Received: 19 November 2021 | Revised: 4 February 2022 | Accepted: 26 February 2022

DOI: 10.1111/jmp.12577

ORIGINAL ARTICLE

WILEY

Role of glycodeoxycholic acid to induce acute pancreatitis in *Macaca nemestrina*

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Abstract

Background: Acute pancreatitis exhibits a rapid clinical progression which makes it difficult to observe in human; hence, an experimental animal model is needed. This preliminary study performed an induction of acute pancreatitis using glycodeoxycholic acid (GDOC) in an experimental macaque model.

Methods: GDOC injections (initial dose of 11.20 mg/kg) were administered in an escalating manner at specific time points. The injection was given along the bilio-pancreatic duct, followed by measurement of vital signs, serum amylase-lipase, TNF-α, procalcitonin, oxidative stress parameters, and microscopic and macroscopic findings.

Results: The results indicated that acute pancreatitis occurred following induction with low-dose GDOC. Serum amylase and lipase levels increased with subsequent GDOC injections. Blood pressure and heart rate were elevated, indicating abdominal pain. Changes in TNF-α, procalcitonin, and oxidative stress values showed active inflammation. We observed histologic features of pancreatitis and as the dose increased, vasodilation of the splanchnic vasculatures was observed.

Conclusions: Small dose GDOC injection in the bilio-pancreatic duct may have a role to induce acute pancreatitis in *Macaca nemestrina*.

KEYWORDS
acute pancreatitis, experimental, glycodeoxycholic acid, *Macaca nemestrina*

1 | INTRODUCTION

Acute pancreatitis (AP) is inflammation of pancreas which commonly presents in daily clinical practice. In general, mortality is observed at 5% which increases according to severity.[1] The common causes of acute pancreatitis are gallstones (biliary pathology) estimated to be 28%–38% and alcohol consumption which accounts for 19%–41%, while the rest are due to other causes (hypertriglyceridemia, idiopathic, drug, and trauma).[2,3]

According to the Atlanta classification, most cases (approximately 80%) are mild, characterized by interstitial change of pancreas without local and systemic complications.[12] The moderate-severe classification is defined by local or systemic transient complication or transient organ failure in less than 48 hours. Severe AP is characterized by permanent organ failure.[1] In order to differentiate from other acute abdominal diseases, the diagnosis of AP is obtained if two of the three following criteria are fulfilled, (1) abdominal pain; (2) Threefold-elevated amylase serum and or lipase; and 3) radiologic imaging consistent of AP.[2,4,5]

Although mild cases are manageable with appropriate early treatment, some cases could progressively worsen and lead to severe cases within 48 h.[6] Although some etiologies of AP have been identified, the exact mechanism of organ failure is not well described.[7] Because of its rapid clinical progression, it is difficult to observe AP in humans. To date, the majority of pathogenesis models of acute pancreatitis are based on experimental study using animal models.[6,7]

Experimental animal studies are important to understand the mechanism of AP. The use of primate as an animal model in this study was based on physiologic similarities with humans which was known to be more valid than other animal models.[8] There is a variation of methods in inducing pancreatitis in animal models. The most common etiology of AP is biliary pathology. Hence, injection of molecule or ligation method can be implemented to induce pancreatitis.[7] Sodium glycodeoxycholic acid Na-GDC or GDOC is a bile acid formed in the liver, a bile acid glycine conjugate of deoxycholic acid,[9] commonly used to induce pancreatitis in various animal models. This preliminary study was performed to induce AP using glycodeoxycholic acid (GDOC) in primate *Macaca nemestrina* as an animal model.

2 | MATERIALS AND METHOD

2.1 | Animal preparation

The experimental study was performed in accordance with the Primate Research Centre (PSSP) Institut Pertanian Bogor (IPB), West Java, Indonesia, an AAALAC-accredited facility. Ethical approval was obtained from the Commission of Supervision of Animal Welfare & Use Research, Testing, Education and Capture of *Institute Pertanian Bogor*. Based on the principle of reduction, a healthy male macaque (*Macaca nemestrina)* with code B151022B, weighing 6.57 kg, was selected as the subject for this study. The experimental animal was born and housed in the facility, where it was quarantined and fed *ad libitum* on a standard diet in a conducive environment. The macaque was fasted 12 hours prior to anesthesia with no water withholding.

