Effect of Intravenous Polyunsaturated Fatty Acids Administration on Gastric Mucosal Integrity in Pig-tailed Macaques with Obstructive Jaundice

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ABSTRACT

Background: *Acute gastric mucosal injury commonly occurs in patients with obstructive jaundice. We studied the effect of intravenous polyunsaturated fatty acids (PUFA) administration on gastric mucosal integrity in pig-tailed macaques (Macaca nemestrina) with obstructive jaundice by ligating common bile duct (CBD).*

Method: *The study was conducted between February 2009 and May 2010 at the Primate Research Center, Bogor Agricultural Institute. Eight selected male pig-tailed macaques with 6.625 ± 0.83 kg of body weight were used and divided into two groups. In both groups, laboratory examination, including liver function tests and upper gastrointestinal endoscopy were performed before CBD ligation and every two weeks after ligation. In the first group, intravenous PUFA with the dose of 2 g/day was administered every day since four weeks post-ligation up to four weeks later, and in the second group, intravenous PUFA was administered since before ligation up to eight weeks later.*

Results: *In both groups, increased total bilirubin, direct bilirubin, indirect bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma glutamyl transpeptidase were obviously found, meanwhile decreased albumin level was detected and the cholinesterase level of both groups remained unchanged. Ulcer formation occurred among the first group during 4 weeks after CBD ligation, and these ulcers showed obvious healing within four weeks after intravenous PUFA administration. In the second group, there was no significant ulcer formation within eight weeks after CBD ligation.*

Conclusion: *The potential appearance of acute gastric mucosal injury which reflected by ulcer formation in pig-tailed macaques with obstructive jaundice was significantly decreased by intravenous PUFA administration. We also have successfully developed animal model of obstructive jaundice by CBD ligation, based on the result of liver function tests.*

Keywords*: acute gastric mucosal injury, intravenous PUFA administration, obstructive jaundice, pig-tailed macaques*

ABSTRAK

Latar Belakang*: Kerusakan mukosa lambung akut biasanya terjadi pada pasien dengan ikterus obstruktif. Pada penelitian ini dipelajari pengaruh pemberian polyunsaturated fatty acids (PUFA) secara intravena terhadap integritas mukosa lambung dengan ikterus obstruktif pada beruk (Macaca nemestrina) yang dilakukan ligasi duktus koledokus.*

Metode*: Penelitian dilakukan pada Februari 2009−Mei 2010 di Pusat Studi Satwa Primata, Institut Pertanian Bogor (IPB). Delapan ekor beruk jantan dengan berat badan 6,625 ± 0,83 kg dibagi menjadi 2 kelompok. Pada kedua kelompok tersebut dilakukan pemeriksaan laboratorium, termasuk pemeriksaan fungsi hati dan dilakukan endoskopi saluran cerna bagian atas sebelum ligasi dan setiap dua minggu setelah ligasi dilakukan. Pada kelompok pertama PUFA diberikan secara intravena dengan dosis 2 g/hari, sejak empat minggu paska ligasi*

sampai empat minggu kemudian. Pada kelompok kedua, PUFA diberikan secara intravena sebelum dilakukan ligasi sampai delapan minggu kemudian.

***Hasil**: Pada kedua kelompok ditemukan peningkatan bilirubin total, bilirubin direk, bilirubin indirek, AST, ALT, alkali fosfatase dan gamma glutamil transpeptidase, sedangkan kadar albumin terdeteksi menurun dan kadar kolinesterase dari kedua kelompok tidak berubah. Pembentukan ulkus terjadi pada kelompok pertama selama empat minggu setelah dilakukan ligasi duktus koledokus, dan ulkus yang terbentuk menyembuh dalam waktu empat minggu setelah pemberian PUFA secara intravena. Pada kelompok kedua, tidak terjadi pembentukan ulkus yang signifikan dalam delapan minggu setelah dilakukan ligasi duktus koledokus.*

***Kesimpulan**: Terjadinya kerusakan mukosa lambung akut yang tercermin dari pembentukan ulkus pada beruk dengan ikterus obstruktif secara signifikan menurun dengan pemberian PUFA secara intravena. Penelitian ini juga telah berhasil mengembangkan hewan model dengan ikterus obstruktif dengan melakukan ligasi duktus koledokus, terbukti dari hasil pemeriksaan fungsi hati.*

