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=== REVIEW ===

Overview on nanoparticulate formulations for 5-fluorouracil delivery in colorectal cancer treatment

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SUMMARY. 5-Fluorouracil (5-FU) is an antimetabolite drug used to treat various cancer types, especially colon cancer. Its mechanism of action includes the inhibition of the biosynthetic processes in proliferating cells by inhibiting the normal function of DNA and RNA. 5-FU has a short half-life since 80% of the administered drug is catabolized in the liver. Moreover, the therapies based on the 5-FU administration are accompanied by severe side effects such as immunosuppression, cardiotoxicity, and neurotoxicity. Therefore, the use of the nanoparticle-encapsulated 5-FU can target efficiently colorectal cancer, could reduce the major drawbacks of conventional administration of 5-FU and also increase the drug lifetime and its tumour accumulation, and finally 5-FU therapeutic index will be enhanced. To this end, this article aims to review the recent research regarding nanoparticles encapsulating 5-FU for colorectal cancer targeted therapy.

Keywords: 5-fluorouracil, colorectal cancer, nanoparticles

Introduction

Colorectal cancer (CRC) is the third most common cause of cancer, with over 1.2 million new cases being diagnosed every year and the second cause of death by cancer worldwide (Jemal *et al.*, 2011). Moreover, CRC has a high incidence in developed countries and affects both genders equally (Jemal *et al.*, 2011). The main therapies applied in the colon cancer include surgery, biological therapy (hormone

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therapy, immunotherapy), radiation therapy, and chemotherapy. Depending on the cancer stage, various combinations of these types of treatments are used to achieve a better response (Krishnaiah *et al.*, 2002). Nevertheless, systemic chemotherapy is the only available treatment in colorectal cancer with advanced, nonresectable tumours (Arias, 2008). Among most active cytostatic drugs used in the management of this malignancy is 5-fluorouracil (5-FU). This cytotoxic agent was first synthetized over 40 years ago (Wigmore *et al.*, 2010) and is a widely used antimetabolite drug for cancer treatment (Longley *et al.*, 2003) including colorectal cancer but also respiratory, breast, head, and neck cancers (Cheng *et al.*, 2012b). The low efficiency of the clinical applicability of 5-FU is caused by its high toxicity associated with the lack of tumour tissue specificity, low bioavailability, and the acquisition of drug resistance in the CRC cells. Therefore, 5-FU targeting to colorectal carcinoma offers the possibility to increase its antitumour efficacy and to reduce strongly many serious side effects associated with tumour therapy. Therefore, the main goal of this review is to offer a brief overview on the 5-FU mechanisms of action and principal causes of its low antitumour efficiency as well as on the current status of the nanoparticulate formulations encapsulating 5-FU, especially for the cytotoxic drug delivery to colorectal tumours.

Limitations of conventional anticancer therapies based on 5-FU administration

Being a fluorinated analogue of uracil, 5-FU can rapidly enter the cells and further be converted intracellularly to its three active metabolites: fluorouridine triphosphate (FUTP), fluorodeoxyuridine monophosphate (FdUMP) or fluorodeoxyuridine triphosphate (FdUTP). These compounds can either disrupt RNA and DNA synthesis by incorporating themselves within these macromolecules or block the action of thymidylate synthase (TS) (Longley *et al.*, 2003). FUTP and FdUTP cause apoptosis as a result of cell cycle arrest (Anitha *et al.*, 2014). Moreover, FUTP is extensively incorporated into RNA, causing high toxicity on these molecules at different levels. FUTP can inhibit pre-mRNA processing and splicing, disrupt post-transcriptional modifications of tRNAs. In conclusion, 5-FU disrupts normal function and processing of RNA, leading to major effects on cellular viability.In addition, FdUMP-induced inhibition of TS decreases deoxythymidyne triphosphate (dTTP) synthesis. As a result uracil-DNA-glycosylase, the nucleotide excision repair enzyme, will preferentially incorporate FdUTP in DNA leading to irreversible DNA damage (Longley *et al.*, 2003).

Besides the multiple possibilities of action shown above 5-FU is rapidly metabolized in the liver. Dihydropyrimidine dehydrogenase is abundantly expressed in the liver and is the rate limiting enzyme that is responsible for the rapid metabolism of 5-FU, with a half-life of about 5-10 minutes (Zhang *et al.*, 2008). Due to the activity of this enzyme, more than 80% of the administered dose of 5-FU is metabolized (Longley *et al.*, 2003). Therefore several strategies were developed to increase 5-FU anticancer efficiency. Thus, it has been noted that long-term administration of 5-FU via