2.2 | Anesthesia procedure

In the preparation room, the macaque was given ketamine 10 mg/kg intramuscularly as premedication followed by insertion of 24G IV catheter and transferred to the procedural room. In the procedural room, noninvasive hemodynamic monitors were equipped, and hemodynamic parameters were recorded. A veterinarian was onsite to monitor the animal condition and perform standard anesthesia technique. The macaque was given Ringer lactate solution 4cc/kg as fluid maintenance, and endotracheal intubation was performed without neuromuscular block. Anesthesia was maintained with isoflurane 1.2 vol%. The laparotomy procedure was performed after ensuring that the macaque was completely unconscious based on minimum hemodynamic response to pain from incision with surgical knife.

2.3 | Pancreatitis induction procedure

An incision was made at the upper abdominal region. After the billiopancreatic duct was identified and the entire pancreas visualized, AP was induced by injection of GDOC using a 3ml syringe needle (Figure 1). Initial dosage of GDOC 11.20 mg/kg was injected along the billio-pancreatic duct by a standard retrograde injection technique. The induction dose was given in an escalating manner at four specific time points (0, 15, 30, and 45 mins).

2.4 | Animal monitoring and blood sample examination

During the administration of GDOC, the following parameters were measured and recorded; (1) vital signs; (2) amylase and lipase levels; (3) TNF-α and procalcitonin levels; (4) oxidative stress parameters (MDA and GSH); and (5) macroscopic findings. Noninvasive hemodynamic monitoring such as blood pressure, mean arterial pressure, heart rate, temperature, and oxygen saturation were measured continuously using the monitor attached to the animal body. Blood samples were withdrawn at 0, 15, 30, 45, 60, 120, and 180 mins. 2cc of blood was withdrawn from the femoral vein of the macaque and was processed in the PSSP IPB Laboratory. The vital signs data were recorded at the same time points as blood samples withdrawing. Fresh blood sample was examined with point-of-care testing (POCT) device (The Abbot I-Stat clinical analyzer by Abbot Laboratories), for sodium (Na), potassium (K), ionized calcium (iCa), glucose (Glu), hematocrit (Hct), hemoglobin (Hgb), blood gas analysis, and lactate.

The remaining whole blood was then centrifuged to obtain serum for measurement of amylase–lipase, tumor necrosis factor-alpha (TNF-α), procalcitonin, malondialdehyde (MDA), and glutathione (GSH) levels. We obtained TNF-α measurement using Cusabio Monkey Tumor Necrosis Factor-α Elisa Kit® and Mybiosource-Monkey Procalcitonin Elisa Kit® for PCT level. MDA levels were measured using the Wills Method with thiobarbituric acid (TBA) reaction and GSH levels were measured using the Elman Method.

2.5 | Organ preparation

After all parameters were obtained, the experimental animal was euthanized with sodium pentobarbital injection following organ collection. We collected part of the pancreas, duodenum, ileum, colon, liver, and kidney from the experimental animal and stored them in paraffin blocks. After slicing the paraffin block, hematoxylin-eosin (HE) staining was carried out to observe the existence of acinar edema, fat necrosis, inflammation (plasma cell, lymphocyte, and granulocyte beyond tissue necrosis and adipose tissue), and pancreatic edema. We calculated the accumulation of pancreatic damage scoring and compare the scoring results of the preparations. We also performed immunohistochemistry (IHC) staining in each preparation to evaluate NF-κB expression using Abcam Anti- NF-κB p65 Antibody Kit®.

