

## Original Article

# Validation of a novel, physiologic model of experimental acute pancreatitis in the mouse

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**Abstract:** Background: Many experimental models of acute pancreatitis suffer from lack of clinical relevance. We sought to validate a recently reported murine model of acute pancreatitis that more closely represents the physiology of human biliary pancreatitis. Methods: Mice (C57BL/6J n=6 and CF-1 n=8) underwent infusion of 50µl of 5% sodium taurocholate (NaT) or 50µl of normal saline (NaCl) directly into the pancreatic duct. Twenty-four hours later, pancreatitis severity was graded histologically by three independent observers, and pancreatic tissue concentration of interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) were determined by ELISA. Results: Twenty four hours after retrograde injection, the total pancreatitis score was significantly greater in mice infused with NaT than in those infused with NaCl ( $6.3 \pm 1.2$  vs.  $1.2 \pm 0.4$ ,  $p < 0.05$ ). In addition, the inflammatory mediators IL-6 and MCP-1 were increased in the NaT group relative to the NaCl group. Discussion: Retrograde pancreatic duct infusion of sodium taurocholate induces acute pancreatitis in the mouse. This model is likely representative of human biliary pancreatitis pathophysiology, and therefore provides a powerful tool with which to elucidate basic mechanisms underlying the pathogenesis of acute pancreatitis.

**Keywords:** Acute pancreatitis, animal model, murine, sodium taurocholate, retrograde injection

## Introduction

Acute pancreatitis is a significant health problem in the United States, with over 240,000 cases annually [1]. Importantly, no specific treatment for acute pancreatitis currently exists [2]. This lack of definitive treatment is due in large part to our relative lack of understanding of the pathophysiological mechanisms underlying the development of pancreatitis. These mechanisms of disease are often uncovered via the extensive study of relevant animal models. Moreover, the use of murine disease models can be particularly advantageous in the current era of transgenic capabilities.

Common murine models of acute pancreatitis, however, suffer from several major limitations. For example, one widely used mouse model involves hyperstimulation with the secretagogue cerulein [3]. Advantages of the cerulein model

include ease of administration and reproducibility of a typically mild pancreatitis. A major disadvantage of cerulein administration is that it is unlikely to mimic any etiologic mechanism responsible for the pathogenesis of human pancreatitis. Other murine models of acute pancreatitis such as intraperitoneal injection of arginine [4] or choline-deficient ethionine-supplemented diets [5] are also unlikely to represent the pathophysiological mechanisms responsible for human pancreatitis. Mechanical models of pancreatitis involve ligation of the pancreatic duct or the duodenum [6,7]. Ligation or obstruction of the common biliopancreatic duct, which may more clearly mimic the pathophysiology of human biliary pancreatitis, produces widely variable degrees of pancreatitis in different species. In addition, models involving the formation of a closed duodenal loop require restoration of gastrointestinal continuity, typically with a gastrojejunostomy, rendering this

technique more suitable for large animals.

A relatively common rat model that more likely approximates the pathogenesis of human biliary pancreatitis involves retrograde infusion of bile acids into the pancreatic duct. The larger size of the rat relative to the mouse makes this procedure feasible; at present, however, genetic manipulation is not possible in rats. In an effort to develop a clinically relevant murine model of acute pancreatitis, Laukkarinen and colleagues have recently published their experience with retrograde infusion of sodium taurocholate in mice [8]. The aim of the current study was to validate this recently reported model of murine acute pancreatitis.

### Materials and methods

All studies were performed with approval of the Indiana University Institutional Animal Care and Use Committee and were in accordance with the National Research Council guide for the care and use of laboratory animals.

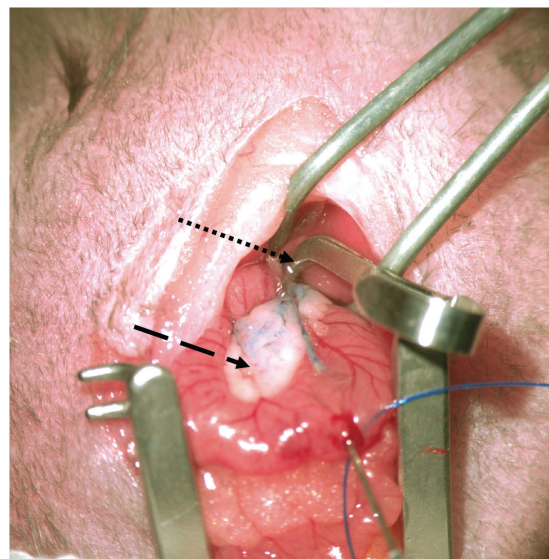
#### Animals

Mice (C57BL/6J and CF-1) were obtained from Jackson Laboratories (Bar Harbor, ME) at 7 weeks of age. The mice were housed in a light (12 hour light:dark) and temperature (22°C) controlled environment, and were fed a diet containing 25% fat (soybean oil + corn oil), 55% carbohydrate (sucrose and cornstarch), and 20% protein derived calories (Dyets Inc., Bethlehem, PA) with water allowed ad libitum.

