Review

Liver fluke-associated cholangiocarcinoma

P. Watanapa and W. B. Watanapa*

Departments of Surgery and *Physiology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10 700, Thailand
Correspondence to: Professor P. Watanapa (e-mail: sipwt@mahidol.ac.th)

Background: Infection with liver flukes has been reported to be associated with bile duct malignancy.
Methods: The review is based on a literature search (Medline) and, in some cases, direct contact with authors or principal investigators.
Results: A large body of evidence indicates that *Opisthorchis viverrini* is a definite cause of human cholangiocarcinoma, whereas *Clonorchis sinensis* is a probable cause. The evidence regarding *Opisthorchis felineus* is insufficient to assess its role in carcinogenesis. Possible mechanisms of carcinogenesis include chronic irritation, nitric oxide formation, intrinsic nitrosation and activation of drug-metabolizing enzymes. Early detection of bile duct malignancy is difficult and not clinically available at present, although cholangiocarcinoma-associated soluble antigen has been reported in an experimental study to be a useful early marker of cancer development. Long-term survival after surgical treatment of liver fluke-associated cancer is similar to that reported in patients without liver fluke infestation.
Conclusion: Liver fluke-associated cholangiocarcinoma is still a health problem in developing countries. Mechanisms of carcinogenesis should be explored further in order to reduce the impact of this disease.

Paper accepted 20 March 2002 British Journal of Surgery 2002, **89,** 962–970

Extent of the problem

According to the 1994 report of the World Health Organization and the International Agency for Research on Cancer (IARC) meeting[1,2], crude estimation of the global number of liver fluke infestations is of the order of 17 million, comprising 9 million with *Opisthorchis viverrini*, 7 million with *Clonorchis sinensis* and 1·5 million with *Opisthorchis felineus*. *O. viverrini* infestation is found predominantly in Thailand, Laos and Cambodia, whereas *C. sinensis* is endemic in southern China, Korea, Taiwan and Vietnam. *O. felineus* is endemic in western Siberia, Kazakhstan, Ukraine and parts of eastern Europe. Recent recovery of *O. felineus* from cyprinoid fish and cats in Germany indicates the persistent existence of the fluke in Europe[3].

Association between liver fluke and cholangiocarcinoma: clinical studies

Evidence of association between *O. viverrini* and bile duct malignancy has emerged from several studies, including hospital-based case series, population-based ecological studies that correlated the incidence of cancer with prevalence of infestation in various geographical areas, and a few case–control studies. The first large case series was reported from Siriraj Hospital, Mahidol University, in Bangkok[4]. The authors analysed 9694 autopsies performed between 1954 and 1965, and 1301 liver biopsies obtained between 1960 and 1962. An unusually high incidence of cholangiocarcinoma was observed in both the autopsy and biopsy materials taken from patients with *O. viverrini* infestation. The ratio between hepatocellular carcinoma and cholangiocarcinoma in autopsies without opisthorchiasis was 8 : 1, whereas the ratio was reversed among those with liver fluke infestation. Similarly, the ratio of these two malignancies in biopsies was 5 : 1 in non-infected patients and 1 : 2 in the presence of the fluke.

A nationwide survey of liver pathology in Thailand by Bunyaratvej and colleagues, who examined 3305 biopsy specimens collected between 1974 and 1978, also demonstrated a high incidence of cholangiocarcinoma in association with opisthorchiasis in the north-east of Thailand[5]. More than two-thirds of the patients were male, and the mean age at diagnosis of cholangiocarcinoma was 48·8 years in males and 48·1 years in females. The results of this study were later confirmed by Srivatanakul *et al.*[6], who showed that the incidence of cholangiocarcinoma was almost twice that of hepatocellular carcinoma in endemic areas of *O. viverrini* in the north-east of the country, and the

incidence in males was 2·4 times that in females. Subsequent study found that the highest proportional incidence rate of cholangiocarcinoma was in the north-eastern region, where the prevalence of *O. viverrini* infestation was also highest[7].

In a population-based study in a fluke-endemic area, the age-standardized incidence rates for the year 1988 were 89·2 per 100 000 in males and 35·5 per 100 000 in females – the highest rates of cholangiocarcinoma in the world. Moreover, the tumour burden appeared to correspond with the intensity of *O. viverrini* infestation[8]. Studying in the same year and in the same region by using hospital-based data, Green *et al.*[9] estimated the age-standardized incidence rates of cholangiocarcinoma to be 135·4 and 40·0 per 100 000 in males and females respectively. The truncated standardized incidence rates (in those aged 35–64 years only) were 334·2 per 100 000 in males and 104·3 per 100 000 in females. Parkin and colleagues[10] performed a case–control study of 103 patients in comparison with controls matched by age and sex. They found that *O. viverrini* infection increased the risk of cholangiocarcinoma fivefold, and that males carried a higher risk than females. They estimated that two-thirds of cases of cholangiocarcinoma in Thailand were caused by *O. viverrini* infestation.

In a longitudinal study of clinical, laboratory and hepatobiliary ultrasonography of 913 voluntary subjects infected with *O. viverrini*, treatment with the antihelminthic drug praziquantel significantly improved clinical symptoms and ultrasonographic changes[11]. A study in Syrian hamsters, however, showed that removal of the parasite load in itself might not be sufficient to block the development of cholangiocarcinoma, particularly if the fluke had already caused significant and permanent changes in the biliary system[12]. There are no concrete data available to confirm the benefit of fluke eradication in terms of reducing cancer death.

An association between *C. sinensis* and cholangiocarcinoma was first made by Katsurada in Japan, just over a century ago[13]. Although interest in the relationship between the fluke and the cancer was initially stimulated by a series of reports by Hou in Hong Kong[14–16], the disease is uncommon in Hong Kong today. By contrast, clonorchiasis-associated cholangiocarcinoma is still a problem in Korea. In Pusan, an area with extremely high prevalence of *C. sinensis*, the fluke increased the risk of cholangiocarcinoma sixfold[17]. More recently, a case–control study[18] in the same area showed that identification of *C. sinensis* in the stool was significantly associated with cholangiocarcinoma, with a relative risk of 2·7. Cases of cholangiocarcinoma associated with *C. sinensis* have also been reported among Asian immigrants to the USA[19,20].