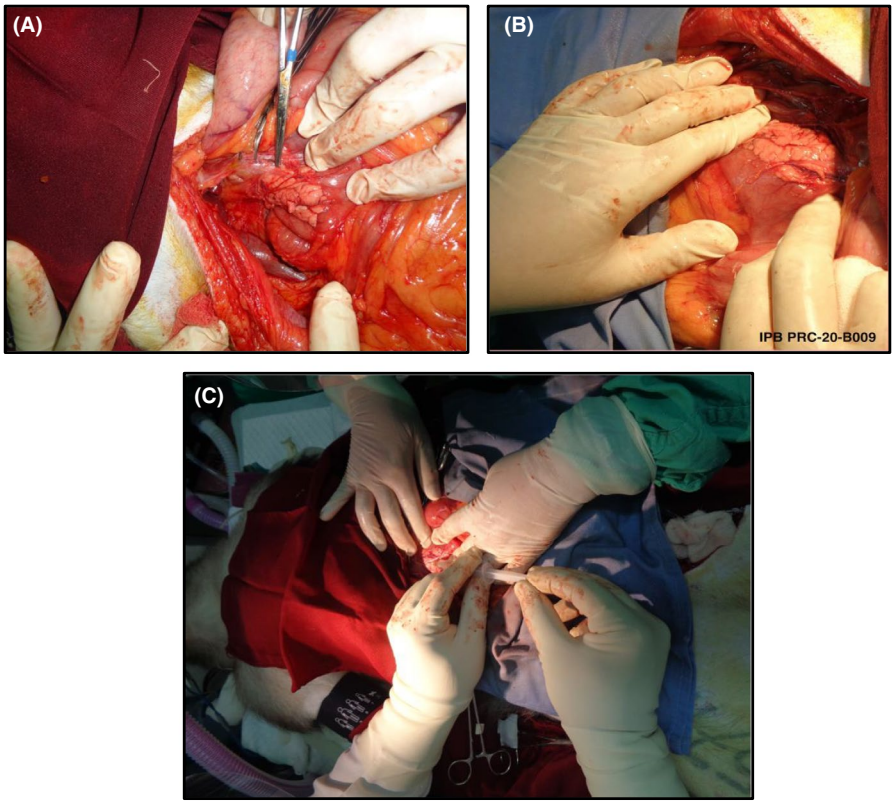

FIGURE 1 Surgery documentation. Annotations: The figure consist of three moments in the surgical procedure of the experimental animal. (A) identification of the bilio-pancreatic duct; (B) visualization of the pancreas; and (C) retrograde injection of GDOC

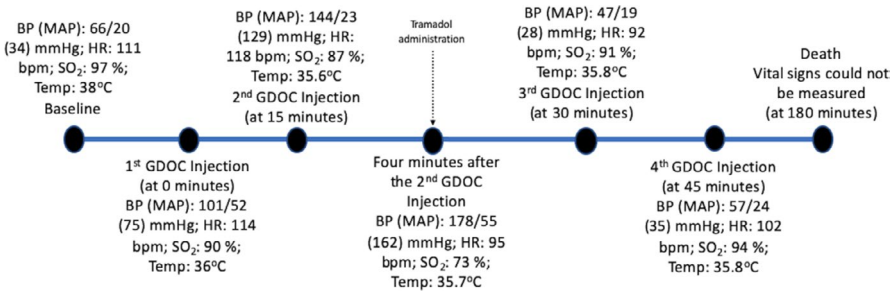

FIGURE 2 Flowchart of vital signs and alteration throughout GDOC injections

3 | RESULTS

3.1 | Vital signs

The measurement of vital signs was performed constantly alongside the induction of GDOC. We observed that blood pressure began to increase following the initial dose of GDOC. Eventually after the second dose injection, the blood pressure began to increase significantly in comparison with baseline value (178/55 mm Hg). Peak levels were observed 4 mins following the second dose. Meanwhile, oxygen saturation levels continued to diminish. To avoid undesirable events, tramadol injection was administered in response to the high blood pressure. Afterwards, the blood pressure rapidly went down, in line with the oxygen saturation level that increased over time. Vital signs were maintained close to the baseline value until the animal was euthanized (Figure 2).

3.2 | Amylase and lipase results

The measurements of amylase and lipase levels were obtained in seven specific time points. Baseline level of serum amylase (prior induction with initial dose of GDOC) was 237 U/L. An initial increase of serum amylase levels was observed at 15 mins following the initial dose injection. Although GDOC was last administered at 45 mins, serum amylase continued to increase until it reached its peak at 180 mins. Similar finding was observed for serum lipase. Baseline serum lipase was measured at 8 U/L. 15 mins after initial GDOC injection, serum lipase levels increased about 23 times compared with baseline. Upon the second injection, an increase in the serum lipase levels was continuously observed and reached peak levels at 120 mins. However, differing from amylase, lipase levels decreased at 180 mins. (Table 1).