***Kata kunci**: kerusakan mukosa lambung akut, pemberian PUFA secara intravena, ikterus obstruktif, beruk*

INTRODUCTION

In obstructive jaundice, gastric mucosal injury is frequently found in the form of erosion or ulcer. Both conditions usually lead to bleeding or even perforation. Gastric mucosal injury is caused by imbalance between aggressive and defensive factors. Aggressive factors may be external (i.e. non-steroid anti-inflammatory drugs, alcohol, and *Helicobacter pylori)* or internal (gastric acid, bile salt and its components). Moreover, defensive factors include mucus barrier, bicarbonate, prostaglandins, and gastric mucosal blood flow. The defensive factors have role in maintaining gastric mucosal integrity against various aggressive factors.[1,2] There are numerous mechanisms for pathogenesis of acute gastric mucosal injury in patients with obstructive jaundice including the role of oxidative stress such as lipid peroxidation and bile acid in the blood circulation.[3,4,5,6,7] Obstructive jaundice may occur due to mechanical obstruction in the extrahepatic biliary duct caused by various etiologies. It is characterized by increased bilirubin blood level, which causes yellow eyes and yellow skin. Data from Cipto Mangunkusumo hospital Jakarta in 2007 regarding the results of endoscopic retrograde cholangiopancreatography (ERCP) associated with obstructive jaundice demonstrated that most cases are caused by choledocholithiasis (54%), tumors of papilla of Vater (17%), pancreatic head tumor (13%), common bile duct structure (5%), cholangiocarcinoma (2%), Klatskin tumor (2%) and the rest (7%) with unknown etiology.[8] The above mentioned facts are assumed to be associated with the alteration of phospholipid plasma or tissue level.

Phospholipid has important role in gastric mucosal integrity, i.e. as part of cell membrane, one of factors that form mucus barrier,[1] as well as primary source of prostaglandin through arachidonic acid pathway, both in blood circulation and body tissues including the gastric mucosa. In addition, the cells of gastrointestinal mucosa (enterocytes) also need nutritional elements which are obtained by oral or enteral and parenteral route. In obstructive jaundice, there is impaired absorption of enteral lipid due to diminished or reduced bile salts and its components which have very important role in lipid metabolism.[9,10] Since poly-unsaturated fatty acids (PUFA) is one of main elements in the formation of phospholipids, hence the PUFA administration is assumed to have effect on gastric mucosal integrity.[11,12]

The aim of this study was to review the effect of intravenous PUFA administration on gastric mucosal integrity in pig-tailed macaques (*Macaca nemestrina)* with obstructive jaundice by ligating common bile duct (CBD). The pig-tailed macaques were selected as the animal experimental model based on their similar gastrointestinal system to human. Furthermore, the nutrient requirements are comparable to human.[15,16,17] Up to now, studies about obstructive jaundice have only been conducted in other experimental animal model such as rats, rabbits and dog.[18,19] This study may provide advantage on preventive efforts against gastric mucosal injury in patients with obstructive jaundice.

METHOD

The study was conducted between February 2009 and May 2010 at the Primate Research Center, Bogor Agricultural Institute after having approval by the Animal Care and Use Committee (ACUC) number P.10-08-IR dated October 20th, 2008. Eight adult male pig-tailed macaques were used in our study; their age was between 4-6 years with body weight of 5.5-7.5 kg. Those animals were adapted to the experimental

