Experimental model of obstructive jaundice in mongrel dog: study of subsequent pathological changes in liver

HEMANT KOCHER, RAVINDRA BAPAT, AMITA JOSHI *

Departments of Gastroenterology Surgical Services and *Pathology, Sir G S Medical College and K E M Hospital, Mumbai 400 012

Background: Attempts at creating an experimental model of obstructive jaundice in large animals have been unsuccessful because of frequent fistulation of the ligated bile duct. Objectives: To produce a model of obstructive jaundice in mongrel dogs and to study subsequent serial pathological changes in the liver. Methods: Four techniques were evaluated in four dogs to produce obstructive jaundice. After creation of a successful model, subsequent clinical and biochemical outcome and serial pathological changes in the liver were studied in another 14 dogs. Results: Complete extrahepatic biliary dissection produced a model of persistent obstructive jaundice. Serial histological changes included inflammation and edema in the first 14 days followed by lymphocytic infiltration at 28 days. Fibrosis started by day 7 and resulted in architectural distortion by day 21. Conclusion: An acute, complete and irreversible model of obstructive jaundice can be produced by extrahepatic biliary tree excision and ligation. Serial pathological changes showed acute inflammation, followed by fibrosis and architectural distortion. [Indian J Gastroenterol 1997; 17: 6-9]

Key words: Bile duct obstruction - extrahepatic

A large animal model of obstructive jaundice would enable us to understand the pathophysiology of this condition and to correlate it with clinical manifestations. This would enable us to time interventions in patients with obstructive jaundice, possibly before irreversible changes occur. However, use of ductal ligation to produce a model of obstructive jaundice in dogs fails because fistulae frequently develop between the ligated common bile duct (CBD) and adjoining normal bile duct or duodenum.

We undertook this experimental study in mongrel dogs to establish such a model and study the biochemical and histologic sequelae of ductal obstruction.

Methods:

Eighteen adult mongrel dogs (8 male, 10 female) weighing 13-24 (16 ± 2.1) Kg, free of dermatological or systemic infection or worm infestation, were maintained on standard laboratory diet and allowed free access to food and water. They were kept in separate kennels in hygienic conditions and all ethical considerations in animal care were adhered to.

Part I

A pilot study of four dogs was carried out to evaluate the following four techniques to create a model of obstructive jaundice:

1. Simple ligation of the CBD at its duodenal end (dog 1)
2. Excision of 1 cm length of CBD at its duodenal end and ligation of both the free ends (dog 2)
3. Incision of CBD and cannulation with 1 cm of 1 no. nylon and ligation of the CBD at two points, proximal and distal to the incision, thus incorporating the nylon sutures (dog 3)
4. Complete excision of extrahepatic biliary tree along with the gall bladder and ligation of the individual lobar hepatic ducts (dog 4)

Biochemical investigations were carried out at intervals (days 0, 3, 6, 9 in all dogs and also on day 18 in dog 4) to assess the progress of biliary obstruction produced. Re-exploration was carried out whenever the biochemical parameters normalized or at a later date if they worsened beyond day 15.

Part II

Fourteen other dogs were subjected to the technique carried out in dog 4 above as only this technique proved successful. All primary operations were carried out through an upper abdominal midline incision under general endotracheal anesthesia. Repeated operations, through a right subcostal incision under general anesthesia, were carried out at weekly intervals to obtain liver tissue. Liver biopsies (approximately 1 cm x 1 cm) were taken from 2 sets each comprising 3 of the 6 liver lobes, alternating at each surgery, including first surgery (baseline liver biopsy). Blood samples for biochemical investigations were collected at the time of induction of anesthesia for surgery.

All dogs were given perioperative antibiotics (ampicillin 30-40 mg/Kg, gentamicin 2-4 mg/Kg, and metronidazole 30-40 mg/Kg in divided doses) and analgesics (diclofenac 0.5 mg). They received intravenous fluids for 1-2 days postoperatively, till they started taking oral foods. No measures were taken to combat coagulation defects or endotoxin effects, in order to study the natural clinicopathological evolution.

All blood samples were tested for total and direct bilirubin, AST, ALT, alkaline phosphatase, blood urea nitrogen and serum creatinine. All dogs were studied till the time of death and postmortem examinations were carried out. Specimens for histology were embedded in paraffin blocks and see-
tions stained with hematoxylin-eosin. Special stains, viz., reticu-
lin and Masson’s trichrome were used to identify fibrin
deposition in appropriate specimens. The pathologist study-
ing the specimen was unaware of the timing or source of speci-
men.

Results

Part I (Table 1)
Simple ligation of the CBD (dog 1) caused a small rise in
bilirubin and transaminases which returned to normal within
nine days. Excision and ligation of the CBD (dog 2) caused a
rise in alkaline phosphatase and a small rise in bilirubin which
started regressing by day 9. Cannulation and ligation of CBD
(dog 3) caused a rise in bilirubin and liver enzymes which
returned to normal by the ninth day. Complete dissection and
removal of the extrahepatic biliary tree (dog 4) resulted in a
rise in bilirubin and alkaline phosphatase which persisted till
the eighteenth day.