TABLE 1 Results of amylase and lipase levels

Lab test	Laboratory results time point (mins)						
Amylase (U/l)	M−0* (GDOC 11.20 mg/kg)	M−15 (GDOC 22.40 mg/kg)	M−30 (GDOC 44.80 mg/kg)	M−45 (GDOC 89.60 mg/kg)	M−60	M−120	M−180
	237	346	475	555	644	891	909
Lipase (U/l)	M−0* (GDOC 11.20 mg/kg)	M−15 (GDOC 22.40 mg/kg)	M−30 (GDOC 44.80 mg/kg)	M−45 (GDOC 89.60 mg/kg)	M−60	M−120	M−180
	8	184	288	400	390	570	440

Note: Annotations: M-0, at 0 minute; M-15, at 15 mins; M-30, at 30 mins; M-45, at 45 mins; M-60, at 60 mins; M-120, at 120 mins; M-180, at 180 mins.

*Baseline.

3.3 | TNF-α and procalcitonin levels

We observed baseline level value of TNF-α at 3584.10 pg/ml. At 15 mins, the value increased to 3739.08 pg/ml. Further increase was observed at 30 mins, TNF-α value was observed at 4035.76 pg/ml. At 45 mins, TNF-α levels decreased to 3401.96 pg/ml. TNF-α values then gradually increased, reaching peak levels at 180 mins (Table 2). Procalcitonin exhibited a similar trend to TNF-α. Procalcitonin baseline level was measured at 214.38 pg/ml. Procalcitonin levels kept increasing until the 45 mins time point, in where a decrease was observed at 60 mins, and peak level was observed at 120 mins (544.65 pg/ml) (Table 2).

3.4 | Oxidative stress parameters

MDA baseline value was obtained at 1.204 nmol/L. MDA levels exhibited a decreasing trend until the 120 mins time point where it increased to 1.305 nmol/L, while the final measurement at 180 mins was obtained at 0.877 nmol/ml (Table 2). We observed no clear trend in GSH levels in plasma as levels fluctuated with the lowest measured at 1.508 ug/ml and highest 1.712 ug/ml with a baseline level of 1.533 ug/ml (Table 2).

3.5 | Microscopic and macroscopic findings

We obtained nine different histopathology preparations from different parts of the pancreas (caput, corpus, and caudal), duodenum, ileum, colon, cecum, kidney, and liver. Under the microscope, we calculated the histological scoring for AP as presented in Table 3.

Signs of inflammation and severity of inflammation were found highest in the head of pancreas (caput) preparation. We observed that acinar necrosis was found in more than a third of the preparations. We also observed moderate infiltration of inflammatory process and intercellular edema in more than two lobules in the head of pancreas. We also observed that other organs showed signs of inflammation and necrosis. Other than the pancreas, other organs nearby the pancreas such as the duodenum, cecum, liver, and kidney all exhibit a degree of cellular damage similar to the pancreas. (Table 2 and Figure 3).

We also observed the expression of NF-κB using IHC staining (Figure 4). The positive expression of NF-κB was classified into strong, moderate, and weak. All preparations exhibited positive expression for NF-κB. From the head of pancreas, a moderate expression of NF-κB in the lobules (about 20%–50% of the area) was found, while a strong expression of NF-κB was observed in the Langerhans islet (about 50%–80% of the area). From the corpus of the pancreas, we found moderate expression in the lobules (about <20% of the area) and moderate expression as well in the Langerhans islet (about 80% of the area). From the tail of the pancreas, we found moderate expression in the lobules (<20% of the area) and strong expression in the Langerhans islet (about >80% of the area). We also observed NF-κB in nearby organs with varying degrees. Strong expression was observed in more than 80% of the preparation area in the ileum sample. Moderate expression was observed in more than 80% of the preparation of the duodenum, colon, and cecum samples. From the liver sample, we observed moderate expression in 50–80% of the preparation area. Lastly, we observed strong expression in the tubules of the kidney (50%–80% of the preparation area) with none in the glomerular space.

During the laparotomy procedure, we visualized the pancreas and surrounding organs, such as the duodenum and colon. We observed that as the dose increases, the splanchnic vasculature became more dilated. As seen on pictures, the mesenteric vessels are vasodilated (Figure 5).