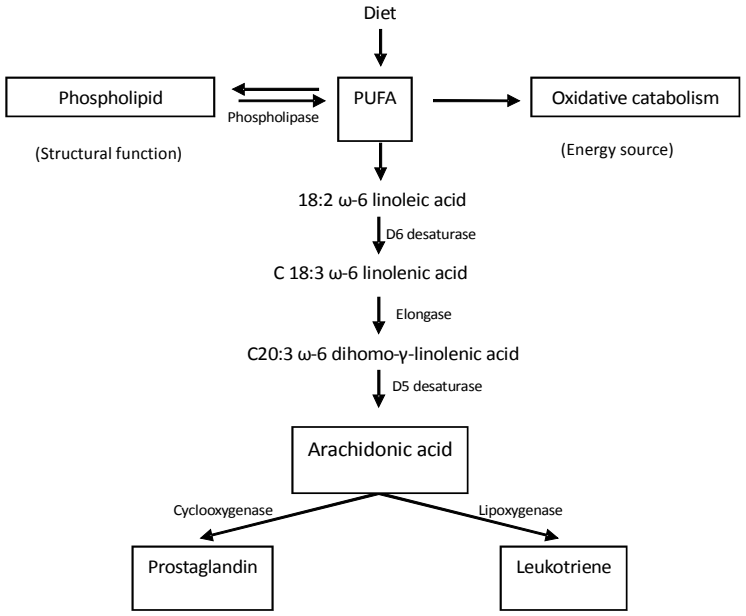

Figure 1. Metabolism pathway of PUFA and their signaling molecules (adapted from Sessler[13] and Coste[14])

situation and placed in individual cage. Monkey chow biscuits (enriched with protein, lipid, mineral, fiber, starch, calcium and phosphate) and fruits were provided; while water was provided sufficiently.

Instruments utilized in our study were gastrointestinal endoscope from Olympus® Excera 160 type including its accessories (light source, video monitor, printer, suction pump and biopsy forcep), a laparatomy surgical instrument set and laboratory instruments as well as the reagents for blood biochemistry analysis, i.e. total protein (TP) level, albumin (Alb), globulin (Glob), total bilirubin (TB), direct bilirubin (DB), indirect bilirubin (IB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT) and cholinesterase (CHE) level.

After 2-weeks adaptation, the experimental animals were randomly categorized into two groups, included four animals each of group. In group I, upper gastrointestinal (GI) endoscopy were performed following the biochemistry analysis which included TP, Alb, Glob, TB, DB, IB, AST, ALT, ALP, GGT, CHE in order to evaluate the macroscopic appearance of gastric mucosa on the presence of erosion or ulcer. Subsequently, CBD was ligated through laparotomy surgery. It was ligated distal to the opening of cystic duct but proximal to the point of entry of pancreatic duct. In addition to daily observation on the experimental animals, routine observations were performed every 2 weeks, including body weight evaluation, upper GI endoscopy to observe macroscopic appearance of gastric mucosa and blood biochemistry analysis. Following the second routine observation (4 weeks after CBD ligation), intravenous PUFA with the dose of 2 g/day was administered. Routine observations were performed every two weeks up to 8 weeks post CBD ligation. Afterward, euthanasia was performed for all animals.

In group II, similar evaluation procedures as the first group were also performed including the upper GI endoscopy, blood biochemistry analysis and routine observation every 2 weeks up to 8 weeks post CBD ligation. Intravenous PUFA administration was given at the dose of 2 g/day since prior to ligation up to 8 weeks following the CBD ligation. Then, euthanasia was performed for all animals.

The observed variables were biochemistry level of liver function test (TP, Alb, Glob, TB, DB, IB, AST, ALT, ALP, GGT, and CHE) and macroscopic evaluation of gastric mucosa by upper GI endoscopy on experimental animals that had undergone CBD ligation in each group.

RESULTS

The study results of eight male pig-tailed macaques with average 6.625 ± 0.83 kg of body weight and age range between 4 and 6 years, is presented in the form of biochemistry analysis of liver function test and macroscopic appearance of gastric mucosa. The results of biochemistry analysis on liver function test of all experimental animals are presented on tables below, i.e. general liver function test (Table 1) and a more specific liver function test associated with biliary obstruction (Table 2).

The results of 8-weeks observations, biochemistry analysis on liver function test of all experimental animals both in group I and group II demonstrated an increase pattern of bilirubin blood level, decreased albumin level and increased level of liver enzymes, i.e. AST, ALT, ALP, and GGT, as well as decreased

Table 1. Mean value and variation coefficient on general biochemistry analysis results of liver function tests