There are several case reports or case series of cholangiocarcinoma in patients with *O. felineus* infestation[21–23], but fewer than a handful of systematic studies have been conducted to identify the actual relationship between the two conditions[1].

Association between liver fluke and cholangiocarcinoma: experimental studies

An experimental model successfully demonstrating an association between liver fluke and cholangiocarcinoma was obtained using a combination of liver fluke infestation and continuous administration of a hepatocarcinogen in the drinking water or the basal diet of hamsters. Examples are dimethylnitrosamine (DMN) in the drinking water for 10 weeks plus *O. viverrini*[24,25]; DMN in drinking water for 8 weeks plus *C. sinensis*[26]; 0·03 per cent *N*-2-fluorenylacetamide in the basal diet for 40 weeks plus *C. sinensis*[27]. In hamsters receiving DMN (0·0025 per cent in drinking water) and 100 metacercariae of *O. viverrini*, more than 50 per cent of the hepatic parenchyma was occupied by cholangiocellular lesions and all animals developed cholangiocarcinoma[24]. Similar increases in cholangiocarcinoma, as well as preneoplastic hepatocellular lesions, were also found in hamsters receiving continuous administration of combined nitrite and aminopyrine in drinking water and *O. viverrini*[28]. The report of DMN formation in the stomach by nitrite and aminopyrine may explain such findings[29].

Based on a critical review of existing evidence from several clinical and experimental studies, the working group of IARC concluded that *O. viverrini* is a definite cause of bile duct cancer in humans; *O. viverrini* infestation is therefore classified as a carcinogen. The review reported that *C. sinensis* is a probable cause; *O. felineus* has not yet been studied sufficiently to assess its carcinogenic role[1].

Pathological studies

Infestation of liver flukes occurs when humans or other mammals ingest raw fish containing the infective cysts of the fluke (metacercariae) in the muscle and connective tissue. After being eaten by humans, the infective metacercariae excyst in the duodenum. The newly excysted flukes move into the bile duct through the ampulla of Vater and develop into adults in the intrahepatic bile ducts, and occasionally in the pancreatic duct or gallbladder, within 1 month. The life cycles of *O. viverrini*, *C. sinensis* and *O. felineus* are similar. The adult fluke lays eggs that enter the biliary system and are excreted in the faeces, to be ingested by snails of *Bithynia* species, the first intermediate hosts. The eggs hatch inside the snail and mature, eventually leaving the snail in the form of freely swimming cercariae that find and penetrate cyprinoid fish, the second intermediate hosts, where they

develop into metacercariae in the muscle and connective tissue. The lifespan of *O. viverini* and *C. sinensis* has been reported to be 25–30 years[30–32].

Pathological consequences of *O. viverrini* infection appear to be similar in both animals and humans[33,34]. Early pathological changes consist of an acute inflammatory reaction involving the bile ducts of the second order and the portal connective tissue, particularly the large veins. Death of bile duct epithelial cells and hepatocytes, and evidence of cell regeneration, occur early after liver fluke infestation[33,35]. As the flukes develop into adults, they induce hyperplasia of the bile duct epithelium and adenoma formation. The adult flukes and ova also induce a granulomatous reaction. Resolution of such granulomas leads to periductal and portal scarring. Bile ductule proliferation associated with periportal fibrosis and linking up between portal areas by fibrous bands results in the appearance of multilobular cirrhosis in most hepatic lobules. The periportal and periductal fibrosis in hamsters with chronic infection of *O. viverrini* has been shown to correlate with a marked increase in the synthesis and hepatic content of type I and type III collagen[36]. In the liver of patients with opisthorchiasis, the subcapsular bile ducts are usually dilated, with prominent fibrotic wall[34,37]. Microscopically, pathological changes in the liver are confined to the biliary tree, particularly the large and medium-sized bile ducts where the flukes are usually found[34]. Moreover, obstruction of bile ducts due to large numbers of the flukes in the ductal lumen is frequently seen. This obstruction may lead to suppurative cholangitis with abscess formation, and cholangiohepatitis may subsequently develop, particularly when rupture of abscess occurs[35].

In hamsters receiving both the carcinogen DMN (0·0025 per cent in drinking water) and 100 metacercariae of *O. viverrini*, all animals developed mucin-producing cholangiocarcinomas, with or without cholangiofibrosis[24]. When hamsters received a subcarcinogenic oral dose of *N*-nitrosodimethylamine (1·6 mg) and 50 metacercariae of *O. viverrini*, 20 per cent of the animals developed cholangiocarcinoma. All tumours were of intrahepatic type and were found most frequently in the right lobe[38]. In humans, however, the tumour location has been reported to be of central type (60 per cent), peripheral type (20 per cent) and diffused type (20 per cent)[40]. Of the extrahepatic lesions, 60 per cent are located in the proximal ductal region and are of the scirrhous type, with an occasional papillary component. Nodular and papillary types are found in the middle and distal ductal region[40,41]. The histopathology is almost always adenocarcinoma and occasionally the flukes are found in the tumour or in the adjacent liver parenchyma (*Fig. 1*). Direct invasion and local metastasis via lymphatics, blood vessels and perineural are common, especially in extrahepatic cancer[42,43]. Among 29 non-jaundiced patients with opisthorchiasis and intrahepatic or peripheral-type cholangiocarcinoma, 23 had tumours in the right lobe[41].

