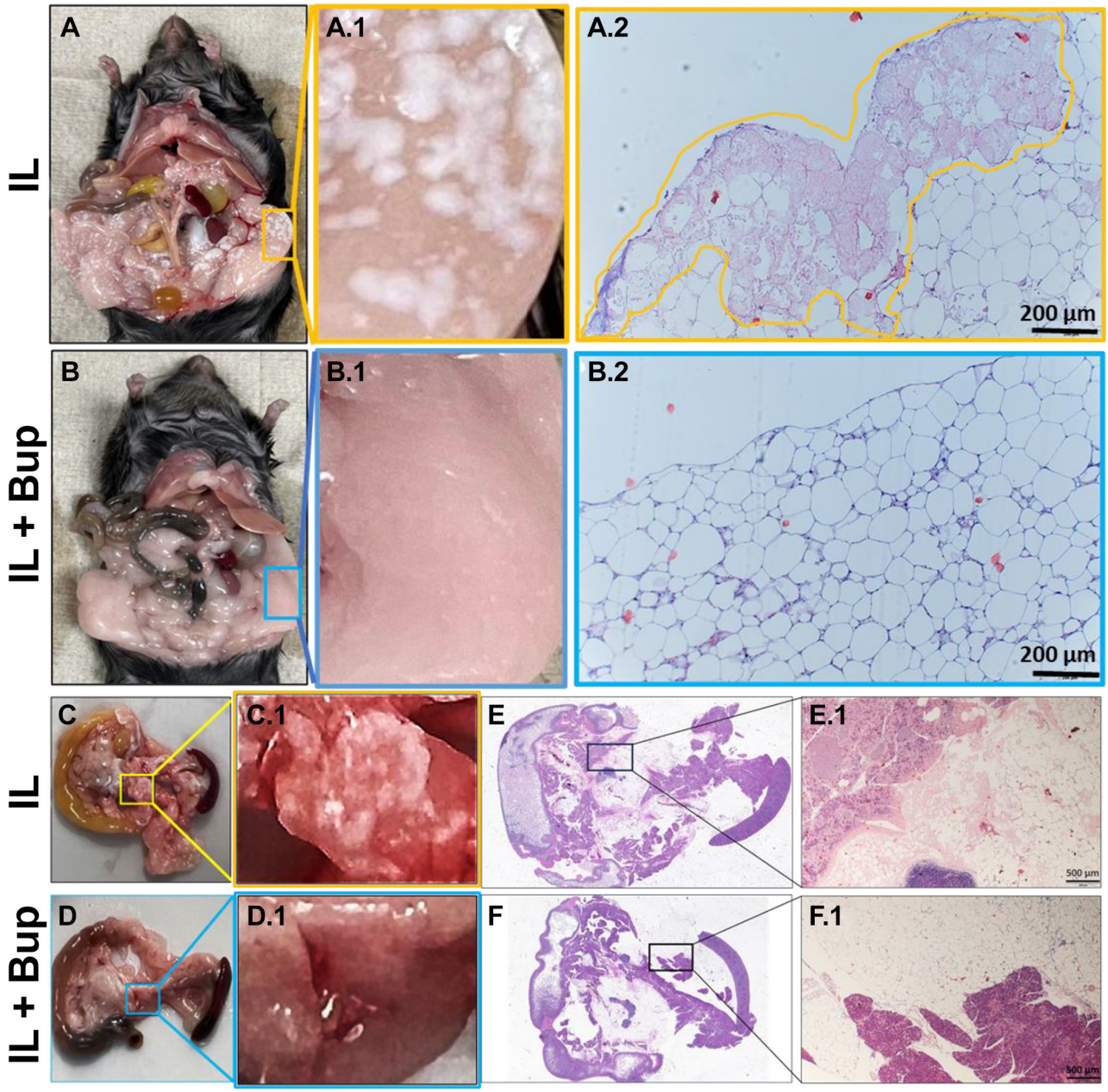

Figure 2. Effects of buprenorphine on fat necrosis in acute pancreatitis. Images of intraperitoneal cavity of mice injected with IL12,18 alone (*A*) and IL12,18 with buprenorphine (*B*) with magnified areas of fat pads highlighting the differences in numerous foci of fat necrosis in IL12,18 alone (*A.1*) compared with to IL12,18 with buprenorphine (*B.1*). H&E-stained sections of fat pads at ×10 magnification in IL12,18 AP (*A.2*) and with buprenorphine (*B.2*) comparing histological fat necrosis, seen as diffuse bluish-pink areas in adipocytes outlined in yellow. Gross images of pancreas in IL12,18 AP (*C*) and AP with buprenorphine (*D*), and magnification of boxed areas highlighting areas of fat necrosis in IL12,18 alone (*C.1*) compared with buprenorphine (*D.1*). Low power H&E images, at ×2 magnification of pancreas in IL12,18 AP (*E*) and AP with buprenorphine (*F*). Boxed areas in ×4 magnification showing acinar and peripancreatic fat necrosis bordering on the acinar necrosis in IL12,18 AP (*E.1*) and AP with buprenorphine (*F.1*). AP, acute pancreatitis; H&E, hematoxylin-eosin; IL, interleukin.

initiation was evidenced by a less pronounced elevation of serum pancreatic enzymes and less acinar necrosis on histology in the buprenorphine-treated group. Interference in worsening was evidenced by less fat necrosis in pancreatic fat and surrounding fat, an earlier increase in BUN, along with respiratory depression, and a less pronounced drop in blood pressure and body temperature in the buprenorphine-treated group compared with the group not given analgesic. In addition, we noted epidermal thinning and injury at the site of injection.