Table 1: Serial biochemistry in dogs studied in Part I

<table>
<thead>
<tr>
<th>Dog</th>
<th>Day</th>
<th>Total bilirubin (mg/dL)</th>
<th>Direct bilirubin (mg/dL)</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>Alkaline phosphatase (IU/L)</th>
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(Normal values: total bilirubin 0.1-0.6 mg/dL, direct bilirubin 0.05-0.12 mg/dL, AST 5-45 IU/L, ALT 5-57 IU/L, alkaline phosphatase 11-101 IU/L)

At re-exploration, the CBD had regained continuity de-
spite intact initial sutures in dogs 1, 2 and 3. In dog 4, how-
ever, no continuity was seen at re-exploration on day 19; all
lobar hepatic ducts were minimally dilated.

Part II (Table 2)
Four of the 14 dogs died before day 7 and were excluded. No
single cause of death could be determined at the postmortem
examination in these dogs but there was no evidence of hemor-
rhage or sepsis.

Four dogs died by day 15; autopsy revealed intra-abdomi-
nal sepsis but no evidence of excessive bleeding. Other
systems were normal. Six dogs who died after day 15 had
bleeding tendency (ecchymoses on the skin, mucosal surfaces,
Fig 2: Day 14: Portal edema (H & E, 160X)

Fig 3: Day 21: Extensive parenchymal fibrosis (Masson trichrome, 160X)

Fig 4: Day 21: Centrilobular cholestasis and focal fatty change (H & E, 160X)

Fig 5: Day 28: Hemorrhagic necrosis (H & E, 160X)

one had wound dehiscence. Wound infection was treated using standard surgical principles of drainage under local or general anesthesia and appropriate antibiotics. Wound dehiscence was treated with re-suturing under general anesthesia after freshening the wound edges.

Pathology

Day 7: Polymorphonuclear cell infiltration (Fig 1) and edema of the portal tracts were seen in 6/10 dogs. Cholestasis in zones 1 and 2 was seen in all specimens, and microabscesses in 2/10. Hepatocytes showed impending necrosis (7/10) in zone 1 and Kupffer cells were prominent (3/10). Portal tracts showed fibrosis (3/10).

Day 14: Edema had increased in all the eight dogs studied (Fig 2) but inflammation remained unchanged. The fibrosis was marked by now (5/8) but there was no architectural distortion. All dogs had zones 1 and 2 cholestasis, similar in degree to that observed on day 7. Hepatocytes showed impending necrosis (7/8) in zone 1 and Kupffer cells were prominent.

Day 21: Inflammation and edema started decreasing (3/3). Distortion because of fibrosis was seen (2/3) on Masson trichrome stain (Fig 3). Cholestasis decreased and some fatty changes were also seen (3/3) (Fig 4). There was no necrosis of hepatocytes. Kupffer cells were not prominent.

Day 28: Only dog was studied. Polymorphonuclear cells were replaced by lymphocytes. Cholestasis was restricted to
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Kocher, Bapat, Joshi

to acute inflammation, the probable initiating agents being biliary products. The initial deaths may be thus related to acute inflammation. Later deaths may be a result of liver dysfunction due to replacement by fibrosis, mainly perportal, and hemorrhagic necrosis. Our dogs did not survive long enough to show cirrhotic changes.

We have thus successfully created a dog model of obstructive jaundice and have studied serial events in the liver as a consequence. This model may be of use in future experimental studies and may help unravel the clinico-pathologic correlates of this condition.

References

BOOK REVIEW

Baillière's Clinical Gastroenterology was published prior to 1986 as Clinics in Gastroenterology, the series had established its reputation as an academic storehouse from those early days. The present issue is a worthy addition.

The book begins with an Introduction that leads the uninitiated through the terminology. The actions of growth factors and cytokines, their relationship and their classification, are explained simply.

The first chapter is a review of cytokines, their sources, receptors and signalling. This is followed by a review of cytokines in gastrointestinal diseases. Subsequent chapters deal with epidermal growth factor, transforming growth factors α and β, insulin-like growth factor, basic fibroblast growth factor, platelet-derived growth factor, and trefoil peptides. The final chapters review their role in inflammatory, ulcerative and neoplastic gastrointestinal diseases. In fact, each chapter dwells to some extent on these varieties of gastrointestinal diseases.

One strong feature of these issues is that the text is fully referenced, the references of course being up-to-date. Large print size, simple line diagrams, and quality packaging are features we have come to expect from this series.

It is obvious that a title such as this cannot have broad appeal. The average clinician will find the cellular and molecular sketches uninteresting. But, equally certainly, the academician and researcher will find a wealth of information here. To the extent that every doctoral student in the field of gastroenterology must have read this book at least once, details may be of more interest to the laboratory researcher, but the gastroenterologist will retain enough from this book to make his knowledge current. Personal copies are not recommended, but no library of a medical school with postgraduate/postdoctoral studies can do without this issue.

Department of Gastroenterology

Philip Abraham
Seth G S Medical College and K E M Hospital, Mumbai 400 012

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