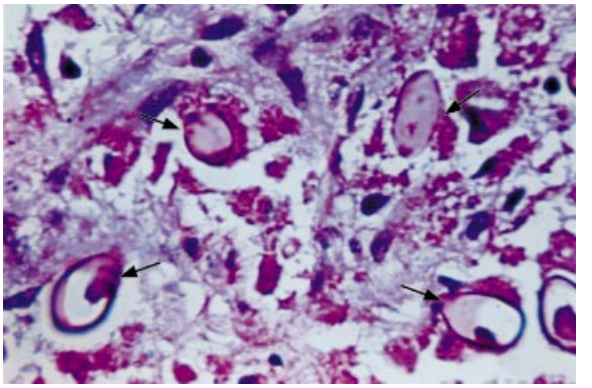

Fig. 1 Histological appearance of peripheral type of *Opisthorchis viverrini*-associated cholangiocarcinoma. Arrows show the eggs of the fluke in the area adjacent to the tumour. (Courtesy of Dr K. Atisook, Department of Pathology, Faculty of Medicine, Siriraj Hospital)

Scanning electron microscopy of a cholangiocarcinoma cell line (HuCCA-1), originally established from an intrahepatic bile duct tumour of a patient seropositive for antibody to *O. viverrini*, showed the surface of the cells to be covered with microvilli of varying size and irregular distribution. Transmission electron microscopy clearly revealed the presence of cytokeratin filaments, an intracytoplasmic lumen, tight junctions at the apices and desmosomes at the lateral surfaces of neighbouring cells, all of which are characteristics of an adenocarcinoma cell. Cells from a tumour mass that developed in a nude mouse following subcutaneous injection of HuCCA-1 cells exhibited some morphological changes. Nearly one-third of the tumour cells, particularly those lining the base of the tumour tubules, formed small foci of cells in sheet-like arrangements and exhibited electron-dense tonofilament bundles typical of squamous cells[44]. These findings are consistent with evidence from a clinicopathological study of an intrahepatic cholangiocarcinoma with a squamous cell carcinoma component[45].

Seventy per cent of *Clonorchis*-associated cholangiocarcinomas are adenocarcinomas and may be found at varying stages of differentiation[14]. Anaplastic tumours of the bile duct epithelium have been reported occasionally, but they are uncommon. They may resemble primary liver cell carcinoma[13]. Newly formed goblet cells are frequently found and mucin production may be abundant, but glycogen and bile pigment are characteristically absent.

Squamous metaplasia is found in 13 per cent of cases[14]. The tumour usually develops in the second-order intrahepatic bile ducts near the hepatic hilum. Adenomatous proliferation of ductal epithelium can often be seen side by side with malignant changes, and flukes are frequently found in bile ducts that are surrounded by tumour[46]. Intrahepatic or peripheral cholangiocarcinoma is common and is found much more often (as in *O. viverrini*-associated cancer) in the right lobe of the liver[47].

Molecular studies of carcinogenesis

Molecular mechanisms of human cholangiocarcinogenesis secondary to liver fluke infestation are still to be investigated, particularly in patients with *O. viverrini* infection. By using the polymerase chain reaction and direct sequencing of the product of c-Ki-*ras* gene, one study showed that five of nine Japanese patients with cholangiocarcinoma in normal liver (without associated opisthorchiasis) had a point mutation at codon 12, whereas none of 12 Thai patients with opisthorchiasis had such a mutation[48]. A more recent study confirmed the findings: the incidence of *ras* mutation differed markedly between the Japanese (seven of 12 patients without opisthorchiasis) and Thai (two of 26 with opisthorchiasis) patients. In contrast, the incidence of *p53* mutation was similar: four of 12 and nine of 26 respectively. All but one of the *ras* mutations occurred at codon 12 or 13 of the c-Ki-*ras* gene. The exceptional case was found to have deletion–insertion in the second exon of the N-*ras* gene. For *p53* mutations, all but one were detected in a highly conserved region, and the predominant form of the mutations was G : C → A : T transition at CpG sites in both the Japanese and Thai patients[49].

Results of such molecular studies on carcinogenesis in patients with liver flukes may pave a way for further investigations to crystallize the actual or causal relationship between the parasite and the tumour. However, the fact that the incidence of cholangiocarcinoma is low, in sharp contrast to the high prevalence of liver fluke infestation, even in fluke-endemic areas, highlights the importance of co-factors. Exploration of the molecular carcinogenesis of the tumour with several plausible aetiological factors will obviously be a lengthy and laborious task.

Possible mechanisms of carcinogenesis

The pathogenesis of liver fluke-associated cholangiocarcinoma may be a complex process, involving several mechanisms. From existing evidence, possible mechanisms may be suggested.

Hyperplasia of bile duct epithelium and carcinogen exposure

Chronic irritation and chronic inflammation caused by the fluke results in hyperplasia and adenomatous changes of bile duct epithelium[33,34]. As previously shown in malignancy of the colon or pancreas, hyperplastic cells are vulnerable to carcinogen because the agent can easily induce DNA damage during active cell proliferation[50,51]. If the DNA damage involves cell cycle control genes, neoplastic changes occur. It has been demonstrated that nitrates, nitrites and even *N*-nitroso compounds are commonly found in several fermented or preserved foods, and that these compounds can act as exogenous carcinogens[52,53]. Moreover, patients with liver fluke infestation have increased rates of endogenous nitrosation[54,55].