Opioids have a complex relationship with pancreatitis. Even though they are commonly used in AP (5), their safety in pancreatitis has been brought into question (39), as preclinical studies have linked morphine to increased intestinal permeability and bacterial translocation in AP (26). Opioids have been attributed to causing sphincter of Oddi dysfunction (40–42), and there are documented cases of opioids such as codeine causing drug-induced pancreatitis (43, 44). Buprenorphine is an opioid with mixed agonist-antagonist activity at classical opioid receptors and is used as

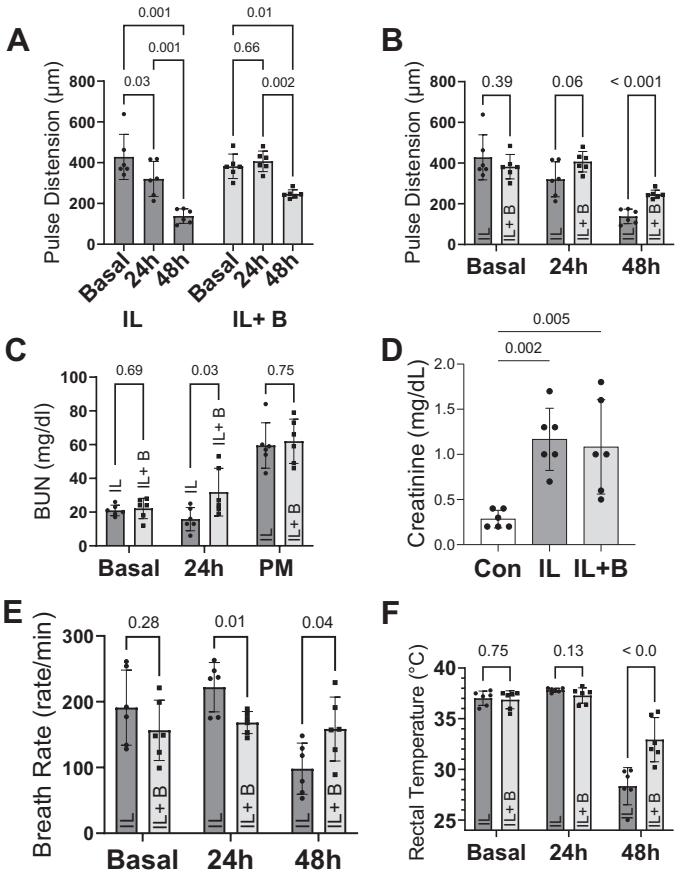

Figure 3. Effects of buprenorphine on the outcomes of acute pancreatitis. *A* and *B*: bar graphs comparing the carotid artery pulse distention as a measure of blood pressure in mice receiving IL 12,18 alone (IL, dark gray bar, $n = 6$) to those with buprenorphine (IL + B, light gray bar, $n = 6$). Comparisons between different time points in the same group (*A*). Comparisons between two groups at individual time points (*B*). *C*: bar graphs comparing serum blood urea nitrogen (BUN) in mice receiving IL 12,18 alone (IL, dark gray bar, $n = 6$) to those with buprenorphine (IL + B, light gray bar, $n = 6$) at baseline, 24 h, and postmortem (PM). *D*: bar graphs comparing postmortem serum creatinine levels in mice receiving IL 12,18 alone (IL, dark gray bar, $n = 6$) and those with buprenorphine (IL + B, light gray bar, $n = 6$) to control mice (Con, white bar, $n = 5$). Time course bar graphs comparing the breath rate (*E*) and rectal temperature (*F*) in mice receiving IL 12,18 alone (IL, dark gray bar, $n = 6$) to those with buprenorphine (IL + B, light gray bar, $n = 6$) at baseline, 24 h, and 48 h. Comparisons were made by *t* tests, *P* values are reported above the pairs of bars. IL, interleukin.

an analgesic (45). Several studies have shown buprenorphine to effectively relieve moderate to severe pain in rodents (46–50), including AP (51). Extended-release buprenorphine can also alleviate postsurgical pain (36, 52).

In animal models, opioid analgesics have been shown to alter the course of induced AP. Barlass et al. (26) noted that morphine, an opioid agonist, increased pancreatic necrosis and inflammatory response. In our study, we observed a masking effect where buprenorphine greatly reduces pancreatic necrosis. Ogden et al. (25) showed that buprenorphine decreases pancreatic edema, decreases amylase concentrations in isolated pancreatic acini, decreases amylase secretion after carbachol stimulation in vivo, and total protein and lipase synthesis in rat caerulein-induced AP. Another study showed that buprenorphine trended to reduce pancreatic enzymes in serum and ascites in 3% sodium taurocholate-induced AP in rats (51). Elevated serum lipase and amylase are used in the diagnosis of AP (2). Whether reduced enzyme synthesis contributes to the reduced serum amylase and lipase levels (Fig. 1, *B* and *C*) or reduced IL12,18-induced pancreatic necrosis in the presence of buprenorphine (Fig. 1, *D*–*F*) results in the muted response remains to be determined. Please note that despite the necrosis on histology, there was no difference in the separation of acini or lobules due to edema in mice with pancreatitis (Fig. 1, *D* and *E*). This is consistent with previous studies noting necrotizing pancreatitis without pancreatic edema in obese mice with IL12,18-induced AP (10, 11).

