MINI-REVIEW

Benefits of Metformin Use for Cholangiocarcinoma

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Abstract

Metformin is an oral anti-hyperglycemic agent, which is the most commonly prescribed medication in the treatment of type-2 diabetes mellitus. It is purportedly associated with a reduced risk for various cancers, mainly exerting anti-proliferation effects on various human cancer cell types, such as pancreas, prostate, breast, stomach and liver. This mini-review highlights the risk and benefit of metformin used for cholangiocarcinoma (CCA) prevention and therapy. The results indicated metformin might be a quite promising strategy CCA prevention and treatment, one mechanism being inhibition of CCA tumor growth by cell cycle arrest in both *in vitro* and *in vivo*. The AMPK/mTORC1 pathway in intrahepatic CCA cells is targeted by metformin. Furthermore, metformin inhibited CCA tumor growth via the regulation of Drosha-mediated expression of multiple carcinogenic miRNAs. The use of metformin seems to be safe in patients with cirrhosis, and provides a survival benefit. Once hepatic malignancies are already established, metformin does not offer any therapeutic potential. Clinical trials and epidemiological studies of the benefit of metformin use for CCA should be conducted. To date, whether metformin as a prospective chemotherapeutic for CCA is still questionable and waits further atttention.

Keywords: Metformin - cholangiocarcinoma - bile duct cancer - gall bladder cancer

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Introduction

Cholangiocarcinoma (CCA) is neoplasms that involve the epithelial cells of the bile duct, also known as one of the most aggressive malignant tumors associated with local invasiveness and a high rate of metastasis. CCA is originated in the bile duct in which drained bile from the liver into the small intestine. Other biliary tract cancers include pancreatic cancer, gall bladder cancer, and cancer of the ampulla of Vater. It is also known to be one of the most common causes of cancer related death in Thailand and it has been reported that Thailand is the highest incident of the world (Green et al., 1991; Sripa et al., 2007; Shin et al., 2010). It has an annual incidence rate of 1-2 cases per 100,000 in the Western world, but rates of CCA have been rising worldwide over the past several decades (Landis et al., 1998; Patel, 2002).

This disease is difficult to diagnose early, as most symptoms present late in the disease. In addition, the specific anatomic position can cause periductal extension and result in a very low radical excision rate and a very poor prognosis. Furthermore, CCA is considered to be an incurable and rapidly lethal disease unless all the tumors can be fully resected. Three-year survival rates of 35% to 50% are achieved only in a subset of patients who have negative histological margins at the time of surgery (Akamatsu et al., 2011). Survival of CCA patients in northeastern Thailand after supportive treatment was reported and indicated that the stage of disease was an important prognosis factor affecting survival of CCA patients who had diagnosis in late stage. The encourage patients to see health personnel at early stage is very important (Thunyaharn et al., 2013). Palliative therapeutic approaches, consisting of percutaneous and endoscopic biliary drainage, have usually been used for these patients because there is no effective chemotherapeutic treatment for this type of cancer.

Chemotherapy agents used to treat CCA include 5-fluorouracil with leucovorin Choi et al., 2000), gemcitabine and irinotecan (Bhargava et al., 2003), combining gemcitabine and capecitabine (Knox et al 2005), gemcitabine as a single agent (Park et al., 2005), or

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gemcitabine plus cisplatin (Giuliani et al., 2006), tyrosine kinase inhibitor erlotinib (Philip et al., 2006). Recently, the benefit of using metformin for chemoprevention of CCA is suggested. The using metformin, was also demonstrated that DM patients who used metformin as DM treatment had a significantly decreased risk of CCA development with an OR 0.40 (95% CI: 0.20-0.90) (Chaiteerakij et al., 2013). In addition, metformin inhibits proliferation and enhances chemosensitivity of intrahepatic CCA cell lines. It may be a prospective chemotherapeutic agent or a chemosensitizer in future intrahepatic CCA treatment (Ling et al., 2014). However, Yang et al. (2015) has been argument that metformin does not improve survival of CCA in persons with diabetes. Furthermore, Santos et al. (2012) has been reported a 56-year-old woman with antecedents of cholelithiasis and ampullary adenocarcinoma who was an overweight diabetic using metformin. From above data, update on metformin using for CCA is need required and concentrate clarified, therefore, mini-review critically analyze the literature behind the potential use of metformin across the spectrum of CCA. The PubMed and Ovid MEDLINE databases were searched, using a combination of relevant text words and MeSH terms: metformin and/or cholangiocarcinoma, bile duct neoplasms, intra-extrahepatic, common bile duct, and gall bladder cancer.