4 | DISCUSSION

The key finding of this study indicated that low-dose GDOC (11.20 mg/kg) successfully induced pancreatitis in animal model. We observed an increasing trend of amylase and lipase values in correlation with GDOC administration. In line with conservative diagnostic method for pancreatitis in humans,[10] serum amylase and lipase can be used to diagnose pancreatitis in animal model. However, normal reference value of serum amylase and lipase are not well defined, as it is not as extensively studied as humans. Thus, the increase of

TABLE 2 Results of TNF-α, procalcitonin, MDA, and GSH levels

No	Time point (Mins)	TNF-α (pg/ml)	Procalcitonin (pg/ml)	MDA (nmol/ml)	GSH (ug/ml)
1	M−0*	3584.10	214.38	1.204	1.533
2	M−15	3739.08	250.25	0.708	1.635
3	M−30	4035.76	355.44	0.809	1.661
4	M−45	3401.96	434.54	0.517	1.482
5	M−60	3557.04	405.50	0.832	1.533
6	M−120	3767.83	544.64	1.305	1.508
7	M−180	4187.08	392.98	0.877	1.712

Note: Annotations: M-0, at 0 minute; M-15, at 15 mins; M-30, at 30 mins; M-45, at 45 mins; M-60, at 60 mins; M-120, at 120 mins; M-180, at 180 mins.

*Baseline.

TABLE 3 Histological scoring for acute pancreatitis

No	Parameters	Preparations								
		Caput P.*	Corpus P.	Caudal P.	Duodenum	Ileum	Colon	Cecum	Liver	Kidney
1	Acinar Necrosis - Nil (0) - <10 single necrosis/lobule (1) - ≥10 single necrosis/lobule (2) - >1/3 plane (3)	3	2	2	3	3	2	3	3	2
2	Fat necrosis - Nil (0) - <1/3 plane (1) - >1/3 plane (2) - ≥2/3 plane (3)	2	2	1	1	0	1	1	0	2
3	Inflammation (plasma cell, lymphocyte, and granulocyte beyond tissue necrosis and adipose tissue) - Nil (0) - Rare infiltration/*loose* (<30 cells/HPF**) (1) - Moderate infiltration/*moderate* (>30 cells; <10 cells/HPF) (2) - Dense Infiltration/*dense* (>100 cells/HPF) (3)	2	1	1	3	2	1	2	2	2
4	Edema - Nil (0) - Interlobular edema (1) - Inter-acinar edema, ≥2 lobule (2) - Intercellular edema, ≥2 lobule (3)	3	1	1	1	2	2	2	3	2
Total Score		10	6	5	8	7	6	8	8	8

*Pancreas; ** High Power Field.

serum amylase and lipase over time compared with baseline was used as the study parameter instead.

In the study, amylase and lipase value immediately increased after first administration of GDOC. This simulates AP in where pancreatic enzymes such as amylase, lipase, and trypsin are released to the bloodstream. One postulated factor is the release of trypsin due to pancreatic acinar cells (PAC) hyperstimulation. Trypsin further induces other pancreatic enzymes activation which leads to pancreatic and peripancreatic inflammation. Among these enzymes, lipase was found to be more sensitive and specific in diagnosing pancreatitis. Longer half-time of lipase creates wider timeframe window of convenient parameter retrieval and better cost-efficiency.[10–12]

Compared with a previous study, based on the most common cause of AP, gallstone obstruction causes biliary sludge to reflux