Increased formation of endogenous carcinogen

Endogenous nitrosation caused by liver fluke infestation has been studied in both animals and humans. In hamsters with *O. viverrini* infestation, induction of nitric oxide synthase in macrophages, mast cells and eosinophils in inflamed areas surrounding the bile ducts has been reported, and the enzyme subsequently increases endogenous nitrosation of thiazolidine-4-carboxylic acid (thioproline)[56]. In a dietary control (low-nitrate diet) study comparing patients with opisthorchiasis and uninfected controls, those with liver fluke infestation had increased endogenous generation of nitric oxide and production of *N*-nitroso compounds, as indicated by increased levels of plasma and urinary nitrate, salivary nitrite and increased nitrosation of proline and thioproline. Such increases were abolished by co-administration of ascorbic acid with proline, and by elimination of the fluke by treatment with the antihelminthic drug praziquantel. Production of *N*-nitroso compounds occurs through a reaction between amines and nitrosating agents derived from the oxidation of nitric oxide outside the stomach[55,57]. It is likely that *N*-nitroso compounds are formed in the area of chronic inflammation around the bile ducts as the result of local generation of nitric oxide by inflammatory cells. Therefore, the bile duct epithelial cells are exposed continuously to high concentrations of *N*-nitroso compounds, leading to neoplastic transformation. To prove this hypothesis, the concentration of *N*-nitroso compounds in the bile ducts should be assayed. However, measuring these compounds in bile is technically difficult at present, because they are labile and other components in bile can affect the assay.

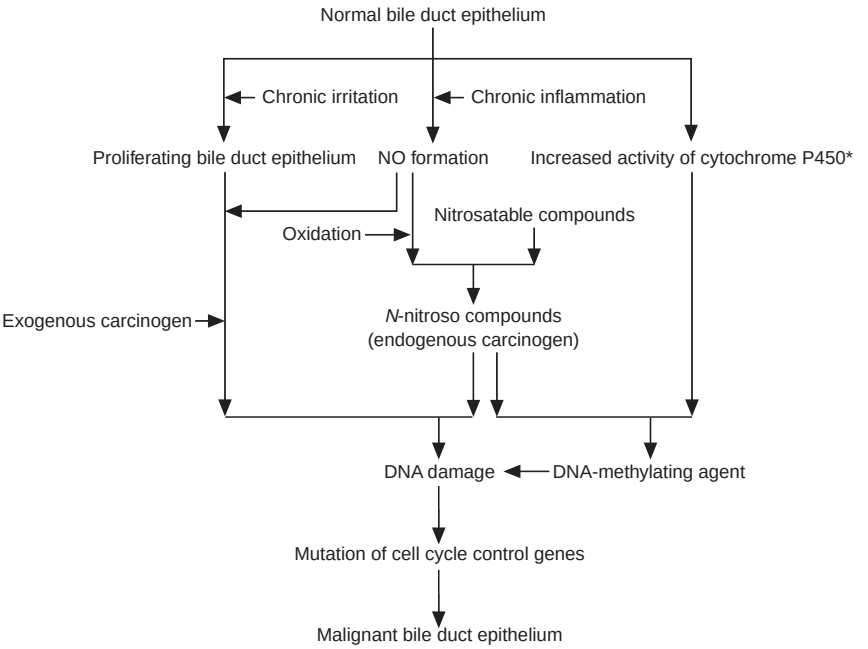

Fig. 2 Possible mechanisms of carcinogenesis of liver fluke-associated cholangiocarcinoma. *Particularly CYP2A6 and CYP2E1. NO, nitric oxide

Activation of drug-metabolizing enzymes

In male hamsters infected by *O. viverrini*, activities of the hepatic cytochrome P450 (CYP) isoenzymes (particularly CYP2E1 and CYP2A6) have been shown to be higher than those of controls, and the highest levels of these two enzymes occur in hepatocytes immediately adjacent to the area of inflammation[58]. Induction of CYP2A6 expression has been demonstrated in patients with opisthorchiasis, and a significant reduction of the enzyme activity was observed 2 months after eradication of the flukes by praziquantel treatment[59]. *N*-nitrosodimethylamine (NDMA), one of the products of endogenous nitrosation formed in the tissue, is significantly metabolized by CYP2E1 and CYP2A6. The product of this metabolism is a DNA-methylating agent that can result in DNA damage, particularly in proliferating bile duct epithelial cells. However, the mechanism of increased activity of these two enzymes in patients with chronic liver fluke infestation is as yet uncertain.

Increased nitric oxide production

In areas of chronic inflammation caused by the liver fluke, macrophages and other cell types (e.g. mast cells, eosinophils), activated by parasite-specific T cells and cytokines, synthesize nitric oxide from L-arginine via the induction of the enzyme nitric oxide synthase[56,60,61]. Nitric oxide is not only cytotoxic, but also genotoxic at physiological concentrations[62,63]. Chronic exposure of the proliferating bile duct epithelium to this genotoxic inflammatory product may create an environment favouring malignant change.

It is probable that the above four mechanisms of carcinogenesis may act in concert during the development of liver fluke-associated cholangiocarcinoma. The possible process of carcinogenesis is outlined in *Fig. 2*.

Early detection of cancer

A soluble antigen for early detection of *O. viverrini*-associated cholangiocarcinoma has been reported in hamsters[64]. This antigen is a 200-kDa glycoprotein that appears to be immunologically distinct from other tumour markers. The level of the antigen in serum and bile was raised markedly in animals with progressive tumours, compared with controls. Serum taken serially from each animal that subsequently developed cholangiocarcinoma showed a gradual but significant increase in the level of antigen as carcinogenesis progressed. Identification of the antigen, and the establishment of a cell line from *O. viverrini*-associated cholangiocarcinoma induced in a hamster model and a cholangiocarcinoma cell line from a patient with liver-fluke infestation, seem to provide an early opportunity for tumour detection[65,66]. However, identifying a tumour marker in serum or bile does not, on its own, solve the problem. The intrahepatic biliary system is a complex tubular structure. Although abdominal computed

tomography (CT) (particularly three-dimensional spiral CT cholangiography) or magnetic resonance imaging can detect cholangiocarcinoma with an accuracy of up to 90 per cent[67,68], the demonstration of a small focus of early cancer change in the liver is difficult. Confirmation of neoplastic change from such small lesions is difficult, particularly when the tumour is beyond the second-order bile ducts.