#### Procedure

Mice were fasted overnight preoperatively. Anesthesia was induced with ketamine/xylazine (100mg/kg / 10mg/kg; Phoenix Pharmaceuticals, St. Joseph, MO) by intraperitoneal injection, and maintained with inhaled 1% isoflurane (Abbot, Chicago, IL). Upper midline mini-laparotomy provided access to the second portion of the duodenum and pancreatic head, which were rotated axially. Using optical magnification, the duodenum was immobilized with a 5-0 traction suture, and the ampulla of Vater was identified. A puncture wound was made with a 19 gauge needle on the antimesenteric surface of the duodenum opposite the papilla. The duodenotomy was then entered with a 30-gauge blunt-tipped catheter, and the ampulla

was carefully cannulated. The catheter was advanced 1mm within the pancreatic duct and secured with a 10-0 suture. The proximal common bile duct was then occluded at the liver hilum with a neuro bulldog clamp (Fine Science Tools, Inc., Foster City, CA). An infusion pump (Harvard Apparatus, Natick, MA) was used to infuse either 50µl of 5% sodium taurocholate (NaT; Sigma, St. Louis, MO) in NaCl or 50µl of 0.9% NaCl into the pancreatic duct over 5 minutes. Infusion was carried out at a constant rate over a defined time interval; no attempt was made to measure intraductal pressures. Methylene blue (Sigma, St. Louis, MO) was



**Figure 1.** Intra-operative photograph of murine retrograde pancreatic duct infusion. Through a small duodenotomy (solid arrow) a 30 gauge catheter was inserted through the papilla into the pancreatic duct (dashed arrow). Note the neuro bulldog clamp applied to the common bile duct (dotted arrow).

added to the infusion solution to confirm proper intraductal placement and perfusion (**Figure 1**).

Following completion of infusion, the bulldog clamp was released, the catheter was removed, and the duodenotomy and fascia were closed. Animals were allowed to recover from anesthesia with close monitoring. Analgesia was maintained via subcutaneous buprenorphine hydrochloride injections (0.1mg/kg; Bedford Labs, Bedford, OH) immediately post operatively and 12 hours later. Mice were allowed water and food ad libitum. Twenty-four hours following the

initial operative procedure, total pancreatectomy was performed. A portion of the pancreatic tissue was preserved in 5% formalin for histologic analysis, and the remaining pancreas was snap frozen in liquid nitrogen and stored at -80 °C for subsequent study.

### Histology

Formalin-fixed pancreatic tissue was embedded in paraffin, sectioned into 5 µm sections, and stained with hematoxylin and eosin. Tissue sections were evaluated using light microscopy at 10X magnification (Leica DM 5000B, Wetzler, Germany). Pancreatitis severity was determined by three separate observers who were unaware of the treatment group. A validated scale incorporating degree of edema, vacuolization, and inflammatory cell infiltrate was used to evaluate the total pancreatitis score [9].

### Biochemical assays

Pancreatic tissue was homogenized in a buffer containing 50mM Tris, 250 mM NaCl, 5 mM EDTA, 1 mM NaF, 20 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, 0.02% NaN<sub>3</sub>, proprietary detergent, and protease inhibitor (Sigma, St. Louis, MO) at a volume of 50µL per gram tissue. Homogenates were centrifuged at 10,000 rpm at 4°C for 15 min, and protein concentration of the supernatant was assayed (Bio-Rad, Hercules, CA). Pancreatic tissue concentrations of the proinflammatory cytokine interleukin-6 (IL-6) and chemoattractant molecule monocyte chemoattractant protein-1 (MCP-1), which are known to be important in the pathogenesis of pancreatitis [10-13], were measured using ELISA (R & D Systems, Minneapolis, MN) according to the manufacturer's instructions.

