Parasitology International 66 (2017) 383–389

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Review

Co-infections with liver fluke and *Helicobacter* species: A paradigm change in pathogenesis of opisthorchiasis and cholangiocarcinoma?

Banchob Sripa [a,*], Raksawan Deenonpoe [a,b], Paul J. Brindley [c]

[a] WHO Collaborating Centre for Research and Control of Opisthorchiasis (Southeast Asian Liver Fluke Disease), Tropical Disease Research Center, Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
[b] Chulabhorn International College of Medicine, Thammasat University, Pathum Thani 12120, Thailand
[c] Department of Microbiology, Immunology and Tropical Medicine, Research Center for Neglected Tropical Diseases of Poverty, School of Medicine & Health Sciences, The George Washington University, Washington, DC 20037, USA

ARTICLE INFO

Article history:
Received 15 November 2016
Received in revised form 27 November 2016
Accepted 27 November 2016
Available online 3 December 2016

Keywords:
Liver fluke
Opisthorchis viverrini
Helicobacter spp.
Co-infection
Pathogenesis
Hepatobiliary diseases
Cholangiocarcinoma

ABSTRACT

Infection with the fish-borne liver fluke *Opisthorchis viverrini* is classified by the International Agency for Research on Cancer as a Group 1 carcinogen: definitely carcinogenic in humans. Cofactors likely contribute to bile duct cancer (cholangiocarcinoma) caused by this infection. Here we review recent findings that address the role of liver fluke associated *H. pylori* in hepatobiliary disease and malignancy. We hypothesize that co-infection by *O. viverrini* and the bacillus *Helicobacter pylori* is central of liver fluke infection associated cholangiocarcinoma.

Contents

1. Introduction

Foodborne trematodiasis caused by infection with the opisthorchiid liver flukes *Opisthorchis viverrini*, *O. felineus* and *Clonorchis sinensis* remains a major public health problem in East Asia and Eastern Europe

* Corresponding author at: Tropical Disease Research Center (TDRC), Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.
E-mail address: banchob@kku.ac.th (B. Sripa).

http://dx.doi.org/10.1016/j.parint.2016.11.016
1383-5769/

where >40 million people are infected [1,2]. *O. viverrini* is endemic in Thailand, Lao People's Democratic Republic (Lao PDR), Vietnam and Cambodia with over 10 million people are infected [1]. Humans acquire the infection by eating raw or undercooked fish harboring infective stage metacercariae (reviewed in [3]). Upon ingestion, the metacercariae excyst in the duodenum and juvenile flukes migrate into the biliary tree. In the bile ducts, the parasites mature over four weeks into adult flukes. Parasites' eggs are shed in the fecal stream to the environment where the eggs are ingested by freshwater snails of the genus *Bithynia*. The parasite undergoes transformations and multiplications within the snail, culminating in the release of cercariae that seek out and penetrate the skin of a freshwater cyprinid fish, completing the cycle. Human infection causes several hepatobiliary abnormalities, including cholangitis, obstructive jaundice, hepatomegaly, periductal fibrosis, cholecystitis and cholelithiasis (see [3]). Both experimental and epidemiological evidence strongly implicates liver fluke infection in the etiology of one of the primary liver cancer subtypes – cholangiocarcinoma (CCA), a fatal bile duct cancer [1,4,5]. Khon Kaen province in north-eastern Thailand where the *O. viverrini* liver fluke is endemic has reported the highest incidence of CCA in the world, >100 cases per 100,000 [6]. However, additional risk factors for hepatobiliary diseases and CCA have been documented including primary sclerosing cholangitis (see [7]), inflammatory bowel disease [8], metabolic syndromes [9], hepatitis virus [10], fluke infection-associated oxysterols [11], and infection with *Helicobacter* spp [12]. The last is attracting increasing research interest [13].

2. *Helicobacter* spp. and extragastric diseases

Infection with *Helicobacter pylori*, a Gram-negative bacillus is the first bacterial infection known to be an etiological agent of gastric diseases including gastric adenocarcinoma [14–17]. Virulence factors of *H. pylori* including cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) contribute to the pathogenesis of *H. pylori*-associated disease [18,19]. Although chronic *H. pylori* infection is associated with the stomach cancer but the possible association with several extragastric complications including hepatobiliary and pancreatic diseases have been proposed [20,21]. There is strong evidence that *H. pylori* seropositivity and biliary tract cancer with overall OR 5.47 and, specifically, for extrahepatic (OR 7.01) and intrahepatic cancer (OR 10.67) but not for hepatocellular carcinoma in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) cohort [22]. For liver fibrosis, prevalence of *cag*A *H. pylori* was directly proportional to severity of liver disease and was more positive in advanced stages of fibrosis (28.2%) compared to early stages (5.9%) in HCV-related chronic hepatitis and cirrhosis [23]. The mechanism by which *H. pylori* induces liver fibrosis may involve increased cytokines, i.e. TGF-β1 and oxidative stress induced pro-inflammatory signaling pathways in hepatic stellate cell line (HSC) [24,25]. Other species of *Helicobacter*, specifically *H. hepaticus* and *H. bilis* also are implicated in hepatobiliary disease [12,13,22].