Treatment and outcome

Surgical treatment

Operative treatment is the best available therapy for cholangiocarcinoma. Not only may it cure a patient with early-stage disease (if the lesion can be located precisely), but it may also provide effective palliation for those with an irresectable tumour. Whenever possible, tumour resection should be performed unless there is clear evidence of advanced disease. A series of 29 non-jaundiced patients with opisthorchiasis and peripheral-type cholangiocarcinoma who underwent liver resection has been reported[41]. Twenty-one patients had tumour only in the right lobe, seven had a tumour in the left lobe, and one had tumour involving both lobes. Operations included right hepatectomy (19), extended right hepatectomy (four), left hepatectomy (two), left lobectomy (two) and medial segmentectomy (two). Fourteen patients had died by the end of the first postoperative year, five by the end of the second year, and a further five by the end of the third year. Five patients survived until the end of the fifth year, resulting in an actual 5-year survival rate of 17 per cent.

In a series of 30 patients with hilar cholangiocarcinoma associated with *O. verrini* infestation, the criteria for diagnosis of opisthorchiasis were (1) a history of previous positive stool examination for *Opisthorchis* or its eggs, (2) identification of *Opisthorchis* or its eggs in the stool or bile, (3) demonstration of a typical bead-like cholangiogram, and (4) histological evidence of the fluke in the specimen[43]. Seven patients had their tumours removed, four with concomitant liver resection. The four patients who underwent hepatectomy were anicteric before operation. Twenty-two patients underwent palliative biliary bypass procedures to a segmental duct (segment III duct in 18 patients and another segmental duct in four). For those undergoing resectional procedures, the actual 1-year survival rate was 86 per cent and the 2-year survival rate was 43 per cent. No patient survived until the end of the third year. The mean survival of patients undergoing palliative biliary bypass procedures was 8 months and the 1-year survival rate was only 26 per cent. All patients died within 2 years. Some jaundiced patients in this series were found to have several abnormal autonomic function test results[69], although the significance of these findings needs further investigation.

Generally, non-jaundiced patients with opisthorchiasis seem to have a better long-term outcome after operation than those who are icteric. Patients with a peripheral-type cholangiocarcinoma have a better prognosis than those with a hilar tumour; similar findings occur in patients with cholangiocarcinoma without associated liver fluke infestation[70]. Successful curative tumour resection is uncommon, although in approximately one-quarter of the patients the tumour can be resected[43]. The fact that up to three-quarters of the patients already have lymphatic or vascular invasion, or peritoneal seedings, at the time of operation makes aggressive surgery unlikely to be beneficial. By and large, survival after operation for cholangiocarcinoma associated with opisthorchiasis is similar to that in patients without liver fluke infestation[70,71]. Although there are no data available for the long-term outcome following surgical treatment of *C. sinensis*- or *O. felineus*-associated cholangiocarcinoma, survival can be expected to be more or less similar to that of the patients with opisthorchiasis described above.

Chemotherapy and immunomodulation

As periductal fibrosis and thickening of the bile duct walls, leading to bile duct obstruction, are consistent pathological findings, and intraluminal obstruction by flukes is frequently seen in the liver of patients with liver fluke infestation regardless of the development of cholangiocarcinoma[35], there is no significant role for available adjuvant chemotherapy after operation because of the risk of cholangitis and sepsis[41,43]. Progress in identifying suitable or new forms of chemotherapeutic agents is slow because this tumour is rare in the developed countries where most research institutes and pharmaceutical companies are located. However, several anticancer drugs, especially from plant products, have been tested against liver fluke-associated cholangiocarcinoma in Thailand[72,73]. Triptolide, a diterpene from *Tripterygium wilfordii*, has been shown to be highly effective against cholangiocarcinoma by *in vitro* cytotoxicity assay and *in vivo* inhibition of tumour growth in hamsters. It can induce apoptosis in several cholangiocarcinoma cell lines, and the appearance of apoptosis and DNA fragmentation occurs within 24 h of exposure to the agent[73]. The fact that the ED_{50} (effective dose that inhibits 50 per cent of cell growth) of triptolide when tested against these cell lines is lower than that of other antitumour compounds suggests that it may have therapeutic potential for development into an effective drug against this form of bile duct cancer[72–74].

More recently, tumour necrosis factor (TNF) α, at a concentration of 760 and 100 pg/ml in the presence of actinomycin D 1 μg/ml, has induced 50 per cent cell death in the two established human cholangiocarcinoma cell lines. The death of these two cell lines has been shown to be through apoptosis, and TNF-α type I receptor is involved in the process by generating sufficient signal to activate caspase group II enzymes, which are key enzymes in initiating the apoptotic event[75]. Pharmacological modulation of this group of enzymes may have a role in the treatment of cholangiocarcinoma in the future.

Conclusion

Although the association between liver fluke and cholangiocarcinoma has been recognized for more than half a century, and the carcinogenesis induced by the fluke has been actively investigated, the actual causal relationship between the parasite and the cancer is still poorly understood. The molecular biology of the tumour must be further studied. Identification of a marker for the tumour at an early stage is essential if the outcome of surgery is to be improved. Similarly, improvement in imaging technology to locate the tumour precisely will be important. New forms of treatment in addition to surgical resection, as well as effective means of controlling liver flukes, should also be explored.

References

1 International Agency for Research on Cancer. Infection with liver flukes (*Opisthorchis viverrini*, *Opisthorchis felineus* and *Clonorchis sinensis*). *IARC Monogr Eval Carcinog Risks Hum* 1994; **61:** 121–75.

2 Møller H, Heseltine E, Vainio H. Working group report on schistosomes, liver flukes and *Helicobacter pylori*. *Int J Cancer* 1995; **60:** 587–9.

3 Hering-Hagenbeck S, Schuster R. A focus of opisthorchiasis in Germany. *Appl Parasitol* 1996; **37:** 260–5.

4 Bhamarapravati N, Virranuvatti V. Liver diseases in Thailand. An analysis of liver biopsies. *Am J Gastroenterol* 1966; **45:** 267–75.