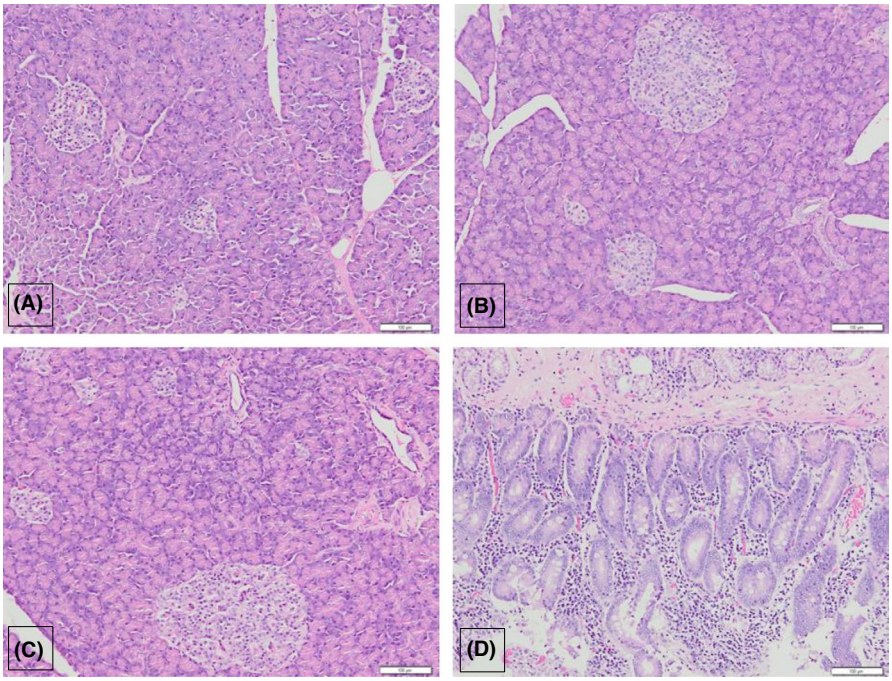

FIGURE 3 Microscopic findings. Annotations: The figure consist of four different HE staining preparations under 100× microscope magnification. Upper left (A) Head of Pancreas (Caput); upper right (B) body of pancreas (corpus); lower left (C) tail of pancreas (caudal); and (D) duodenum

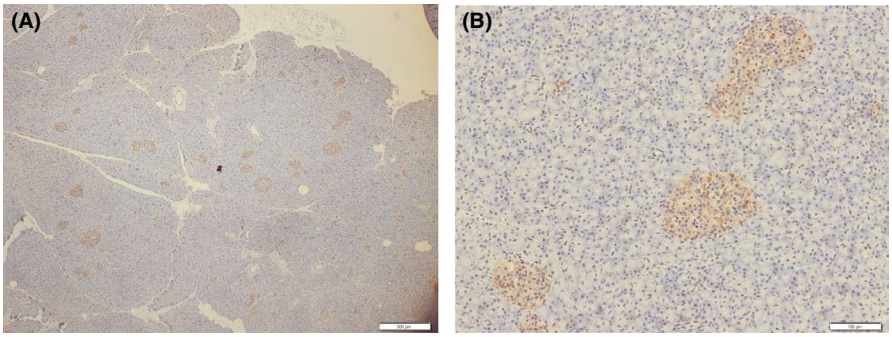

FIGURE 4 NF- κB expression in IHC staining. Annotations: The figure consist of two different preparations of NF-κB expression in IHC staining. From the left (A) head of the pancreas; (B) tail of the pancreas

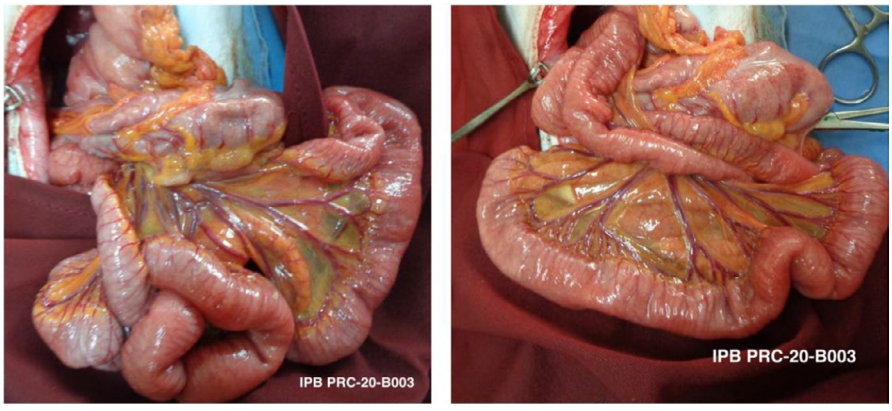

FIGURE 5 Macroscopic view of the vasodilatation of mesenteric vessels

through pancreatic duct and contact with PAC. Bile acid induce PAC injury by overloading intracellular concentration of Ca^{++}, halting intracell mitochondrial ATP production.[7,12] Another in vivo study demonstrated that bile acid exposure induced pancreatic injury due to Ca^{++} generated calcineurin activation.[13] One of the earliest models for studying pancreatitis in animal model was performed by ligation of the bile-pancreatic duct in animal models, the study successfully induced AP in rats.[14] However, this model was unable to assess the particular dose of which bile acids causes PAC injury, and thus, unable to control the extent of induced AP.[7,12]

Bernard et al. in 1956 introduced cannulation to ampulla of vater and retrograde injection of bile acid and olive oil in canines.[12] The method enabled scientists to modify the degree of induced AP and study the dose-dependent correlation of its inducers. Thus, various other chemicals and mixtures were used to induce AP in various animal models, the most common being sodium chenodeoxycholate