Peripancreatic fat necrosis is the most pathognomonic finding in AP (16, 53) and is associated with progression of mild AP to severe AP (12). Again, it remains to be studied whether the reduction of fat necrosis (Fig. 2) was due to buprenorphine interfering with IL12,18-induced pancreatic necrosis, enzyme synthesis, or direct effects on adipocyte responses during fat necrosis.

The effect of buprenorphine on organ failure in AP or physiological parameters such as blood pressure and core body temperature has not been studied before in AP. Hypotension is a marker of organ dysfunction and part of the modified Marshall scoring system for organ failure (2) and is associated with severe AP (54). Hypothermia is a criterion of systemic inflammatory response syndrome (SIRS) (55) and is associated with severe AP and a higher risk of persistent organ failure (56). Therefore, we compared hypotension and hypothermia as indicators of AP severity in our experiments. Although buprenorphine prevented a drop in blood pressure and temperature in AP (Fig. 3, *A* and *B*), it caused an earlier BUN increase (Fig. 3*C*) despite maintaining carotid pulse distention. This is consistent with a clinical case showing buprenorphine can cause renal injury (57). Therefore, although AP caused renal failure (elevated creatinine; Fig. 3*D*) irrespective of buprenorphine and required euthanasia, the mechanisms are likely different; with buprenorphine causing early renal injury, which was perhaps nephrotoxic and unrelated to AP-induced hypotension. The early reduction in respiratory rate by extended-release buprenorphine (Fig. 3*E*) is consistent with the known respiratory depressant effects of opioids (58). Despite muting the changes in respiratory rate and temperature by unknown mechanisms later in the disease (Fig. 3, *E* and *F*), mice given buprenorphine developed organ failure (Fig. 3, *A*–*D*) by unexplained mechanisms requiring euthanasia similar to AP alone. These can be due to the increased translocation and effects on the gut noted by Barlass et al. (26) and are consistent with a higher risk of severe AP reported with longer use of opioids in clinical studies (8).

Analgesics like buprenorphine are extremely important for the humane treatment of experimental animals. Pilot studies are sometimes required under IACUC oversight to analyze extraneous variability introduced by analgesics that may justify withholding them (30). Animal studies also require implementation of the 3 Rs (refinement, reduction, and replacement) for conducting harm-benefit analysis (30, 59). The principle of reduction emphasizes the judicious use of a minimized number of animals whenever feasible. However, if multiple laboratories studying AP independently undertake pilot studies on the same topic, i.e., the impact of buprenorphine in AP, the principle of reduction