5 Bunyaratvej S, Meenakanit V, Tantachamrun T, Srinawat P, Susilaworn P, Chongchitnan N. Nationwide survey of major liver diseases in Thailand; analysis of 3305 biopsies as to year-end 1978. *J Med Assoc Thai* 1981; **64:** 432–9.

6 Srivatanakul P, Sontipong S, Chotiwan P, Parkin DM. Liver cancer in Thailand: temporal and geographic variations. *J Gastroenterol Hepatol* 1988; **3:** 413–20.

7 Srivatanakul P, Parkin DM, Jiang YZ, Khlat M, Kao-Ian U, Sontipong S *et al.* The role of infection by *Opisthorchis viverrini*, hepatitis B virus, and aflatoxin exposure in the etiology of liver cancer in Thailand. A correlation study. *Cancer* 1991; **68:** 2411–17.

8 Vatanasapt V, Tangvoraphonkchai V, Titapant V, Pipitgool V, Virivapap D, Sriamporn S. A high incidence of liver cancer in Khon Kaen province, Thailand. *Southeast Asian J Trop Med Public Health* 1990; **21:** 489–94.

9 Green A, Uttaravichien T, Bhudhisawasdi V, Chartbanchachai W, Elkins DB, Marieng EO *et al.* Cholangiocarcinoma in north east Thailand. A hospital-based study. *Trop Geogr Med* 1991; **43:** 193–8.

10 Parkin DM, Srivatanakul P, Khlat M, Chenvidhya D, Chotiwan P, Insiripong S *et al.* Liver cancer in Thailand. I. A case–control study of cholangiocarcinoma. *Int J Cancer* 1991; **48:** 323–8.

11 Pungpak S, Viravan C, Radomyos B, Chalermrut K, Yemput C, Plooksawasdi W *et al. Opisthorchis viverrini* infection in Thailand: studies on the morbidity of the infection and resolution following praziquantel treatment. *Am J Trop Med Hyg* 1997; **56:** 311–14.

12 Thamavit W, Moore MA, Sirisinha S, Shirai T, Ito N. Time-dependent modulation of liver lesion development in *Opisthorchis*-infected Syrian hamster by an antihelminthic drug, praziquantel. *Jpn J Cancer Res* 1993; **84:** 135–8.

13 Schwartz DA. Helminths in the induction of cancer: *Opisthorchis viverrini*, *Clonorchis sinensis* and cholangiocarcinoma. *Trop Geogr Med* 1980; **32:** 95–100.

14 Hou PC. The relationship between primary carcinoma of the liver and infestation with *Clonorchis sinensis*. *Journal of Pathology and Bacteriology* 1956; **72:** 239–46.

15 Hou PC. Primary carcinoma of bile duct of the liver of the cat (*Felis catus*) infested with *Clonorchis sinensis*. *Journal of Pathology and Bacteriology* 1964; **87:** 239–44.

16 Hou PC. Pathological changes in the intrahepatic bile ducts of cats (*Felis catus*) infected with *Clonorchis sinensis*. *Journal of Pathology and Bacteriology* 1965; **89:** 357–64.

17 Chung CS, Lee SK. An epidemiological study of primary liver carcinomas in Pusan area with special reference to clonorchiasis. *Korean J Pathol* 1976; **10:** 33–64.

18 Shin H-R, Lee C-U, Park H-J, Seol S-Y, Chung J-M, Choi H-C *et al.* Hepatitis B and C virus, *Clonorchis sinensis* for the risk of liver cancer: a case–control study in Pusan, Korea. *Int J Epidemiol* 1996; **25:** 933–40.

19 Sher L, Iwatsuki S, Lebeau G, Zajko AB. Hilar cholangiocarcinoma associated with clonorchiasis. *Dig Dis Sci* 1989; **34:** 1121–3.

20 Ona FV, Dytoc JNO. Clonorchiasis-associated cholangiocarcinoma: a report of two cases with unusual manifestations. *Gastroenterology* 1991; **101:** 831–9.

21 Shain AA. Opisthorchiasis and hepatic cancer among the population of the Hanty-Mansy National District. *Vopr Onkol* 1971; **17:** 34–9.

22 Glumov VY, Kotrikov VV, Tret'yakova NA. Pathogenesis and morphology of primary hepatic cancer developed on a background of opisthorchiasis. *Vopr Onkol* 1974; **20:** 46–50.

23 Iablokov DD, Ordina OM, Taranov SV, Trotsenko BA, Baiusova ZA. Combination of primary liver cancer with opisthorchiasis. *Arkh Patol* 1980; **42:** 95–6.