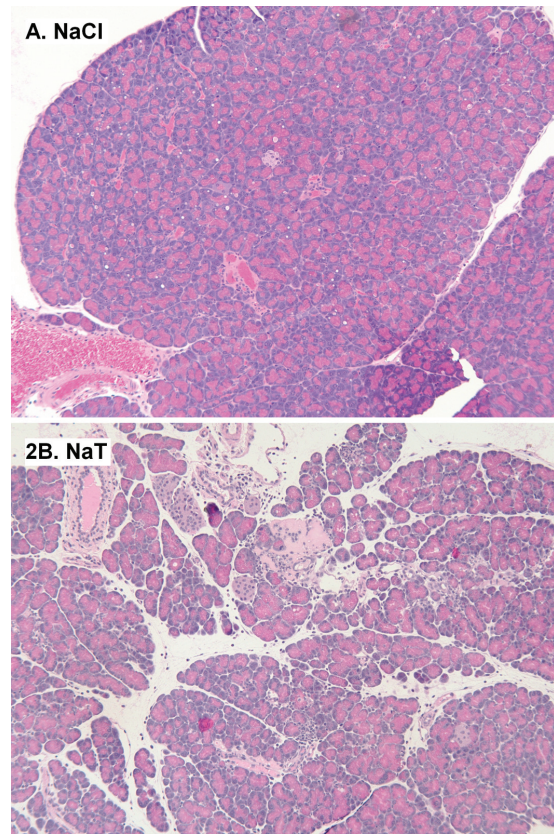
### Statistical analysis

Statistical analysis was performed using Sigma Stat Statistical Software (Jandel Corp., San Rafael, CA). Data are expressed as mean ± SEM. Student's t-test, Fisher's exact test, and ANOVA were applied where appropriate; p value <0.05 was accepted as statistically significant.

## Results

### Development of histologic pancreatitis

Seven mice from the NaT group and 6 mice in



**Figure 2.** Representative micrographs (H&E, 10X) illustrating (a) relatively normal pancreatic histology in a NaCl-infused mouse and (b) increased edema, vacuolization, and inflammatory cell infiltrate in the pancreata of a mouse infused with NaT.

the NaCl group were studied 24 hours after retrograde infusion. **Figure 2** shows representative photomicrographs of pancreata infused with NaT and NaCl. Retrograde NaT infusion generated acute pancreatitis as manifest by edema, cellular vacuolization, inflammatory cell infiltrate, and in some cases pancreatic parenchymal necrosis. Mice injected with NaT developed significantly more severe pancreatitis relative to NaCl injected mice (**Figure 3, Table 1**).

### Pancreatic Inflammatory Mediators

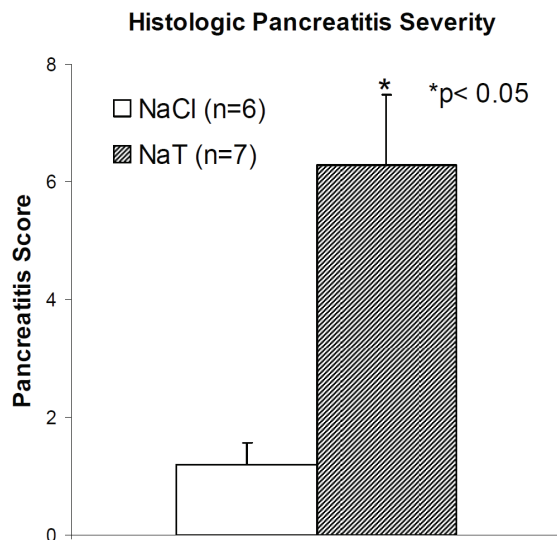
The proinflammatory cytokine interleukin-6 (IL-6) and the chemoattractant molecule monocyte chemoattractant protein-1 (MCP-1) are important inflammatory mediators in acute pancreatitis [10,13]. Pancreatic concentration of both IL-6 and MCP-1 were increased in mice perfused

## NaT pancreatitis

**Table 1.** Histologic pancreatitis score by category for both NaCl and NaT groups

	n	Edema	Inflammation	Vacuolization	Total
NaCl	6	0.7 ± 0.2	0.2 ± 0.2	0.3 ± 0.1	1.2 ± 0.4
NaT	7	2.4 ± 0.5*	2.2 ± 0.4*	1.7 ± 0.7*	6.3 ± 1.2*