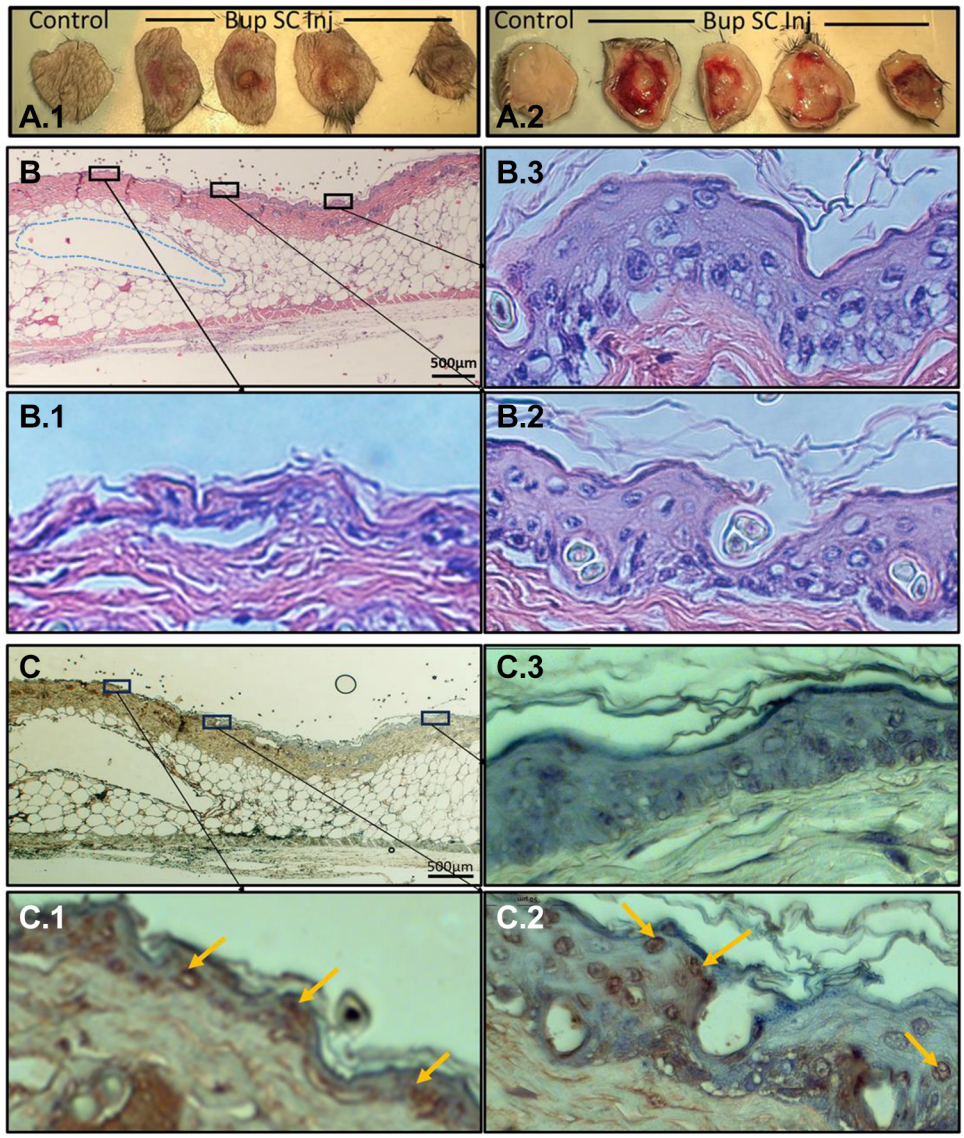

Figure 4. Local effects of extended-release subcutaneous buprenorphine injection (Bup SC Inj). Gross images of skin showing exterior (*A.1*) and interior surfaces (*A.2*) at sites of subcutaneous injections of saline (control) and buprenorphine. Note swelling, erythema, and hemorrhage of skin around buprenorphine depot. Low-power photomicrographs, at ×4 magnification, of H&E-stained sections of skin at the site of injection of buprenorphine (*B*) and corresponding TUNEL-stained section (*C*). Blue dashed line indicates site of buprenorphine injection (*B*). Magnified areas of H&E-stained skin sections from box *B* at ×40 magnification showing epithelium over the site of injection (*B.1*), at the edge (*B.2*), and away from the injection site (*B.3*). Note, the thinning of epithelial cell layers from multilayer away from the injection site to single layer on top of the injection site. Magnified areas of TUNEL-stained skin sections from box *C* at ×40 magnification showing epithelium at the site of injection (*C.1*), at the edge (*C.2*), and away from the injection site (*C.3*). Note yellow arrows pointing to TUNEL-positive nuclei (brown nuclei) in epithelium over (*C.1*) and at the edge (*C.2*) of buprenorphine injection site. H&E, hematoxylin-eosin.

may be compromised. Therefore, the information provided by the current study, that buprenorphine interferes with the course of induced AP by affecting the initiation, progression, and outcomes has relevance in planning future studies in experimental AP.

In summary, we found that the use of buprenorphine as an analgesic in IL12,18 model of experimental AP in mice alters the course of the disease. We noted interference at all stages of AP starting from initiation to progression and outcome. In addition, we noted local damage at the site of buprenorphine injection. As buprenorphine interferes with course experimental AP, the use of alternate forms or methods of pain alleviation should be explored. Researchers interested in investigating AP in animal models should cautiously choose an analgesic that does not interfere with the outcomes of induced AP.

DATA AVAILABILITY

Data will be made available upon reasonable request.

ACKNOWLEDGMENTS

The authors thank the Histology Core, Mayo Clinic Arizona, Scottsdale, AZ, for technical support. Graphical abstract and Fig. 1*A* were created using BioRender.com.

GRANTS

This project was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants RO1DK092460 and R01DK119646 and National Institute on Alcohol Abuse and Alcoholism Grant R01AA031257 (to V.P.S.).

DISCLOSURES

V. P. Singh is an editor of *American Journal of Physiology-Gastrointestinal and Liver Physiology* and was not involved and did not have access to information regarding the peer-review process or final disposition of this article. An alternate editor oversaw the peer-review and decision-making process for this article. None of the other authors has any conflicts of interest, financial or otherwise, to disclose.

AUTHOR CONTRIBUTIONS

V.P.S. conceived and designed the study; B.K., S.J., B.K., N.S., and M.J.S. performed experiments; V.P.S., S.J., B.K., N.S., P.R., B.M., and N.M.G., and V.P.S. analyzed data; V.P.S, S.J., B.K., N.S., P.R., A.N.P., and V.P.S. interpreted results of experiments; S.J., B.K., and N.M.G. prepared figures; V.P.S, S.J., B.K., and V.P.S. drafted manuscript; S.J., B.K., P.R., A.N.P., M.J.S., B.M., S.K., and N.M.G. edited and revised manuscript; S.J., B.K., N.S., P.R., A.N.P., M.J.S., B.M., S.K., N.M.G., and V.P.S. approved final version of manuscript.

