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REVIEW ARTICLE

Chemotherapy-associated hepatotoxicity in colorectal cancer

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Summary

Chemotherapy-associated hepatotoxicity in liver metastatic colorectal cancer is attracting more and more attention for clinicians. This hepatotoxicity heralds an increased risk of morbidity and mortality in patients with colorectal liver metastases, therefore it is important that clinicians have an adequate knowledge of the chemotherapy-associated hepatotoxicity.

Key words: *chemotherapy, colorectal cancer, diagnosis, hepatotoxicity*

Introduction

Colorectal cancer remains one of the most common cancers worldwide. According the American Cancer Statistics, in 2012 an estimated 103,170 new cases of colon cancer and approximately 40,290 cases of rectal cancer will occur, and an estimated 51,690 people will die of colon and rectal cancer combined [1]. Fifty percent of patients with primary colorectal cancer will go on to develop metastatic disease in the liver, and in 25% of the patients, this is present at the time of diagnosis [2-4]. In patients with liver metastases only, the gold standard of treatment is liver resection, however for those patients with inoperable disease, the mainstay of treatment remains systemic chemotherapy.

It has been shown that systemic chemotherapy can increase the progression free survival (PFS) in those who had undergone resection. The EPOC (EORTC40983) study met its primary endpoint of improved 3-year PFS. However, at a median follow-up of 8.5 years this did not translate into a significant difference in overall survival (OS) between the groups, although the study was underpowered for this secondary endpoint [5].

Patients with initially unresectable colorectal cancer can also be converted to resectable status with 5-year survival rates after resection almost as good as those who were resectable at presentation [6]. The advent of modern chemotherapeutics such as oxaliplatin and irinotecan, as well as biological treatments such as bevacizumab and cetuximab, have improved OS rates in patients with inoperable liver metastatic colorectal cancer [7].

Many observational studies have been published claiming that the use of chemotherapy before surgery can lead to injury to the hepatic parenchyma. This injury has been reported to take the form of hepatic steatosis and sinusoidal obstruction syndrome [8-10].

This hepatotoxicity herals an increased risk of morbidity and mortality in those patients who have colorectal liver metastases, therefore it is important that clinicians have an adequate knowledge of the chemotherapy-associated hepatotoxicity.

In this review we focused on the diagnosis of chemotherapy-associated hepatotoxicity and the mechanisms of action of the most commonly used agents, namely 5-fluorouracil (5-FU), irinotecan, oxliplatin, cetuximab/panitumumab.

Definitions

Chemotherapy-induced hepatic injuries (CIHI) are divided into two main groups:(i) chemotherapy-associated fatty liver diseases, the spectrum

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of which includes chemotherapy-associated simple steatosis(CASS) and chemotherapy-associated steatohepatitis (CASH) and (ii) sinusoidal injuries, including sinusoidal dilation and congestion, sinusoidal obstruction syndrome (SOS), haemorrhagic centri-lobular necrosis (HCN) and nodular regenerative hyperplasia (NRH).

Steatosis and steatohepatitis

Nonalcoholic fatty liver disease is being seen with increased frequency, even in patients not receiving chemotherapy. This clinicopathological condition comprises a wide spectrum of liver damage, ranging from simple steatosis to steatohepatitis, occasionally leading to fibrosis and cirrhosis. Steatohepatitis is defined pathologically by the presence of steatosis together with necro-inflammatory activity [11]. Steatosis is distinct from steatohepatitis. The severity of hepatic steatosis is determined by the proportion of involved hepatocytes as judged by histological study of hematoxylin and eosin-stained sections of the liver. A variety of grading systems exist, although the most commonly used is that proposed by Kleiner et al. [11], which classifies steatosis as absent (<5% hepatocytes), mild (5–33% hepatocytes), moderate (>33–66% hepatocytes), and severe (>66% of hepatocytes). This grading system is different from other grading systems, using a cutoff of 30 and 60% to define moderate and severe steatosis, respectively [12,13].

Given the inherent interobserver variability in assessing steatosis, minor differences in these grading systems are unlikely to be significant, and as such, a cut off of 30 or 33 % was considered to be equivalent for the purposes of assessment [14,15].

Vascular sinusoidal injury

Sinusoidal injury is distinct from fatty liver disease. It is characterized by sinusoidal dilatation and erythrocytes' congestion, occasionally accompanied by perisinusoidal fibrosis and fibrotic venular occlusion [16]. Grossly, sinusoidal injury can be manifested intra-operatively as a patchy blue-appearing liver.