3. Associations among *Helicobacter*, opisthorchiasis and cholangiocarcinoma

H. pylori has been reported to be involved in a case series of hepatobiliary diseases in opisthorchiasis endemic Thailand [26]. We first systematically reported an association between *H. pylori*, specifically cagA positive-*H. pylori* and CCA but not hepatolithiasis or normal controls in patients from Northeast Thailand, a region endemic for opisthorchiasis [26,27].CCA cases with *H. pylori* infection exhibit higher portal inflammation and biliary cell proliferation as determined by PCNA immunohistochemistry [26]. Molecular mechanisms integral to *H. pylori* induced hepatobiliary diseases have also been described [28–30]. Since CCA is strongly associated with opisthorchiasis in endemic areas, as noted, *O. viverrini* may have an integral though still cryptic relationship with *H. pylori*. Indeed, we have been aware of this relationship for some time, and recently reported an association between *O. viverrini* and *Helicobacter* spp. in a hamster model [31]. The liver fluke infected hamsters showed significantly higher *H. pylori* and *H. bilis* than control, non-liver fluke-infected hamsters. In addition, *H. pylori* can be detected in the gut epithelium of *O. viverrini* and hence we have concluded that the liver fluke represents a reservoir of *H. pylori* within the biliary system [31]. Similar findings have been seen in humans infected with *O. viverrini*. The higher the liver fluke infection intensity, as determined by *O. viverrini* eggs per gram of feces, the greater the fecal numbers of *H. pylori*. Moreover, we also demonstrated that *cag*A positive *H. pylori* associated with increased risk of periductal fibrosis as determined by ultrasonography in opisthorchiasis (Deenonpoe et al., manuscript submitted). Given our pioneering research in the pathogenesis of liver fluke induced pathology and CCA and over 20 years research experience in this field [2,3,11,29,32,33], we hypothesize that the liver fluke/*H. pylori* co-infection is the central player in biliary disease manifestations including CCA in opisthorchiasis in northeastern Thailand. However, the underlying mechanisms by which *H. pylori* associates with the liver fluke, opisthorchiasis and CCA remain to be established.

4. Pathogenesis of *H. pylori* induced biliary diseases

Pathogenesis and disease outcomes following infection with *H. pylori* are mediated by a complex interplay between bacterial virulence factors, host, and environmental factors. Unfortunately, only a few studies describe the pathogenesis in *H. pylori* induced biliary diseases [26,28,30]. In brief, following entry of *H. pylori* into host tissue, four steps are critical for the bacterium to establish successful colonization, persistent infection, and disease: 1) survival in environment (the acidic stomach or the alkaline bile ducts); 2) movement toward epithelial cells by flagella-mediated motility; 3) attachment to host cells mediated by interactions between bacterial cell adhesins and host cell receptors; and 4) tissue damage following the release of toxins, specifically CagA and VacA [34,35].

5. Survival of *H. pylori* in the bile

H. pylori and other species of *Helicobacter* can survive at the alkaline pH of the bile as they can be detected in bile sampled from biliary diseases including CCA [36,37]. Most of the *H. pylori* and other bacteria in the bile are coccoid form that may reflect responses to bile acids [38]. In addition, biofilm formation by the bacteria in the bile seems facilitate survival in the environment within the human biliary tract [36]. Interestingly, an increased abundance of *H. pylori* virulence genes, i.e. *cagA* and *vacA* was observed in extrahepatic CCA compared to benign biliary diseases [37]. Similarly, significant higher frequencies of *cagA*-positive *H. pylori* have been reported during CCA than cholelithiasis and in bile from healthy individuals [26]. Biliary micro-environmental components such cholesterol may enhance the pathogenicity of *H. pylori* as it acquires host cholesterol for catabolism of lipopolysaccharide (LPS) for cell membranes [39]. In addition, cholesterol promotes growth of *H. pylori* in serum-free media [40]. Together, these findings provide support to the notion that *H. pylori* can be more virulent within the biliary environment.

6. *Opisthorchis* is a reservoir of *H. pylori* and host-bacterial interaction

We recently reported that *O. viverrini* was a reservoir of *H. pylori* (Fig. 1) [31]. The intensity of infection with *H. pylori* within *O. viverrini* infected hamsters was significantly greater than that of uninfected hamsters (Fig. 2). The *H. pylori* bacteria localized on the gut epithelium of fluke and survived in the adult liver flukes in vitro co-cultured with antibiotics for >30 days [31]. These findings indicate the establishment of *H. pylori* benefits from the support of the liver fluke gut. As the pH in the secretions from the gut of *O. viverrini* is approximately pH 5–6,