REFERENCES

1. **Peery AF**, **Dellon ES**, **Lund J**, **Crockett SD**, **McGowan CE**, **Bulsiewicz WJ**, **Gangarosa LM**, **Thiny MT**, **Stizenberg K**, **Morgan DR**, **Ringel Y**, **Kim HP**, **DiBonaventura MD**, **Carroll CF**, **Allen JK**, **Cook SF**, **Sandler RS**, **Kappelman MD**, **Shaheen NJ.** Burden of gastrointestinal disease in the United States: 2012 update. *Gastroenterology* 143: 1179–1187.e3, 2012. doi:10.1053/j.gastro.2012.08.002.
2. **Banks PA**, **Bollen TL**, **Dervenis C**, **Gooszen HG**, **Johnson CD**, **Sarr MG**, **Tsiotos GG**, **Vege SS**; **Acute Pancreatitis Classification Working Group.** Classification of acute pancreatitis–2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 62: 102–111, 2013. doi:10.1136/gutjnl-2012-302779.
3. **Banks PA**, **Conwell DL**, **Toskes PP.** The management of acute and chronic pancreatitis. *Gastroenterol Hepatol (N Y)* 6: 1–16, 2010.
4. **Chaffin H**, **Trivedi S**, **Singh VP.** Impact of abdominal imaging on the diagnosis of acute pancreatitis in patients with painless lipase elevation. *Pancreatology* 22: 547–552, 2022. doi:10.1016/j.pan.2022.04.013.
5. **Basurto Ona X**, **Comas DR**, **Urrutia G.** Opioids for acute pancreatitis pain. *Cochrane Database Syst Rev* 7: CD009179, 2013.
6. **Ebbehøj N**, **Friis J**, **Svendsen LB**, **Bülow S**, **Madsen P.** Indomethacin treatment of acute pancreatitis. A controlled double-blind trial. *Scand J Gastroenterol* 20: 798–800, 1985. doi:10.3109/00365528509088825.
7. **Saini M**, **Samanta J**, **Kumar A**, **Choudhury A**, **Dhar J**, **Jafra A**, **Chauhan R**, **Muktesh G**, **Gupta P**, **Gupta V**, **Yadav TD**, **Kochhar R**, **Capurso G**, **De-Madaria E**, **Facciorusso A.** Buprenorphine versus diclofenac for pain relief in acute pancreatitis: A double-blinded randomized controlled trial. *Clin Gastroenterol Hepatol* 22: 532–541.e8, 2024. doi:10.1016/j.cgh.2023.10.021.
8. **Pandanaboyana S**, **Knoph CS**, **Olesen SS**, **Jones M**, **Lucocq J**, **Samanta J**, **Talukdar R**, **Capurso G**, **de-Madaria E**, **Yadav D**, **Siriwardena AK**, **Windsor J**, **Drewes AM**, **Nayar M**; **PAINAP Collaborative.** Opioid analgesia and severity of acute pancreatitis: An international multicentre cohort study on pain management in acute pancreatitis. *United European Gastroenterol J*, 12: 326–338, 2024. doi:10.1002/ueg2.12542.
9. **Pandol SJ**, **Saluja AK**, **Imrie CW**, **Banks PA.** Acute pancreatitis: bench to the bedside. *Gastroenterology* 132: 1127–1151, 2007 [Erratum in Gastroenterology 133: 1056, 2007]. doi:10.1053/j.gastro.2007.01.055.
10. **Sennello JA**, **Fayad R**, **Pini M**, **Gove ME**, **Ponemone V**, **Cabay RJ**, **Siegmund B**, **Dinarello CA**, **Fantuzzi G.** Interleukin-18, together with interleukin-12, induces severe acute pancreatitis in obese but not in nonobese leptin-deficient mice. *Proc Natl Acad Sci USA* 105: 8085–8090, 2008. doi:10.1073/pnas.0804091105.
11. **Pini M**, **Sennello JA**, **Cabay RJ**, **Fantuzzi G.** Effect of diet-induced obesity on acute pancreatitis induced by administration of interleukin-12 plus interleukin-18 in mice. *Obesity (Silver Spring)* 18: 476–481, 2010. doi:10.1038/oby.2009.263.
12. **Patel K**, **Trivedi RN**, **Durgampudi C**, **Noel P**, **Cline RA**, **DeLany JP**, **Navina S**, **Singh VP.** Lipolysis of visceral adipocyte triglyceride by pancreatic lipases converts mild acute pancreatitis to severe pancreatitis independent of necrosis and inflammation. *Am J Pathol* 185: 808–819, 2015. doi:10.1016/j.ajpath.2014.11.019.
13. **de Oliveira C**, **Khatua B**, **Noel P**, **Kostenko S**, **Bag A**, **Balakrishnan B**, **Patel KS**, **Guerra AA**, **Martinez MN**, **Trivedi S**, **McCullough A**, **Lam-Himlin DM**, **Navina S**, **Faigel DO**, **Fukami N**, **Pannala R**, **Phillips AE**, **Papachristou GI**, **Kershaw EE**, **Lowe ME**, **Singh VP.** Pancreatic triglyceride lipase mediates lipotoxic systemic inflammation. *J Clin Invest* 130: 1931–1947, 2020. doi:10.1172/JCI132767.
14. **Navina S**, **Acharya C**, **DeLany JP**, **Orlichenko LS**, **Baty CJ**, **Shiva SS**, **Durgampudi C**, **Karlsson JM**, **Lee K**, **Bae KT**, **Furlan A**, **Behari J**, **Liu S**, **McHale T**, **Nichols L**, **Papachristou GI**, **Yadav D**, **Singh VP.** Lipotoxicity causes multisystem organ failure and exacerbates acute pancreatitis in obesity. *Sci Transl Med* 3: 107ra110, 2011. doi:10.1126/scitranslmed.3002573.