(Na-CDC), Na-GDC/GDOC, sodium taurodeoxycholate (Na-TDC), sodium taurocholate (Na-TC), and taurolithocholic acid 3-sulfate (TLC-S).[12] These procedures still require simultaneous ligation of pancreatic duct which limits its use in recent studies. Na-TDC was introduced with or without trypsin and causes AP and multiple organ dysfunction syndrome (MODS) in rats. However, its subsequent use in other animal models were limited. Na-TC and TLC-S are most widely used and is known to best characterize bile acid for inducing AP in rabbits, swans, dogs, and pigs. However, Na-TC and TLC-S have never been used in primates.[12]

GDOC is another bile salt which has well characterization in animal model. Its advantage is a well observed dose-dependent effect on inducing AP. GDOC at increasing dose (8.5 mM, 17 mM, and 34 mM) caused progressive, severe, but non-lethal AP in rats. An addition of 200 ng enterokinase further escalated the induced AP to be lethal. A previous study by Wan et al.[12] studied GDOC (5 mM or 10 mM) combined with IV cerulein injection to induce moderate AP that lasted ≥24 h. The longer time window provides potential of severity modulation through other substance injection such as hypothetical drugs.[12] These advantages have justified GDOC use in recent studies which focuses on evaluation of new therapeutic modalities.

Previous animal studies in inducing AP were mostly done in rats, mouse, dog, rabbit, and pig. No previous studies at the time of conception of this study were ever performed on primates such as Macaques as this study.[12] Though primates such as Macaques are genetically closer to humans; ethical clearance, clinician skills, and facilities to intervene primates had become the primary challenges to conduct such studies. The scarcity of primate model in publication and the success of inducing AP in this animal model confirm the strength and novelty of this study.

Another finding corroborating the presence of AP is the changes in vital signs. Unlike in human, animals are unable to express pain in words. In a conscious state, we are able to observe pain response in animals by observing behavioral changes such as guarding or avoiding walking because of injured limb or simply by moaning.[15] However, in this study, our subject was under general anesthesia. Thus, we observed changes in vital signs instead of behavioral changes.

At the first GDOC injection, we observed an increase in blood pressure and heart rate. The blood pressure reached its peak following the second dose. The perception of acute pain plays an adaptive role as a measure to prevent tissue damage. The activation of the ascending nociceptive spinal reflex triggers the sympathetic nervous system which manifests as increased peripheral resistance, heart rate, and stroke volume.[16] Thus, it can be presumed that the increased of blood pressure and rapid pulse response are manifestations of pain.

TNF-α levels exhibited an increasing trend in correlation with the addition of GDOC injections. TNF-α is an inflammatory mediator hypothesized to play an important role as a predictor of inflammation in acute pancreatitis. Several in vitro and experimental studies have suggested its role in pancreatitis.[17] However, there is a lack of studies confirming this, especially in primates. TNF-α is a pleiotropic cytokine that acts as a central regulator of inflammation. It is secreted by monocytes, macrophages or even by acinar cells in AP. An in vitro study by Manohar et al.[18] suggested that the acinar cells of the pancreas are able to produce, release and even respond to TNF-α. Furthermore, it suggested that TNF-α could activate NF-κB based on the fact that the inhibition of NF-κB led to the decrease of inflammation response in AP.[18]

The increasing trend of TNF-α levels is considered to be related to the inflammatory process in our experimental animal. The use of TNF-α serum level as an evaluation predictor in AP has been challenging, attributed to the fact that it is rapidly cleared in the blood stream. Thus, it makes the measurement of TNF-α serum level time dependant.[17] Kiyici et al.[19] indicates that both acute and chronic pancreatitis show an increase in serum TNF-α. A study from Japan also showed that the use of anti-TNF-α therapy in AP showed promising results.[20] These facts indicate a strong association between TNF-α and AP.

Procalcitonin is a calcitonin pro-peptide which is associated with severe infection and inflammation.[21] In our study, serum procalcitonin fluctuated but tend to increase along with subsequent GDOC injections. There is still a lack of studies regarding procalcitonin in primates. One prospective study by Dias B et al,[22] aimed to evaluate the prognostic efficacy of procalcitonin plasma value in 50 AP human patients. It observed that the cutoff value of procalcitonin >2ng/mL showed 100% sensitivity and 100% specificity in predicting AP. Another cutoff procalcitonin value studied was >19 ng/ml which showed 70% sensitivity and 65% specificity in predicting the progression of mild acute pancreatitis (MAP) to severe acute pancreatitis (SAP).[22]