A key feature of the sinusoidal obstruction syndrome is sinusoidal dilatation with associated hepatocyte atrophy. Later changes include the development of perisinusoidal fibrosis and nodular regenerative hyperplasia. Most commonly, sinusoidal dilatation is graded according to the method of Rubbia-Brandt et al. [17] (0=absent,1=mild, 2=moderate, 3=severe), and a higher score is thought to reflect a more severe injury to the hepatic sinusoid.

The mechanisms of action of the most commonly used agents

5- fluorouracil: Mechanism of action

5-FU is a fluoropyrimidine antimetabolite. Its structure is analogue to uracil with the substitution of a fluorine atom in place of hydrogen at the C5 position. It enters cells via a facilitated transport mechanism. Its antitumor properties were first studied in the 1950s following a work on hepatomas in rats [18]. Its cytotoxicity derives from the misincorporation of fluoronucleotides into the RNA and DNA of host cells and from the inhibition of the nucleotide synthetic enzyme thymidylate synthase (TS) [19].

5-FU is rapidly metabolized inside the cells into 3 main active metabolites: fluorodeoxyuridine monophosphate (FdUMP); fluorodeoxyuridine triphosphate(FdUTP); and fluorouridine triphosphate (FUTP). The rate-limiting step in 5-FU catabolism is the enzyme dihydropyrimidine dehydrogenase (DPD) that converts 5-FU to dihydrofluorouracil (DHFU). About 80% of this process occurs in the liver due to the high levels of DPD expressed there. The TS enzyme is essential in producing thymidylate for DNA replication and repair. It involves the reductive methylation of deoxythymidine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) with a reduced folate, CH2THF, as the methyl donor. FdUMP, as a metabolite of 5-FU, in conjunction with CH2THF, forms a suicide blockade of the nucleotide binding site on TS, preventing dUMP from binding and being converted to dTMP. The resulting reduction in the amount of thymidylate available causes cell death by reducing the amount of thymine available and causing cell death secondary to lack of thymine. The function of the enzyme thymidine kinase (TK) to produce thymidylate has been suggested as a potential source of resistance to 5-FU [19].

The reduced folate, leucovorin, is often given in combination with 5-FU to help increase its potency. The remaining two active metabolites, FUTP and FdUTP have been implicated in cell death by causing direct damage to DNA and RNA by misincorporation into them causing disruption of the strands [20,21].

The DNA repair enzyme uracil-DNA-glycosylase (UDG) is ineffective due to the levels of metabolites and this leads to further DNA strand

damage and ultimately cell death [22].

Hepatotoxicity and 5-fluorouracil

Although the exact mechanisms of chemotoxicity are still poorly understood, there are some widely accepted hypotheses. Breaking 5-FU down to DHFU, the rate-limiting enzyme DPD also produces catabolites such as fluoro-beta-alanine (FABL) that are metabolized in hepatocytes. It has been shown that FABL remains in hepatocytes long after cessation of therapy, suggesting that the pathways involved are easily saturated [23].

The resulting reduced capacity to metabolize drugs and fat is thought to lead to accumulation of intracellular lipids. 5-FU is also associated with collapse of the mitochondrial membrane leading to impaired oxidation of fatty acids and increased production of reactive oxygen species (ROS) mediated by cytochrome p450 enzymes. The resulting damage from the ROS and impaired beta-oxidation leads to lipid accumulation and steatosis [24] .

It has been shown that body mass index has a significant correlation with the development of steatosis when receiving chemotherapy. Makowiec et al. [25] reported that patients with a BMI>25 had a >20% increased risk (p=0.02), while those with a BMI>30 were at increased risk of severe steatosis (p=0.03). Other patient factors such as age, gender or diabetes did not affect the risk of developing steatosis.

The oral pro-drug of 5-FU, capecitabine, is often used as an alternative to 5-FU, avoiding the need of infusion and removing the need for an indwelling venous catheter. It has been reported that capecitabine can cause a similar hepatic steatosis to 5-FU [21] and a large phase III trial comparing the two drugs showed similar typical toxicity profiles although a significant increase in incidence of grade 3/4 episodes of hyperbilirubinaemia was found (p<0.0001) [26].

Irinotecan: Mechanism of action

Irinotecan is an analogue of camptothecin, a naturally occurring cytotoxic extracted from camptotheca acuminate and was developed as an anticancer drug in the early 1970s [27]. It is a topoisomerase 1 inhibitor that binds to the DNA/topoisomerase 1 complex during DNA replication, preventing resealing of the single strand during DNA coiling and uncoiling. This results in double-strand DNA breaks, leading to apoptotic cell death [28].