24 Thamavit W, Bhamarapravati N, Sahaphong S, Vajrasthira S, Angsubhakorn S. Effects of dimethylnitrosamine on induction

of cholangiocarcinoma in *Opisthorchis viverrini*-infected Syrian golden hamsters. *Cancer Res* 1978; **38:** 4634–9.
25 Thamavit W, Kongkanuntn R, Tiwawech D, Moore MA. Level of *Opisthorchis* infestation and carcinogen dose-dependence of cholangiocarcinoma induction in Syrian golden hamsters. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1987; **54:** 52–8.
26 Lee JH, Rim HJ, Bak UB. Effect of *Clonorchis sinensis* infection and dimethylnitrosamine administration on the induction of cholangiocarcinoma in Syrian golden hamsters. *Korean J Parasitol* 1993; **31:** 21–30.
27 Iida H. Experimental study of the effects of *Clonorchis sinensis* infection on induction of cholangiocarcinoma in Syrian golden hamsters administered 0.03 percent *N*-2-fluorenylacetamide (FAA). *Jpn J Parasitol* 1985; **34:** 7–16.
28 Thamavit W, Moore MA, Hiasa Y, Ito N. Generation of high yields of Syrian hamster cholangiocellular carcinomas and hepatocellular nodules by combined nitrite and aminopyrine administration and *Opisthorchis viverrini* infection. *Jpn J Cancer Res* 1988; **79:** 909–16.
29 Lijinsky W, Greenblatt M. Carcinogen dimethylnitrosamine produced *in vivo* from nitrite and aminopyrine. *Nature New Biology* 1972; **236:** 177–8.
30 Viranuvatti V, Stitnimankarn T. Liver fluke infection and infestation in southeast Asia. In: Popper H, Schaffner F, eds. *Progress in Liver Disease*. New York: Grune and Stratton, 1972: 537–47.
31 Hou PC, Pang LSC. *Clonorchis sinensis* infestation in man in Hong Kong. *Journal of Pathology and Bacteriology* 1964; **87:** 245–50.
32 Attwood HD, Chou ST. The longevity of *Clonorchis sinensis*. *Pathology* 1978; **10:** 153–6.
33 Bhamarapravati N, Thamavit W, Vajrasthira S. Liver changes in hamsters infected with a liver fluke of man, *Opisthorchis viverrini*. *Am J Trop Med Hyg* 1978; **27:** 787–94.
34 Riganti M, Pungpak S, Punpoowong B, Bunnag D, Harinasuta T. Human pathology of *Opisthorchis viverrini* infection, a comparison of adults and children. *Southeast Asian J Trop Med Public Health* 1989; **20:** 95–100.
35 Thamavit W, Pairojkul C, Tiwawech D, Shirai T, Ito N. Strong promoting effect of *Opisthorchis viverrini* infection on dimethylnitrosamine-initiated hamster liver. *Cancer Lett* 1994; **78:** 121–5.
36 Chotigeat W, Ruenwongsa P. Types of collagen in *Opisthorchis viverrini* infected hamster liver. *Mol Biochem Parasitol* 1986; **18:** 377–87.
37 Hitanant S, Trong DT, Damrongsak C, Chinapak O, Boonyapisit S, Plenvanit U *et al.* Peritoneoscopic findings in 203 patients with *Opisthorchis viverrini* infection. *Gastrointest Endosc* 1987; **33:** 18–20.
38 Flavell DJ, Lucas SB. Promotion of *N*-nitrosodimethylamine-initiated bile duct carcinogenesis in the hamster by the human liver fluke, *Opisthorchis viverrini*. *Carcinogenesis* 1983; **4:** 927–30.
39 Uttaravichien T, Buddhisawasdi V, Pairojkul C. Bile duct cancer and the liver fluke: pathology, presentation and surgical management. *Asian J Surg* 1996; **19:** 267–70.
40 Flavell DJ. Liver fluke infection as an aetiological factor in bile-duct carcinoma of man. *Trans R Soc Trop Med Hyg* 1981; **75:** 814–24.
41 Uttaravichien T, Buddhisawasdi V. Experience of non-jaundiced cholangiocarcinoma. *Hepatogastroenterology* 1990; **37:** 608–11.
42 Sinawat P, Hemsrichart V. A histopathologic study of 61 cases of peripheral intrahepatic cholangiocarcinoma. *J Med Assoc Thai* 1991; **74:** 448–53.
43 Watanapa P. Cholangiocarcinoma in patients with opisthorchiasis. *Br J Surg* 1996; **83:** 1062–4.
44 Sriurairatana S, Tengchaisri T, Sririsinha S. Ultrastructural characteristics of liver fluke associated human cholangio-carcinoma cell lines. *Southeast Asian J Trop Med Public Health* 1996; **27:** 57–62.
45 NakajimaT, Kondo Y. A clinicopathologic study of intrahepatic cholangiocarcinoma containing a component of squamous cell carcinoma. *Cancer* 1990; **65:** 1401–4.
46 Sun T. Pathology and immunology of *Clonorchis sinensis* infection of the liver. *Ann Clin Lab Sci* 1984; **14:** 208–15.
47 Choi BI, Park JH, Kim YI, Yu ES, Kim SH, Kim CY *et al.* Peripheral cholangiocarcinoma and clonorchiasis: CT findings. *Radiology* 1988; **169:** 149–53.
48 Tsuda H, Satarug S, Bhudhisawasdi V, Kihana T, Sugimura T, Hirohashi S. Cholangiocarcinomas in Japanese and Thai patients: difference in etiology and incidence of point mutation of the c-Ki-*ras* proto-oncogene. *Mol Carcinog* 1992; **6:** 266–9.
49 Kiba T, Tsuda H, Pairojkul C, Inoue S, Sugimura T, Hirohashi S. Mutations of the *p53* tumor suppressor gene and the *ras* gene family in intrahepatic cholangiocellular carcinomas in Japan and Thailand. *Mol Carcinog* 1993; **8:** 312–18.
50 Williamson RCN, Rainey JB. The relationship between intestinal hyperplasia and carcinogenesis. *Scand J Gastroenterol* 1984; **19**(Suppl 104): 57–76.
51 Watanapa P, Williamson RCN. Experimental pancreatic hyperplasia and neoplasia: dietary and surgical manipulation. *Br J Cancer* 1993; **67:** 877–84.
52 Migasena P, Reaunsuwan W, Changbumrung S. Nitrates and nitrites in local Thai preserved protein foods. *J Med Assoc Thai* 1980; **63:** 500–5.
53 Mitacek EJ, Brunnemann KD, Suttajit M, Martin N, Limsila T, Ohshima H *et al.* Exposure to *N*-nitroso compounds in a population of high liver cancer regions in Thailand: volatile nitrosamine (VNA) levels in Thai food. *Food Chem Toxicol* 1999; **37:** 297–305.
54 Srivatanakul P, Ohshima H, Khlat M, Parkin M, Sukaryodhin S, Brouet I *et al.* Endogenous nitrosamines and liver fluke as risk factors for cholangiocarcinoma in Thailand. *Int J Cancer* 1991; **48:** 821–5.
55 Satarug S, Haswell-Elkins MR, Tsuda M, Mairiang P, Sithithaworn P, Mairiang E *et al.* Thiocyanate-independent nitrosation in humans with carcinogenic parasite infection. *Carcinogenesis* 1996; **17:** 1075–81.
56 Ohshima H, Bandaletova TY, Brouet I, Bartsch H, Kirby G, Ogunbiyi F *et al.* Increased nitrosamine and nitrate