	G	N	Observation (week)					Normal
			0	2	4	6	8	
TP	I	4	6.80 (6.62)	7.05 (7.94)	7.45 (9.13)	7.15 (7.97)	6.90 (3.77)	7.64 ± 1.24
(g/dL)	II	4	7.27 (7.29)	7.52 (6.65)	6.92 (5.78)	7.25 (1.38)	7.20 (11.11)	
Alb	I	4	5.32 (11.09)	4.25 (10.12)	4.72 (12.50)	4.25 (11.29)	3.80 (35.00)	4.40 ± 0.35
(g/dL)	II	4	5.15 (6.02)	4.35 (8.51)	4.12 (11.89)	4.12 (8.98)	4.20 (16.20)	
Glob	I	4	1.47 (55.10)	2.8 (26.43)	2.72 (16.91)	2.90 (32.07)	3.10 (34.52)	
(g/dL)	II	4	2.12 (31.13)	3.2 (23.97)	2.80 (17.14)	3.12 (9.29)	3.00 (48.00)	
AST	I	4	49.7 (21.13)	156.2 (19.14)	137.0 (28.98)	194.2 (31.98)	148.70 (8.41)	49.78 ± 12.85
(U/L)	II	4	47.9 (30.69)	181.7 (45.18)	167.7 (47.11)	221.7 (33.42)	242.70 (28.43)	
ALT	I	4	36.0 (26.11)	283.5 (32.66)	292.3 (16.97)	306.5 (21.34)	297.8 (15.88)	52.18 ± 33.27
(U/L)	II	4	47.5 (20.42)	340.3 (23.39)	345.8 (42.37)	446.0 (53.36)	430.0 (36.09)	
CHE	I	4	18.6 (32.8)	15.3 (24.15)	14.95 (21.54)	15.95 (23.11)	15.18 (26.35)	
(U/L)	II	4	13.31 (73.5)	11.6 (58.24)	11.63 (61.31)	13.31 (56.95)	12.34 (49.35)	

G: Group; TP: total protein; Alb: albumin; Glob: globulin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CHE: cholinesterase

Table 2. Mean value and variation coefficient on biochemistry analysis results of liver function tests associated with biliary obstruction

	G	N	Observation (week)					Normal
			0	2	4	6	8	
TB	I	4	0.77 (44.16)	12.8 (32.55)	14.22 (6.12)	16.07 (13.63)	16.32 (17.95)	0.39 ± 0.20
(mg/dL)	II	4	0.87 (37.93)	9.20 (25.54)	14.77 (40.01)	15.17 (12.59)	15.15 (6.14)	
DB	I	4	0.42 (52.38)	8.77 (9.81)	8.25 (23.15)	11.27 (14.64)	12.74 (7.46)	
(mg/dL)	II	4	0.52 (36.54)	6.45 (20.47)	10.20 (34.31)	9.58 (26.41)	10.37 (8.78)	
IB	I	4	0.35 (54.29)	3.98 (101.26)	5.97 (46.23)	4.80 (18.96)	3.58 (60.61)	
(mg/dL)	II	4	0.35 (57.14)	2.75 (55.64)	4.58 (54.59)	5.60 (33.93)	4.77 (11.53)	
ALP	I	4	1,126 (80.99)	9,873 (57.91)	10,962 (75.67)	15,890 (33.36)	16,251 (34.69)	728.65 ± 524.00
(U/L)	II	4	1,617 (27.52)	9,775 (75.76)	12,311 (45.13)	16,842 (50.76)	14,797 (35.78)	
GGT	I	4	90.50 (19.34)	259.7 (17.02)	280.20 (30.19)	372.0 (27.23)	357.5 (7.22)	74.55 ± 31.38
(U/L)	II	4	98.5 (109.85)	268.0 (67.01)	411.00 (51.34)	481.0 (37.86)	459.0 (35.27)	

G: group; TB: total bilirubin; DB: direct bilirubin; IB:indirect bilirubin; ALP: alkaline phosphatase; GGT: gamma glutamyl transpeptidase

CHE level. Meanwhile, the globulin level in almost all experimental animals was likely increasing. Elevated bilirubin level indicated that there was total obstruction of CBD as expected in our study. Such results are consistent with increased biochemistry results on liver function enzymes, i.e. AST, ALT, ALP and GGT levels.

Macroscopic Appearance of Gastric Mucosa

By upper GI endoscopy following CBD ligation in group I, we found 19 corpus ulcers after 2 weeks which turned out to be 26 ulcers after 4 weeks. Moreover, we found 4 ulcers at the antrum after 2 weeks and it became 15 ulcers within 4 weeks. In group II, we found 2 ulcers in the corpus and it became 3 ulcers after 4 weeks period; while in the antrum, we found no ulcer at all either on the 2-week evaluation or 4 weeks after CBD ligation.