\*p<0.05 vs. NaCl; Values are mean ± SEM

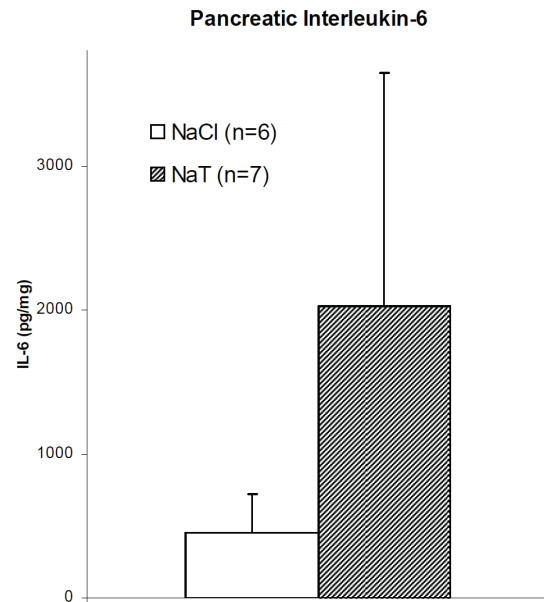


**Figure 3.** Histologic pancreatitis score in lean mice treated with pancreatic duct infusion of either NaCl (white bars) or NaT (hatched bars). Mice treated with NaT developed a significantly higher severe pancreatitis score.

with NaT relative to those infused with NaCl, though at the 24 hour time point these differences did not reach statistical significance (Figures 4 and 5). This observation may be due, in part, to the temporal release of cytokines and other inflammatory mediators over the course of an inflammatory response. These biochemical data complement the histologic (gold standard) findings and indicate that retrograde pancreatic duct infusion of NaT results in changes consistent with acute pancreatitis.

### Discussion

The results of our experiments validate a recently reported technique inducing pancreatitis by retrograde pancreatic duct infusion of sodium taurocholate in the mouse. Mice infused with NaT developed severe acute pancreatitis, as shown histologically, the gold standard in

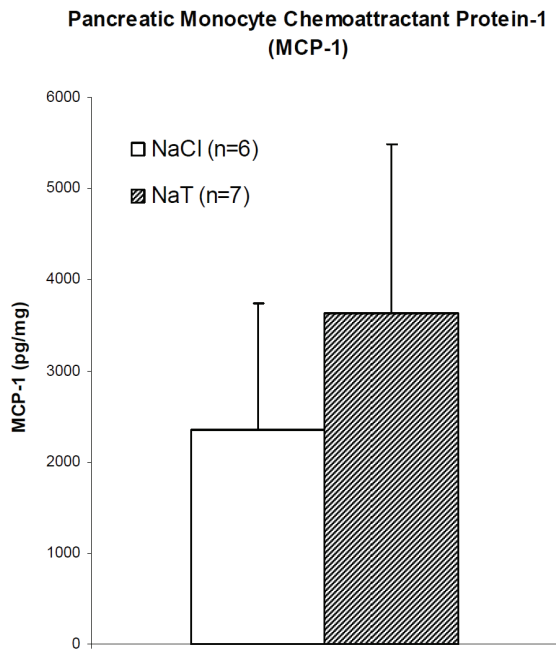


**Figure 4.** Pancreatic concentration of the proinflammatory cytokine IL-6 in lean mice treated with NaCl (white bars) or NaT (hatched bars). Induction of pancreatitis with NaT resulted in increased pancreatic IL-6 concentration compared with NaCl-infused mice. Data are mean ± SEM.

diagnosis. In addition, pancreatic parenchyma in NaT-infused mice demonstrated increased tissue concentrations of the proinflammatory cytokine IL-6 and the marker of neutrophil infiltration MCP-1. This model presents some technical challenges and is associated with a discrete learning curve. Nevertheless, the development of a physiologically relevant murine model of acute pancreatitis is clearly important, given the potential for precise manipulation of the murine genome.

Despite over 100 years of research, current understanding of acute pancreatitis remains rudimentary [3,14,15]. Largely because of this lack of understanding, no specific treatment for acute pancreatitis currently exists. Our knowl-





**Figure 5.** Pancreatic concentration of the chemoattractant molecule MCP-1 in lean mice treated with NaCl (white bars) or NaT (hatched bars). Data are mean  $\pm$  SEM.

edge of acute pancreatitis pathophysiology comes primarily from data generated in *in vivo* animal models. Acute pancreatitis has been studied in both large animal (dog, cat, opossum) and small animal (rat, mouse) models; the latter offer several discrete advantages.