Another study conducted in India by Madhu et al.,[21] proved that in all of their 40 human patients with AP, there was an increase of serum procalcitonin. 7 out of 40 patients were observed to have a procalcitonin value of >0.5 ng/ml. However, the study did state that procalcitonin was not recommended to be used reliably as a predictor for severity of AP.[21] In contrary, a systematic review of 24 studies conducted by Modifi et al.[23] suggested that serum procalcitonin may be of value in predicting severity of AP.[23]

Besides the involvement of cytokines, free radicals play an essential role in inflammation. In AP, acinar cells, pancreatic stellate cells produce reactive oxygen species (ROS) and reactive nitrogen species. ROS induces pro-inflammatory cytokine via the signaling pathway.[24] Oxidative stress can be measured by anti-oxidants such as GSH or degradation products like MDA. In our study, we managed to detect the presence of free radicals in our experimental animal. MDA and GSH levels tend to fluctuate over subsequent GDOC injections in our experimental animal. This finding was not aligned with our hypothesis, as in high degree or acute inflammation, the antioxidant GSH is expected to be lower, while MDA, a degradation product is expected to increase overtime.

In Hilal M. et al.,[25] a study which involved 51 AP human patients, 23 of 51 AP patients presented with severe acute pancreatitis. The study showed that an increase in the levels of MDA in severe acute pancreatitis patients was similar to those with mild acute pancreatitis

patient. The study also studied superoxide dismutase levels which was found to be decreased.[25] Rau B et al.[26] conducted an experimental study with 200 Wistar mouses, in where acute pancreatitis was inducted with 3% sodium taurocholate and found that there was an increase of MDA expression in all study subjects.

Based on our histopathologic preparations, varying degrees of inflammation in the pancreas and surrounding organs was observed. Our results indicate that the head of pancreas is the most affected part in AP. This finding may suggest that the degree of inflammation in each part of the pancreas may vary. The inflammation of surroundings organs reflects organ necrosis and might indicate organ failure. This may be attributed to systemic inflammation induced by pancreatitis which in turn leads to organ failure. Pro-inflammatory cytokines produced by AP triggers the release of NF-κB, and we were, in turn, able to observe a positive NF-κB expression in all of our preparations.[18,27,28] In our study, GDOC was administered directly to the pancreatic duct, thus it is very unlikely that GDOC itself was the cause of necrosis in surrounding organs. Necrosis in surrounding organs were most likely attributed to the release of TNF-α, mediating a cytokine cascade reaction that promotes systemic inflammatory reaction syndrome (SIRS) causing inflammation, ischemia, and necrosis in surrounding organs.[29]

Macroscopically, we observed the splanchnic vasculatures became dilated. As inflammation of the pancreas increased, surrounding vasculature and organs were affected as well. The changes of both macro and micro circulation are thought to contribute to disease progression. Capillary permeability increases, leading to a decrease in circulating blood flow, in particular the splanchnic blood flow which can be observed as the dilatation we observed. Other vascular complications of severe pancreatitis may include intestinal ischemia, hepatic hyperemia, subcutaneous fat necrosis, and vascular retinopathy.[30,31]

A limitation of this study was that histopathologic preparations were not obtained in every time point. Considering the small volume of pancreas in the macaque, constant tissue biopsies would lead to bleeding and endanger the animal's hemodynamics. Samples taken at every time point would have provided more insight to histopathologic changes in the process of acute pancreatitis. Further studies could be conducted with a larger experimental animal in where multiple biopsies would be feasible.

5 | CONCLUSION

In conclusion, GDOC injections in an escalating manner to the biliopancreatic duct successfully induced the development of acute pancreatitis in an experimental macaque model. These results could be used as future development of experimental model of acute pancreatitis to better understand the pathogenesis of acute pancreatitis.

ACKNOWLEDGMENT

We gratefully thank Prof. dr. Azis Rani, Prof. dr. Suhendro, and Prof. dr. Marcellus Simadibrata for the valuable discussion and critical reading on the manuscript; Prof. drh. Dondin (deceased) for the guidance and support; drh. Permana for performing the surgery and for dr. Rosvitha Amanda for assistance in writing the manuscript.

CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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How to cite this article: Fauzi A, Suhendro, Simadibrata M, et al. Role of glycodeoxycholic acid to induce acute pancreatitis in *Macaca nemestrina*. *J Med Primatol*. 2022;51:134–142. doi:10.1111/jmp.12577