Cholangiocarcinoma and its Associated Risk Factors

A number of risk factors for the development of CCA have been described. Multifactorial is associated to develop CCA. The 3 main factors have been hypothesized, including carcinogen agents, infection, and other factors. Caroli's disease, choledocal cyst, liver fluke infection, gallstones, hepatolithiasis, sclerosinf cholangitis, thorotrast, and ulcerative colitis, are strongly associated with CCA development. While, Asbestos, isoniazid, methyldopa, oral contraceptive, polychlorinated biphenyls are the possibly associated to develop CCA (Yeo et al., 1990; Sripa et al., 2005). The most common of these is primary sclerosing cholangitis and an inflammatory disease of the bile ducts which is itself closely associated with ulcerative colitis (Chapman, 1999). Alcoholic liver disease, or cirrhosis of the liver due to other causes, are at significantly increased risk of CCA (Sorensen et al., 1998; Shaib et al., 2005). The presence of hepatolithiasis, intrahepatic stones, which is rare in the West, but common in parts of Asia, has been strongly associated with CCA (Su et al., 1997; Donato et al., 2001; Lee et al., 2002). Caroli's syndrome or choledochal cysts, congenital liver abnormalities, have been associated with an approximately 15% lifetime risk of developing CCA (Dayton et al., 1983; Lipsett et al., 1994). The rare inherited disorders Lynch syndrome II has also been found to be associated with CCA (Mecklin et al., 1992; Lee et al., 2004). Carcinogens have been described that are the main factor to develop CCA. Exposure to Thorotrast has been linked to the development of CCA as late as 30-40 years after exposure; Thorotrast was banned in the United States in the 1950s due to its carcinogenicity (Sahani et al., 2003; Zhu et al., 2004). Dimethylnitrosamine is the caused of primary carcinoma and could induced CCA development in both animal model and human epidemic data. Nitrates and nitrites were found in local Thai preserved protein foods that strongly positive associated to develop CCA (Herrold 1967; Migasena and Changbumrung, 1974; Migasena et al., 1980).

Infections are associated with the development of CCA, mainly liver flukes, *Opisthorchis viverrini* (Watanapa and Watanapa, 2002; Sripa et al., 2007; Kaewpitoon et al., 2008; Sripa et al., 2010), *O. felineus* (Maksimova et al., 2015), *Clonorchis sinensis* (Hong and Fang, 2012; Rustagi and Dasanu, 2012), and viral hepatitis (e.g. hepatitis B or hepatitis C) (Kobayashi et al., 2000; Lu et al., 2000; Yamamoto et al., 2004). In Thailand, the experimental and epidemiological evidences strongly indicated that *O. viverrini* infection in the etiology of CCA (Thamavit et al., 1978; IARC, 1994; Sripa et al., 2007). There has been a strong, positive correlation between opisthorchiasis-associated CCA and infection with *Helicobacter*. Infection with *H. bilis* and *H. hepaticus* species can cause biliary cancer (Chang and Parsonnet, 2010). Other factors are associated with CCA development, mainly smoking (Mitacek et al., 1999), obesity, and diabetes mellitus (DM) (Saengboonmee et al., 2015). The data from several epidemiological studies strongly indicate that DM is a risk factor of both intrahepatic and extrahepatic CCA, including gall bladder cancer (Ren et al., 2011; Jing et al., 2012; Palmer and Patel, 2012; Zhang et al., 2013). There is no epidemiological study of the association of DM and *O. viverrini* associated CCA in Thailand. However, the association of DM and CCA in Thailand, has been reported by Saengboonmee et al. (2015) that almost all provinces with a high mortality rate of DM also had a high mortality rate of liver and biliary tract cancer.

The Benefit of Metformin Used in Cholangiocarcinoma?

Metformin is an oral anti-hyperglycemic agent of the biguanide family, which is the most commonly prescribed medication in the treatment of type-2 diabetes mellitus. It exerts its prevailing, glucose-lowering effect by inhibiting hepatic gluconeogenesis and opposing the action of glucagon. The inhibition of mitochondrial complex I results in defective cAMP and protein kinase A signaling in response to glucagon. Stimulation of 5'-AMP-activated protein kinase, although dispensable for the glucose-lowering effect of metformin, confers insulin sensitivity, mainly by modulating lipid metabolism (Pernicova and Korbonits, 2014). In addition, Pernicova and Korbonits, (2014) suggested that metformin might influence tumourigenesis, both indirectly, through the systemic reduction of insulin levels, and directly, via the induction of energetic stress; however, these effects require further investigation. More recently, the use of metformin has shown potential as a preventive and therapeutic agent for a broad spectrum of conditions, including cancers. It is purportedly associated with a reduced risk for various

cancers, mainly the anti-proliferation effect on various human cancer cell types, such as pancreas (Akinyeke et al., 2013), prostate (Nair et al., 2013), breast (Alimova et al., 2009), stomach (Kato et al., 2012) and liver (Petrushev et al., 2012).