Murine models have great potential in developing our insight into the mechanisms of disease because of the current knowledge of and ability to manipulate the murine genome. For example, conditional expression of a single oncogene (i.e. k-ras) leads to spontaneous development of pancreatic neoplasia in the mouse [16,17]. In addition, the use of this model in genetically obese mice may elucidate mechanisms by which obesity worsens pancreatitis [18-20].

Existing murine models of pancreatitis, however, each have limitations. In general, models that are convenient and reproducible, such as cerulein hyperstimulation and diet-induced pancreatitis, may not be clinically relevant, while models with more clinical relevance (i.e. duodenal exclusion) often require complex surgical manipulation. An ideal murine model of acute pancreatitis is clinically relevant, technically feasible, and reliable (Table 2).

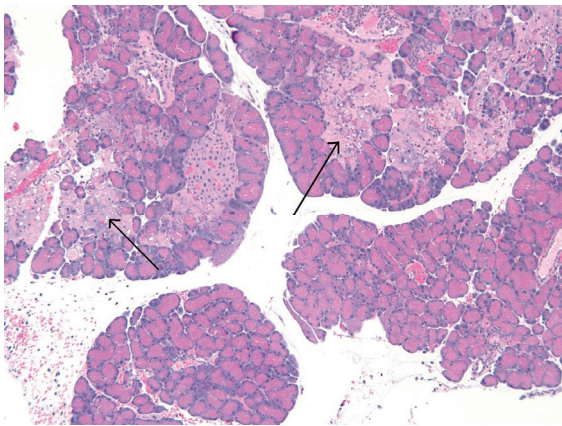
This current model of retrograde pancreatic duct infusion of sodium taurocholate meets these requirements. This model reliably produces acute pancreatitis that can be validated histologically, which is the gold standard in diagnosis. In addition, upregulation of pancreatic tissue inflammatory mediators illustrates the potential to evaluate local biochemical changes of acute pancreatitis. For example, both the cytokine IL-6 and the chemoattractant molecule MCP-1 are known to be accurate markers and important mediators of human and experimental pancreatitis [21]. Time-course experiments will further define the rise and fall of these molecules. Finally, introduction of bile acids into the pancreatic milieu is likely a much closer representation of biliary pancreatitis pathophysiology than other currently available models.

Some challenges of this model must be recognized. Placing a small catheter into the mouse pancreatic duct requires a degree of technical expertise, although it should be noted that we found this to be less technically challenging than anticipated. A discrete learning curve is involved with any operative procedure, including technical and anatomical understanding, as well as clinical adjustments. For example, we found that warming the mice intra- and post-operatively improved survival. In addition, a post-operative subcutaneous fluid bolus of 0.02ml/g (500 $\mu$ l in most mice) also improved recovery. The latter finding is not entirely surprising given the increased fluid requirements seen in patients with acute pancreatitis.

**Table 2.** Advantages and disadvantages of current animal models of pancreatitis

Model	Clinical Relevance	Technical Ease	Low Variability	Genomics
Cerulein	-	+	+	+
Dietary	-	++	+	+/-
Arginine	-	+	-	+/-
Mechanical	+	--	+/-	-
Murine NaT	++	+/-	+	+

It is important to recognize that the NaT model generates relatively severe pancreatitis, and as such may not be suitable for use in all strains of mice. For example, congenitally obese (*ob/ob*<sup>-/-</sup> and *db/db*<sup>-/-</sup>) mice are physiologically fragile, and do not tolerate general anesthesia or laparotomy well. In pilot studies using these genotypes, we observed particularly severe pancreatitis with significant pancreatic necrosis (**Figure 6**); unfortunately, this was accompanied by nearly uniform early (< 24 hours) mortality. The severity of pancreatitis is a particularly important consideration given the variability of



**Figure 6.** Histologic section (H&E,10X) of obese *db/db*<sup>-/-</sup> pancreas infused with NaT. Notable are numerous areas of necrosis (arrows).

pancreatitis severity observed among different strains of mice.

In summary, our experiments have validated a novel murine model of acute pancreatitis: sodium taurocholate infusion into the pancreatic duct causes severe acute pancreatitis in the mouse. This model is physiologically relevant in that it likely approximates the pathophysiology of human biliary pancreatitis. In the context of genetic manipulation, this model clearly provides an extremely powerful tool with which to investigate the mechanisms of acute pancreatitis.

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