Irinotecan is a pro-drug that is converted to the active metabolite SN-38 via the human carboxylesterases CES1 and CES2. The cytochrome p450 enzyme CYP3A4 also converts irinotecan to its inactive metabolite APC (7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin). The active SN-38 is inactivated to SN-38G by glucuronidation via the enzyme UDP-glucuronosyltransferase. The most active form is the UGT1A1 enzyme and interindividual variation in expression of this enzyme, due a relatively common polymorphism, has been linked to differing responses to, and toxicity from, the drug [29]. Its major toxicity and side effects are diarrhoea and neutropenia although hepatotoxicity is increasingly recognised.

Hepatotoxicity and irinotecan

Vauthey et al. demonstrated a clear association between the administration of systemic irinotecan and steatohepatitis. The presence of steatohepatitis translated into a significant increase in 90-day postoperative mortality for liver resection [30].

The precise mechanism of irinotecan hepatotoxicity is unclear although it is thought to involve a 2-hit process. The first "hit" is accumulation of fat within the hepatocytes with oxidative stress caused by chemotherapy and, the second "hit" results in the development of hepatotoxicity. It is thought that mitochondrial dysfunction is at the core of the process [31].

Mitochondrial function and that of the mitochondrial respiratory chain is reliant on the expression of several polypeptides encoded by the mitochondrial DNA (mtDNA) that is located within the mitochondrial matrix. This undergoes continuous replication and constant levels are required for it to function. If the level of mtDNA drops by 20–40% below basal levels, global mitochondrial dysfunction can develop [24] .

The dysfunction causes increased production of ROS through the damaged respiratory chain, increased lipid peroxidation and impairment of beta-oxidation. This can trigger release of pro-apoptotic (TNF-alpha) and pro-fibrotic (TGF-beta) cytokines by Kupffer cells leading to cell death, inflammation and fibrosis [32]. All these are the focus of ongoing research for novel therapies to prevent chemotherapy-induced liver injury. It has also been suggested that impairment of mitochondrial topoisomerases and subsequent inhibition of mtDNA replication could be a potential mechanism of irinotecan-induced hepatotoxicity. Preclinical work has shown that mtDNA contains

Type 1 topoisomerase in a form similar to nuclear DNA [33]. It has also been shown that this mtDNA is sensitive to camptothecin, from which irinotecan was developed [34,35]. Combined with the knowledge that depleted levels of mtDNA can lead to mitochondrial dysfunction and steatohepatitis, this could explain the mechanism involved in irinotecan-induced steatohepatitis (CASH) [24].

Oxaliplatin: Mechanism of action

The cytotoxic activity of oxaliplatin is from direct DNA damage. It undergoes biotransformation *in vivo* into a number of metabolites that all contain a diaminocyclohexane (DACH) ring [36]. DACH-Pt DNA adducts are formed by cross-linking of the DNA strands. There seems to be a preference for nuclear DNA over mtDNA [37]. These primary lesions block DNA replication and transcription causing cell damage and leading to cell death and apoptosis.

Hepatotoxicity and oxaliplatin

Several studies have shown that systemic chemotherapy containing oxaliplatin can cause sinusoidal dilatation in up to 78% of the patients [17]. It is graded depending on the severity of sinusoidal obstruction from grade 1 (mild) to grade 3 (severe).

Ultrastructural abnormalities in the liver after exposure to oxaliplatin have shown that there is an increased rate of endothelial cell apoptosis leading to leaky vessel walls. This leads to extravasation of erythrocytes into the Disse's space and deposition of extracellular matrix components, including collagen fibrosis leading to peri-sinusoidal fibrosis. The dilatation of Disse's space and blebs from the endothelial cells bulging into the sinusoidal lumen lead to the obstructive syndrome [38].

The underlying mechanisms of oxaliplatin-induced sinusoidal obstruction syndrome continue to be poorly understood. It is thought that increased generation of ROS and glutathione depletion from sinusoidal endothelial cells causes increased apoptosis in these cells, allowing the damage to occur. Upregulation and increased activity of matrix metallopeptidase-9 (MMP-9) have also been implicated in the process [8].

Anti-EGFR therapy - Cetuximab/Panitumumab

Mechanisms of action

Cetuximab is a chimeric (mouse/human) monoclonal antibody which inhibits the Epidermal Growth Factor Receptor (EGFR). Panitumumab is a fully human monoclonal antibody that also acts against EGFR. EGFR is found on most colorectal cells and is involved in signalling pathways that are deregulated in cancer cells. Inhibition of the receptor inhibits growth, causes complement activation and mediates antibody-dependent cellular cytotoxicity [39].