Many studies have been reported that metformin might be a quite promising strategy for CCA prevention and treatment. Presently, Bhat et al. (2013) reveal that metformin, which represses mechanistic/mammalian target of rapamycin (mTOR) signaling, the mTOR pathway plays a critical role in cellular metabolism, growth, and proliferation and has been evaluated as a target for therapy in various malignancies. The mTOR pathway is a major tumor-initiating pathway in hepatocellular carcinoma, with up-regulation seen in up to 50% of tumors, by activating adenosine monophosphate-activated protein kinase, has been shown to decrease liver carcinogenesis in population studies. There is also evidence of mTOR pathway activation in CCA, although its biological significance in initiating and promoting tumor progression remains ambiguous. Meanwhile, Jiang et al. (2015) have been reported that metformin inhibited CCA tumor growth by cell cycle arrest *in vitro* and *in vivo*. The expression of six miRNAs (mir124, 182, 27b, let7b, 221 and 181a), which could directly target cell-cycle-regulatory genes, was altered by metformin *in vitro* and *in vivo*. These miRNAs were dysregulated in CCA and promoted the CCA genesis and metformin exactly modulated these carcinogenic miRNAs expression to arrest the cell cycle and inhibit the proliferation. These miRNAs expression changes correlated with the tumor volume and postoperative survival of CCA patients and could be used to predict the prognosis. This study indicated that metformin inhibited CCA tumor growth via the regulation of Drosha-mediated multiple carcinogenic miRNAs expression and comprehensive evaluation of these miRNAs expression could be more efficient to predict the prognosis. Ling et al. (2014) have been reported 1,828 potential intrahepatic CCA patients, metformin use was significantly associated with a 60% reduction in intrahepatic CCA risk in diabetic patients. It exhibited a dose- and time-dependent anti-proliferation effect on intrahepatic CCA cell lines, by mechanisms including apoptosis induction and cell cycle arrest. Metformin targeted the AMPK/mTORC1 pathway in intrahepatic CCA cells. Furthermore, metformin sensitized intrahepatic CCA cells to certain chemotherapeutic agents, such as sorafenib, 5-fluorouracil and As2O3 by targeting the AMPK/mTOR/HIF-1α/MRP1 pathway and ERK. These data suggest that metformin may be a prospective chemotherapeutic agent or a chemosensitizer in future Intrahepatic CCA treatment.

The benefit of metformin is for treating chronic liver diseases, particularly in the context of insulin resistance and inflammation. There is Level III evidence for a chemopreventive role in patients with diabetes and chronic liver disease, with decreased incidence of hepatocellular carcinoma and CCA. The use of metformin seems to be safe in patients with cirrhosis, and provides a survival benefit. Once hepatic malignancies are already established, metformin does not offer any therapeutic potential (Bhat et al., 2015). The case-control study evaluated risk factors for intrahepatic CCA and explored the effects of metformin on intrahepatic CCA risk in a clinic/hospital-based cohort has been reported. Factors associated with increased intrahepatic CCA risk included biliary tract diseases (adjusted odds ratio [AOR]: 81.8; 95% confidence interval [CI]: 11.2-598.8; P<0.001), cirrhosis (AOR, 8.0; 95% CI: 1.8-36.5; P=0.007), diabetes (AOR, 3.6; 95% CI: 2.3-5.5; P<0.001), and smoking (AOR, 1.6; 95% CI:1.3-2.1; P<0.001). Compared to diabetic patients not treated with metformin, the odds ratio (OR) for intrahepatic CCA for diabetic patients treated with metformin was significantly decreased (OR, 0.4; 95% CI: 0.2-0.9; P=0.04). This study confirmed treatment with metformin was significantly associated with a 60% reduction in intrahepatic CCA risk in diabetic patients (Chaiteerakij et al 2013). However, Yang et al. (2015) have been reported that metformin does not improve survival of CCA in persons with diabetes. This study was performed a retrospective analysis of 250 diabetic patients with newly diagnosed CCA seen at Mayo Clinic in Rochester, Minnesota, between January 2001 and December 2012. Survival of patients with DM and CCA taking metformin versus not taking metformin was calculated from the CCA diagnosis date to the last follow-up date and found that among the 250 patients, median survival was 9.5 months. The survival of 49 patients who continued taking metformin after CCA diagnosis was not different from that of 165 patients who had never taken metformin (9.1 versus 9.2 months; HR50.8, 95% CI 0.6-1.2; P50.31). A history of any metformin use before CCA diagnosis also did not affect survival. This study suggested that metformin exerts a chemotherapeutic effect on cancer by reducing cell proliferation and inducing cell cycle arrest and apoptosis. However, metformin did not improve the survival of CCA patients with DM. Santos et al. (2012) has been reported a 56-year-old woman was admitted with jaundice, and laboratory data were indicative of pancreatitis, which recurred in spite of adequate clinical and nutritional management. The patient was an overweight diabetic using metformin, who had antecedents of cholelithiasis and recent cholecystectomy. The histopathology study of the transpapillary biopsy confirmed the ampullary adenocarcinoma.

Conclusion

That metformin decreased the risk of CCA as chemoprevention is clearly demonstrated in many studies, however, the argument of metformin did not improve the survival of CCA patients with DM is described. Therefore, The clinical trial and epidemiological studies the benefit of metformin use for CCA should be more investigated. To date, whether metformin as a prospective chemotherapeutic CCA is still questionable and is waiting for discovery.

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