15. **Vela S**, **Guerra A**, **Farrell G**, **Trivedi S**, **Chaffin H**, **Rood C**, **Singh R**, **Kostenko S**, **Chang Y-H**, **Snozek C**, **Patel K**, **Khatua B**, **Singh VP.** Pathophysiology and biomarker potential of fatty acid ethyl ester elevation during alcoholic pancreatitis. *Gastroenterology*, 161: 1513–1525, 2021. doi:10.1053/j.gastro.2021.07.029.
16. **Nordback I**, **Lauslahti K.** Clinical pathology of acute necrotising pancreatitis. *J Clin Pathol* 39: 68–74, 1986. doi:10.1136/jcp.39.1.68.
17. **Bakker OJ**, **van Santvoort H**, **Besselink MGH**, **Boermeester MA**, **van Eijck C**, **Dejong K**, **van Goor H**, **Hofker S**, **Ahmed Ali U**, **Gooszen HG**, **Bollen TL**; **Dutch Pancreatitis Study Group.** Extrapancreatic necrosis without pancreatic parenchymal necrosis: a separate entity in necrotising pancreatitis? *Gut* 62: 1475–1480, 2013. doi:10.1136/gutjnl-2012-302870.
18. **Shyu JY**, **Sainani NI**, **Sahni VA**, **Chick JF**, **Chauhan NR**, **Conwell DL**, **Clancy TE**, **Banks PA**, **Silverman SG.** Necrotizing pancreatitis: diagnosis, imaging, and intervention. *Radiographics* 34: 1218–1239, 2014. doi:10.1148/rg.345130012.
19. **Acharya C**, **Cline RA**, **Jaligama D**, **Noel P**, **Delany JP**, **Bae K**, **Furlan A**, **Baty CJ**, **Karlsson JM**, **Rosario BL**, **Patel K**, **Mishra V**, **Dugampudi C**, **Yadav D**, **Navina S**, **Singh VP.** Fibrosis reduces severity of acute-on-chronic pancreatitis in humans. *Gastroenterology* 145: 466–475, 2013. doi:10.1053/j.gastro.2013.05.012.
20. **Durgampudi C**, **Noel P**, **Patel K**, **Cline R**, **Trivedi RN**, **DeLany JP**, **Yadav D**, **Papachristou GI**, **Lee K**, **Acharya C**, **Jaligama D**, **Navina S**, **Murad F**, **Singh VP.** Acute lipotoxicity regulates severity of biliary acute pancreatitis without affecting its initiation. *Am J Pathol* 184: 1773–1784, 2014. doi:10.1016/j.ajpath.2014.02.015.
21. **Noel P**, **Patel K**, **Durgampudi C**, **Trivedi RN**, **de Oliveira C**, **Crowell MD**, **Pannala R**, **Lee K**, **Brand R**, **Chennat J**, **Slivka A**, **Papachristou GI**, **Khalid A**, **Whitcomb DC**, **DeLany JP**, **Cline RA**, **Acharya C**, **Jaligama D**, **Murad FM**, **Yadav D**, **Navina S**, **Singh VP.** Peripancreatic fat necrosis worsens acute pancreatitis independent of pancreatic necrosis via unsaturated fatty acids increased in human pancreatic necrosis collections. *Gut* 65: 100–111, 2016. doi:10.1136/gutjnl-2014-308043.
22. **Durst M**, **Graf TR**, **Graf R**, **Kron M**, **Arras M**, **Zechner D**, **Palme R**, **Talbot SR**, **Jirkof P.** Analysis of pain and analgesia protocols in acute cerulein-induced pancreatitis in male C57BL/6 Mice. *Front Physiol* 12: 744638, 2021. doi:10.3389/fphys.2021.744638.
23. **Stumpf F**, **Algül H**, **Thoeringer CK**, **Schmid RM**, **Wolf E**, **Schneider MR**, **Dahlhoff M.** Metamizol relieves pain without interfering with cerulein-induced acute pancreatitis in mice. *Pancreas* 45: 572–578, 2016. doi:10.1097/MPA.0000000000000483.
24. **Tang G**, **Nierath W-F**, **Palme R**, **Vollmar B**, **Zechner D.** Analysis of animal well-being when supplementing drinking water with tramadol or metamizole during chronic pancreatitis. *Animals (Basel)* 10: 2306, 2020. doi:10.3390/ani10122306.
25. **Ogden JM**, **Modlin IM**, **Gorelick FS**, **Marks IN.** Effect of buprenorphine on pancreatic enzyme synthesis and secretion in normal rats and rats with acute edematous pancreatitis. *Dig Dis Sci* 39: 2407–2415, 1994. doi:10.1007/BF02087658.
26. **Barlass U**, **Dutta R**, **Cheema H**, **George J**, **Sareen A**, **Dixit A**, **Yuan Z**, **Giri B**, **Meng J**, **Banerjee S**, **Banerjee S**, **Dudeja V**, **Dawra RK**, **Roy S**, **Saluja AK.** Morphine worsens the severity and prevents pancreatic regeneration in mouse models of acute pancreatitis. *Gut* 67: 600–602, 2018. doi:10.1136/gutjnl-2017-313717.
27. **Song AM**, **Bhagat L**, **Singh VP**, **Van Acker GGD**, **Steer ML**, **Saluja AK.** Inhibition of cyclooxygenase-2 ameliorates the severity of pancreatitis and associated lung injury. *Am J Physiol Gastrointest Liver Physiol* 283: G1166–G1174, 2002. doi:10.1152/ajpgi.00370.2001.
28. **Ozer Cakir O**, **Esen H**, **Toker A**, **Ataseven H**, **Demir A**, **Polat H.** Effects of diclofenac sodium and octreotide on treatment of caerulein-induced acute pancreatitis in mice. *Int J Clin Exp Med* 8: 17551–17564, 2015.
29. **El Kurdi B**, **Imam Z**, **Abonofal A**, **Babar S**, **Shah P**, **Pannala R**, **Papachristou G**, **Echavarria J**, **Pisipati S**, **Jahangir S**, **Rajalingamgari**