Its effect is dependent on the status of the KRAS (Kirsten rat sarcoma viral oncogene) protein status of the tumor. Patients with wild type KRAS tumors have been shown to benefit from the administration of the targeted antibodies cetuximab and panitumumab.

The large CRYSTAL trial randomized patients to FOLFIRI ± cetuximab [40]. Retrospective analysis of the KRAS status was performed on 1063 patients [41] and found response rates of 57.3 vs 39.7% for FOLFIRI and cetuximab compared to FOLFIRI alone in wild type KRAS patients. By contrast, cetuximab offered no survival advantage to the KRAS mutant group [40] .

For this reason, cetuximab has until now been reserved for use only in patients who are KRAS wild-type. However, this is now being challenged with growing evidence that a subgroup of patients with KRAS G13-D mutant tumors may respond to cetuximab, allowing for its potential use alongside cytotoxic chemotherapy in these patients [42].

Hepatotoxicity

To date no significant hepatotoxicity has been reported with cetuximab [43]. Studies have shown a higher rate of grade 3/4 side effects between wild type KRAS vs mutant KRAS (79.3 vs 61%) but these have mostly been due to skin reactions and diarrhoea associated with cetuximab [44].

Discussion

A metaanalysis [45] of published studies has demonstrated that the nature of the parenchymal injury that results from preoperative chemotherapy cannot be generalized as a global effect but rather is a regimen-specific phenomenon-that is, irinotecan-based regimens are associated with steatohepatitis whereas oxaliplatin-based regimens are associated with sinusoidal obstruction.

Chemotherapy-induced hepatotoxicity may have significant impact on the patients if they develop it and therefore its prevention poses a chal-

lenge for both the oncologist and the surgeon. The oncologist has the challenge of preventing the development of the injury in the first place with the use of different agents, varying the duration of therapy and using novel biomarkers to guide therapy and detect toxicity, while the surgeon has the challenge of dealing with its impact on the operation, varying strategies to counter any hepatotoxicity encountered.

The precise mechanisms of chemotherapy-induced hepatotoxicity remain poorly understood and this area is currently the focus of much research. Developing a better understanding of the mechanisms involved may lead to possible therapies being developed to help counter the effect of chemotherapy on the liver. An example of this is the human monoclonal antibody bevacizumab (avastin). This antibody inhibits angiogenesis by affecting the vascular endothelial growth factor A (VEGF-A) and has been shown to be an effective therapy in the treatment of metastatic colorectal cancer. Recognizing the role of angiogenesis in the sinusoidal obstruction syndrome, researchers have explored the impact of bevacizumab in preventing the condition with some encouraging early results [38].

Such developments could have a major impact on outcomes following surgery. Diagnosing hepatotoxicity preoperatively remains a challenge. Currently, the best practice is to monitor the patients' liver enzymes (ALT/AST) as a predictor of developing hepatotoxicity. Unfortunately, despite this, patients are still being found to have chemotherapy-damaged liver only at the time of laparotomy. In order to detect toxicity earlier, different biomarkers must be found. The ideal biomarker should be easy to perform and to expose the patient to as little risk as possible. Non-invasive tests are ideal. Overman et al. have identified that 86% of the patients treated with oxaliplatin had CT evidence of splenic enlargement on post-chemotherapy imaging [46].

This has been proposed as a potential non-invasive biomarker of SOS, but only 22% of patients developed SOS. Therefore, >60% of patients who develop increased splenic size have not developed hepatotoxicity, meaning its use as a biomarker is not yet useful on its own. The potential use of microRNA as a more sensitive marker of hepatotoxicity than current liver enzymes is also of some potential interest [47,48].

The gold standard for diagnosis of hepatotoxicity is liver biopsy. This is associated with some considerable risk and therefore is not practicable in every patient. The ideal situation would be that high risk patients are identified through a combination of novel biomarkers and this small subgroup could undergo biopsy to allow for personalized treatment in the form of alteration in the oncological or surgical management plan. Accurate biomarkers for toxicity are essential if this approach is to be successful and this field is currently the focus of much research and development.

Conclusions

Concerns regarding chemotherapy-associated hepatotoxicity may negatively impact the ability to offer potentially curative therapy or increase morbidity in some patients. Although previously the domain of the oncologist, it is becoming increasingly important that the surgeon is aware of the mechanism of action and hepatotoxicity of these agents in order to predict and anticipate potential problems when the patient comes to surgery. This should be borne in mind when planning multimodal treatment for patients with colorectal liver metastases. The choice of therapy should be individualized based on resectability status, extent of hepatic resection required, and associated comorbid conditions. In short, management of these increasingly complex patients requires a multidisciplinary approach and good communication among the management team.

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