In group II, the upper GI endoscopy showed a clear reduction in the number of ulcers at the corpus, i.e. from 26 to 9 cases within 2 weeks after the administration of intravenous PUFA, which turned out to be only one ulcer after 4 weeks of intravenous PUFA administration. In addition, at the antrum, there was reduction in the number of ulcers from 15 into 4 ulcers within 2 weeks after the administration of intravenous PUFA, and no ulcer was found at all within 4 weeks. Furthermore, in this group, no ulcer was found at 6-week and 8-week evaluation following the CBD ligation, neither in corpus nor antrum region. The macroscopic changes of gastric mucosa at week 0, 2, 4, 6 and 8 are illustrated on Figure 1 and 2. The observation results on the number of corpus and antrum ulcer are presented on Figure 3 and 4 that did not receive intravenous PUFA.

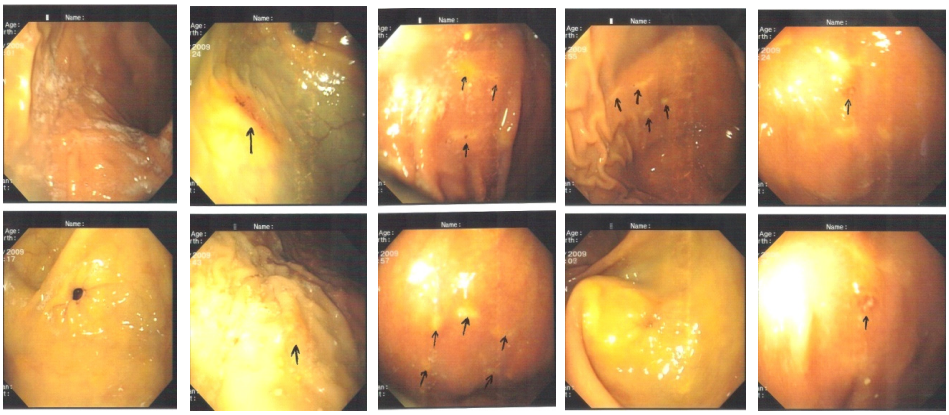

Figure 1. Macroscopic observation of gastric mucosa in experimental animals of group I

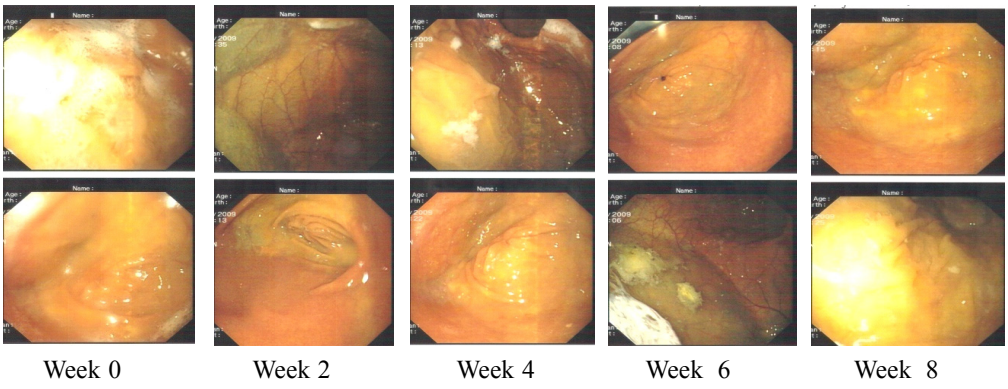

Figure 2. Macroscopic observation of gastric mucosa in experimental animals of group II