P, **Chang Y-HH**, **Singh VP.** NSAIDs do not reduce severity among post-ERCP pancreatitis patients. *Pancreatology* 24: 14–23, 2024. doi:10.1016/j.pan.2023.11.003.
30. **Mohan S**, **Huneke R.** The role of IACUCs in responsible animal research. *ILAR J* 60: 43–49, 2019. doi:10.1093/ilar/ilz016.
31. **Institute for Laboratory Animal Resources, Commission on Life Sciences National Research Council.** *Guide for the Care and Use of Laboratory Animals (8th ed.).* Washington, DC: National Academies Press, 2011.
33. **Khatua B**, **El-Kurdi B**, **Patel K**, **Rood C**, **Noel P**, **Crowell M**, **Yaron JR**, **Kostenko S**, **Guerra A**, **Faigel DO**, **Lowe M**, **Singh VP.** Adipose saturation reduces lipotoxic systemic inflammation and explains the obesity paradox. *Sci Adv* 7: eabd6449, 2021. doi:10.1126/sciadv.abd6449.
34. **Cartin-Ceba R**, **Khatua B**, **El-Kurdi B**, **Trivedi S**, **Kostenko S**, **Imam Z**, **Smith R**, **Snozek C**, **Navina S**, **Sharma V**, **McFayden B**, **Ionescu F**, **Stolow E**, **Keiser S**, **Tejani A**, **Harrington A**, **Acosta P**, **Kuwelker S**, **Echavarria J**, **Nair GB**, **Bataineh A**, **Singh VP.** Evidence showing lipotoxicity worsens outcomes in Covid-19 patients and insights about the underlying mechanisms. *iScience* 25: 104322, 2022. doi:10.1016/j.isci.2022.104322.
35. **Kostenko S**, **Khatua B**, **Trivedi S**, **Pillai AN**, **McFayden B**, **Morsy M**, **Rajalingamgari P**, **Sharma V**, **Noel P**, **Patel K**, **El-Kurdi B**, **Borges da Silva H**, **Chen X**, **Chandan V**, **Navina S**, **Vela S**, **Cartin-Ceba R**, **Snozek C**, **Singh VP.** Amphipathic liponecrosis impairs bacterial clearance and causes infection during sterile inflammation. *Gastroenterology* 165: 999–1015, 2023. doi:10.1053/j.gastro.2023.05.034.
36. **Saenz M**, **Bloom-Saldana EA**, **Synold T**, **Ermel RW**, **Fueger PT**, **Finlay JB.** Pharmacokinetics of sustained-release and extended-release buprenorphine in mice after surgical catheterization. *J Am Assoc Lab Anim Sci* 61: 468–474, 2022. doi:10.30802/AALAS-JAALAS-22-000025.
37. **Khatua B**, **Yaron JR**, **El-Kurdi B**, **Kostenko S**, **Papachristou GI**, **Singh VP.** Ringer's lactate prevents early organ failure by providing extracellular calcium. *J Clin Med* 9: 263, 2020. doi:10.3390/jcm9010263.
38. **Khatua B**, **Trivedi RN**, **Noel P**, **Patel K**, **Singh R**, **de Oliveira C**, **Trivedi S**, **Mishra V**, **Lowe M**, **Singh VP.** Carboxyl ester lipase may not mediate lipotoxic injury during severe acute pancreatitis. *Am J Pathol* 189: 1226–1240, 2019. doi:10.1016/j.ajpath.2019.02.015.
39. **Singh VP.** High on drugs: lessons from opiates in pancreatitis. *Gut* 67: 600–602, 2018. doi:10.1136/gutjnl-2017-314506.
40. **Thompson DR.** Narcotic analgesic effects on the sphincter of Oddi: a review of the data and therapeutic implications in treating pancreatitis. *Am J Gastroenterol* 96: 1266–1272, 2001. doi:10.1016/S0002-9270(00)02329-7.
41. **Helm JF**, **Venu RP**, **Geenen JE**, **Hogan WJ**, **Dodds WJ**, **Toouli J**, **Arndorfer RC.** Effects of morphine on the human sphincter of Oddi. *Gut* 29: 1402–1407, 1988. doi:10.1136/gut.29.10.1402.
42. **Sharma SS.** Sphincter of Oddi dysfunction in patients addicted to opium: an unrecognized entity. *Gastrointest Endosc* 55: 427–430, 2002. doi:10.1067/mge.2002.121600.
43. **Hastier P**, **Buckley MJM**, **Peten EP**, **Demuth N**, **Dumas R**, **Demarquay J-F**, **Caroli-Bosc F-X**, **Delmont J-P.** A new source of drug-induced acute pancreatitis: codeine. *Am J Gastroenterology* 95: 3295–3298, 2000. doi:10.1016/S0002-9270(00)02006-2.