biosynthesis mediated by nitric oxide synthase induced in hamsters infected with liver fluke (*Opisthorchis viverrini*). *Carcinogenesis* 1994; **15:** 271–5.
57 Haswell-Elkins MR, Satarug S, Tsuda M, Mairiang E, Esumi H, Sithithaworn P *et al.* Liver fluke infection and cholangiocarcinoma: model of endogenous nitric oxide and extragastric nitrosation in human carcinogenesis. *Mutat Res* 1994; **305:** 241–52.
58 Kirby GM, Pelkonen P, Vatanasapt V, Camus AM, Wild CP, Lang MA. Association of liver fluke (*Opisthorchis viverrini*) infestation with increased expression of cytochrome P450 and carcinogen metabolism in male hamster liver. *Mol Carcinog* 1994; **11:** 81–9.
59 Satarug S, Lang MA, Yongvanit P, Sithithaworn P, Mairiang E, Mairiang P *et al.* Induction of cytochrome P450 2A6 expression in humans by the carcinogenic parasitic infection, *Opisthorchis viverrini*. *Cancer Epidemiol Biomarkers Prev* 1996; **5:** 795–800.
60 Billiar TR, Curran RD, Stuehr DJ, Stadler J, Simmons RL, Murray SA. Inducible cytosolic enzyme activity for the production of nitrogen oxides from L-arginine in hepatocytes. *Biochem Biophys Res Commun* 1990; **168:** 1034–40.
61 Ohshima H, Brouet IM, Bandaletova T, Adachi H, Oguchi S, Iida S *et al.* Polyclonal antibody against an inducible form of nitric oxide synthase purified from the liver of rats treated with *Propionibacterium acnes* and lipopolysaccharide. *Biochem Biophys Res Commun* 1992; **187:** 1291–7.
62 Wink DA, Kasprzak KS, Maragos CM, Elespuru RK, Misra M, Dunams TM *et al.* DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science* 1991; **254:** 1001–3.
63 Nguyen T, Brunson D, Crespi CL, Penman BW, Wishnok JS, Tannenbaum SR. DNA damage and mutation in human cells exposed to nitric oxide *in vitro*. *Proc Natl Acad Sci U S A* 1992; **89:** 3030–4.
64 Prempracha N, Tengchaisri T, Chawengkirttikul R, Boonpucknavig S, Thamavit W, Duongchawee G *et al.* Identification and potential use of a soluble tumor antigen for the detection of liver-fluke-associated cholangiocarcinoma induced in a hamster model. *Int J Cancer* 1994; **57:** 691–5.
65 Sirisinha S, Tengchaisri T, Boonpucknavig S, Prempracha N, Ratanarapee S, Pausawasdi A. Establishment and characterization of a cholangiocarcinoma cell line from a Thai patient with intrahepatic bile duct cancer. *Asian Pac J Allergy Immunol* 1991; **9:** 153–7.
66 Tengchaisri T, Prempracha N, Thamavit W, Boonpucknavig S, Sriurairatana S, Sirisinha S. Establishment and characterization of cell lines from liver fluke-associated cholangiocarcinoma induced in a hamster model. *Southeast Asian J Trop Med Public Health* 1995; **26:** 231–9.
67 Park SJ, Han JK, Kim TK, Choi BI. Three-dimensional spiral CT cholangiography with minimal intensity projection in patients with suspected obstructive biliary disease: comparison with percutaneous transhepatic cholangiography. *Abdom Imaging* 2001; **26:** 281–6.
68 Maetani Y, Itoh K, Watanabe C, Shibata T, Ametani F, Yamabe H *et al.* MR imaging of intrahepatic cholangiocarcinoma with pathologic correlation. *AJR Am J Roentgenol* 2001; **176:** 1499–507.
69 Watanapa WB, Watanapa P. Autonomic function test in patients with obstructive jaundice. *Siriraj Hosp Gaz* 1997; **49:** 114–19.
70 Nakeeb A, Pitt HA, Sohn TA, Coleman J, Abrams RA, Piantadosi S *et al.* Cholangiocarcinoma. A spectrum of intrahepatic, perihilar, and distal tumors. *Ann Surg* 1996; **224:** 463–75.
71 Uenishi T, Hirohashi K, Kubo S, Yamamoto T, Hamba H, Tanaka H *et al.* Histologic factors affecting prognosis following hepatectomy for intrahepatic cholangiocarcinoma. *World J Surg* 2001; **25:** 865–9.
72 Shamon LA, Pezzuto JM, Graves JM, Mehta RR, Wangcharoentrakul S, Sangsuwan R *et al.* Evaluation of the mutagenic, cytotoxic, and antitumor potential of triptolide, a highly oxygenated diterpene isolated from *Tripterygium wilfordii*. *Cancer Lett* 1997; **112:** 113–17.
73 Tengchaisri T, Chawengkirttikul R, Rachaphaew N, Reutrakul V, Sangsuwan R, Sirisinha S. Antitumor activity of triptolide against cholangiocarcinoma growth *in vitro* and in hamsters. *Cancer Lett* 1998; **133:** 169–75.
74 Sampson LK, Vickers SM, Ying W, Phillips JO. Tamoxifen-mediated growth inhibition of human cholangiocarcinoma. *Cancer Res* 1997; **57:** 1743–9.
75 Utaisincharoen P, Ubol S, Tangthawornchaikul N, Chaisuriya P, Sirisinha S. Binding of tumour necrosis factor-alpha (TNF-α) to TNF-RI induces caspase(s)-dependent apoptosis in human cholangiocarcinoma cell lines. *Clin Exp Immunol* 1999; **116:** 41–7.