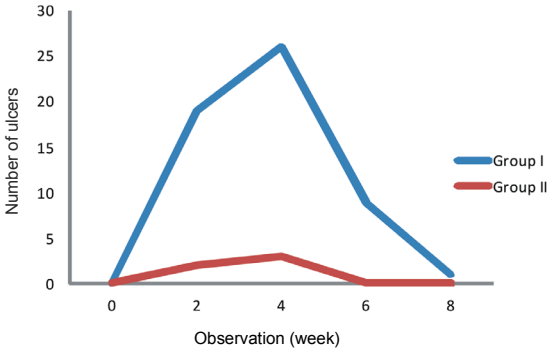

Figure 3. Changes in the number of corpus ulcer

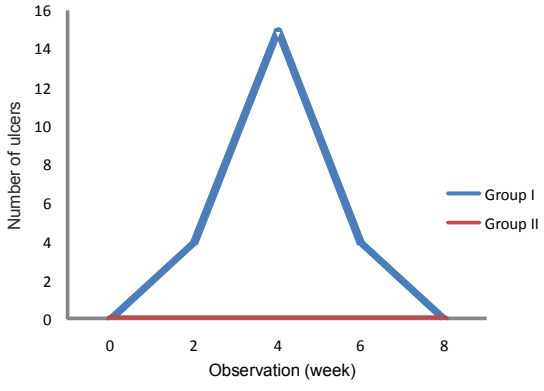

Figure 4. Changes in the number of antrum ulcer

DISCUSSION

Increased bilirubin plasma level may occur in obstructive jaundice, particularly the direct bilirubin which goes into the systemic circulation and fails to enter the intestine. Stools are often pale because less bilirubin reaches the intestine. At certain level, bilirubin retention in the circulation (hyperbilirubinemia) may be followed by bilirubin excretion in the urine.[20]

In this study, the permanent increase of transaminase enzymes level (AST and ALT) suggested that chronic liver disease have been occurred followed by damage of liver cells that causes lysis of the cells and may leak transaminase enzymes into blood stream. Furthermore, our study also demonstrated a very high increase in ALP and GGT level. ALP and GGT elevation more than 3 times the normal level may suggest extra-hepatic obstruction as expected in this study. Total extra-hepatic obstruction causes damage to liver cells which is followed by leakage of ALP and GGT enzymes into blood stream. In normal condition, both enzymes are localized in the biliary membrane of liver cells (hepatocytes). The propensity of reduced albumin level along with elevated globulin level indicated that the process of chronic liver damage had

been developed in the experimental animals, which reduced the synthesis function of the liver, especially albumin synthesis. Elevated plasma globulin level may occur due to the body response against inflammatory process caused by biliary obstruction and should be due to chronic liver disease condition caused by chronic biliary obstruction. In advanced liver damage, it is usually followed by reduced CHE plasma level, which suggests lesser capacity of liver cells which still function normally.[20,21] In this study, the CHE level in both groups at 8 weeks after CBD ligation did not show any obvious reduction. It is assumed that there has not been enough time to cause advanced liver damage in this obstructive jaundice condition, which is known as biliary cirrhosis.

This study results showed that obstructive jaundice in *Macaca nemestrina* may cause acute gastric mucosal injury as expressed in the greater number of ulcers along with time. In obstructive jaundice, the development of gastric ulcer may be prevented by administration of intravenous PUFA. Furthermore, our study demonstrated that gastric ulcer that had been developed in pig-tailed macaques with obstructive jaundice was healed by administration of intravenous PUFA. So far, no study has reported about the effect of intravenous PUFA on gastric mucosal integrity in obstructive jaundice.

The mechanism of PUFA to prevent the development of ulcer in obstructive jaundice of this study is still unclear. We assume that it is associated with the function of phospholipids that maintain the gastric mucosal integrity as the primary source of prostaglandin production, as an integral part of cell membrane and as the part of mucus barrier producer of the gastric mucosa. Prostaglandins have role in maintaining the gastric mucosal integrity by increasing the mucus and bicarbonate secretion as well as increasing the mucosal blood circulation. The number of ulcer produced at the corpus region was greater than the number of ulcer at the antrum. It is assumed that it may be due to greater thickness of mucus layer at the antrum compared to the corpus. In the experimental animals Group II, ulcer still developed with much lesser amount compared to the Group I. Such fact demonstrated that other than the role of phospholipids that maintain the integrity of gastric mucosa in obstructive jaundice, there are also other factors that play role as aggressive factors.

CONCLUSION

This study had proven that intravenous PUFA administration is potential to prevent the development of acute gastric mucosal injury in pig-tailed macaques with obstructive jaundice. Ulcers that developed due to acute gastric mucosal injury in pig-tailed macaques may be healed by the administration of intravenous PUFA.

The CBD ligation in experimental animals of our study has successfully developed the animal model for obstructive jaundice in keeping with the biochemistry evaluation results of liver function test.

SUGGESTION

Further studies are needed to identify the effect of intravenous PUFA administration on the gastric mucosal integrity in experimental animals with obstructive jaundice by evaluation of blood phospholipids level, blood and tissue prostaglandin level as well as the histopathological findings by using electron microscope to observe the characteristics of cell membrane.

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