44. **Moreno Escobosa MC**, **Amat López J**, **Cruz Granados S**, **Moya Quesada MC.** Pancreatitis due to codeine. *Allergol Immunopathol (Madr)* 33: 175–177, 2005. doi:10.1157/13075703.
45. **Lutfy K**, **Cowan A.** Buprenorphine: a unique drug with complex pharmacology. *Curr Neuropharmacol* 2: 395–402, 2004. doi:10.2174/1570159043359477.
46. **Foley PL**, **Liang H**, **Crichlow AR.** Evaluation of a sustained-release formulation of buprenorphine for analgesia in rats. *J Am Assoc Lab Anim Sci* 50: 198–204, 2011.
47. **Adamson TW**, **Kendall LV**, **Goss S**, **Grayson K**, **Touma C**, **Palme R**, **Chen JQ**, **Borowsky AD.** Assessment of carprofen and buprenorphine on recovery of mice after surgical removal of the mammary fat pad. *J Am Assoc Lab Anim Sci* 49: 610–616, 2010.
48. **Carbone ET**, **Lindstrom KE**, **Diep S**, **Carbone L.** Duration of action of sustained-release buprenorphine in 2 strains of mice. *J Am Assoc Lab Anim Sci* 51: 815–819, 2012.
49. **Chum HH**, **Jampachairsri K**, **McKeon GP**, **Yeomans DC**, **Pacharinsak C**, **Felt SA.** Antinociceptive effects of sustained-release buprenorphine in a model of incisional pain in rats (Rattus norvegicus). *J Am Assoc Lab Anim Sci* 53: 193–197, 2014.
50. **Guarnieri M**, **Brayton C**, **DeTolla L**, **Forbes-McBean N**, **Sarabia-Estrada R**, **Zadnik P.** Safety and efficacy of buprenorphine for analgesia in laboratory mice and rats. *Lab Anim (NY)* 41: 337–343, 2012. doi:10.1038/laban.152.
51. **Wereszczynska-Siemiatkowska U**, **Nebendahl K**, **Pohl U**, **Otto J**, **Groene HJ**, **Wilms H**, **Lankisch PG.** Influence of buprenorphine on acute experimental pancreatitis. *Res Exp Med (Berl)* 187: 211–216, 1987. doi:10.1007/bf01852085.
52. **Traul KA**, **Romero JB**, **Brayton C**, **DeTolla L**, **Forbes-McBean N**, **Halquist MS**, **Karnes HT**, **Sarabia-Estrada R**, **Tomlinson MJ**, **Tyler BM**, **Ye X**, **Zadnik P**, **Guarnieri M.** Safety studies of post-surgical buprenorphine therapy for mice. *Lab Anim* 49: 100–110, 2015. doi:10.1177/0023677214554216.
53. **Kloppel G**, **Dreyer T**, **Willemer S**, **Kern HF**, **Adler G.** Human acute pancreatitis: its pathogenesis in the light of immunocytochemical and ultrastructural findings in acinar cells. *Virchows Arch A Pathol Anat Histopathol* 409: 791–803, 1986. doi:10.1007/bf00710764.
54. **Garcia M**, **Calvo JJ.** Cardiocirculatory pathophysiological mechanisms in severe acute pancreatitis. *World J Gastrointest Pharmacol Ther* 1: 9–14, 2010. doi:10.4292/wjgpt.v1.i1.9.
55. **Bone RC**, **Balk RA**, **Cerra FB**, **Dellinger RP**, **Fein AM**, **Knaus WA**, **Schein RM**, **Sibbald WJ.** Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 101: 1644–1655, 1992. doi:10.1378/chest.101.6.1644.
56. **Singh VK**, **Wu BU**, **Bollen TL**, **Repas K**, **Maurer R**, **Mortele KJ**, **Banks PA.** Early systemic inflammatory response syndrome is associated with severe acute pancreatitis. *Clin Gastroenterol Hepatol* 7: 1247–1251, 2009. doi:10.1016/j.cgh.2009.08.012.
57. **Zuin M**, **Giorgini A**, **Selmi C**, **Battezzati PM**, **Cocchi CA**, **Crosignani A**, **Benetti A**, **Invernizzi P**, **Podda M.** Acute liver and renal failure during treatment with buprenorphine at therapeutic dose. *Dig Liver Dis* 41: e8–e10, 2009. doi:10.1016/j.dld.2007.12.014.
58. **Dahan A.** Opioid-induced respiratory effects: new data on buprenorphine. *Palliat Med* 20: 3–8, 2006. doi:10.1191/0269216306pm1126oa.
59. **Curzer HJ**, **Perry G**, **Wallace MC**, **Perry D.** The three Rs of animal research: What they mean for the Institutional Animal Care and Use Committee and Why. *Sci Eng Ethics* 22: 549–565, 2016. doi:10.1007/s11948-015-9659-8.