continuous infusion exerts better response than its bolus injection (van Kuilenburg *et al.*, 2000). Moreover, 5-FU antitumour activity can be improved if the cytotoxic drug is administered in combination with folinic acid (leucovorin) (Dhawale *et al.*, 2010). Consequently, the modulation strategies developed over the last 20 years have increased 5-FU efficacy with up to 40-50% (Longley *et al.*, 2003). However, the limited anticancer efficiency and the low bioavailability of this chemotherapeutic agent require the usage of high dosages that is accompanied by severe adverse effects such as hematopoietic bone marrow suppression, small bowel ulceration, vascular toxicity, cardiotoxicity, and neurotoxicity (Fata *et al.*, 1999; Arias, 2008; Wigmore *et al.*, 2010; Cheng *et al.*, 2012b). Another major disadvantage of the conventional therapy with 5-FU is the development of cancer cell resistance to the treatment (Ortiz *et al.*, 2012). Therefore, tumour-targeted therapies based on 5-FU delivery systems would be capable to overcome the major drawbacks of the conventional administration of this drug (Nair *et al.*, 2011).

Nanoparticles for 5-FU delivery to tumors

Nanoparticles are nanosized drug delivery systems that exhibit the so-called "nanosize effect" (Yassin *et al.,* 2010). Nanosized particles possess different properties than their bulk counterparts, which are caused by their reduction to the nanoscale. This in turn makes the matter to follow quantum mechanics as opposed to Newtonian physics (Leopold, 2009). Due to this effect they have the capacity to make complexes with variety of drugs including 5-FU (Yassin *et al.*, 2010b). Moreover "sterically stabilzed" nanoparticles can extravasate through the leaky pathological vasculature and thereby accumulate in malignant tissue. This effect is referred to as the "enhanced permeability and retention (EPR) effect" and is enabled by the leaky vasculature and poor lymphatic drainage of tumours, which allows nanoparticles to efficiently accumulate in the tumours as a result of their small size (Banciu, 2007; Nair *et al.*, 2011). While in most normal tissues the aperture of vascular endothelial cells is 2 nm, the non-continuous blood vessels in tumours have a much greater aperture, ranging between 100 nm and 780 nm (Cheng *et al.*, 2012b). Therefore, modulation of nanoparticle size allows the drug delivery systems to agglomerate 5-FU passively in the desired area (Nair *et al.*, 2011; Cheng *et al.*, 2012b). Noteworthy, since previous data demonstrated that continuous infusion of the drug induces cell death more effectively than a single injection of 5-FU, nanoparticle-based sustained release of 5-FU would furthermore improve its therapeutic efficacy (Nair *et al.*, 2011). Therefore, several studies described efficient encapsulation of 5-FU in a huge variety of nanoparticles such as those based on polymers (alginate, Poly(ε-caprolactone), chitosan, eudragit, guar gum, Poly(alkylcyanoacrylates), Poly(glutaraldehyde), methacrylic acid, Poly(α -malic acid), Poly(methilidenemalonate 2.1.2), Polyacrylamide, Poly(ortho-ester)s, Poly(D,L-lactide) (PLA) and poly(D,L-lactide-co-glycolide), hydrogels), lipids (liposomes,

niosomes), lipoproteins, inorganic biomaterials (magnetic drug delivery systems, nanoparticles based on clay minerals and anionic clays, metals) and ion exchange resins (Arias, 2008; Zhang *et al.*, 2008; Yan *et al.*, 2010; Yassin *et al.*, 2010b; Nair *et al.*, 2011; Cheng *et al.*, 2012a; Cheng *et al.*, 2012b; Ortiz *et al.*, 2012; Clares *et al.*, 2013; Mishra *et al.*, 2014; Subudhi *et al.*, 2015).

Colorectal specific targeting with nanoformulations containing 5-FU

Nanoparticles based on different drug delivery mechanisms for CRC-specific drug targeting have been developed (Subudhi *et al.*, 2015). Although, most of them had pH-dependent mechanisms, several systems delivered cytotoxic drugs via time-dependent or microflora activated mechanisms (Subudhi *et al.*, 2015).

The pH-sensitive drug delivery systems provide a high controlled release of the cytostatic agent in the colon by taking advantage of the pH variation along the gastrointestinal tract (Dhawale *et al.*, 2010; Subudhi *et al.*, 2015). However, these drug delivery systems did not ensure a specificity for colon region as the pH of the colon is very similar to the pH of the small intestine (Subudhi *et al.*, 2015). Nevertheless, some pH-dependent colon targeted nanoencapsulated formulations containing 5-FU are described in the literature (Yassin *et al.*, 2010a; Mishra *et al.*, 2014; Subudhi *et al.*, 2015). One of them is based on stimuli-responsive hydrogels, such as poly[2-(methacryloyloxyethyl)trimethylammonium chloride-co-methacrylic acid] (Mishra *et al.*, 2014) hydrogels (PMAAc). These hydrogel-based nanoparticles that encaspulated 5-FU were shown to induce apoptosis of HCT116 cells (Mishra *et al.*, 2014). An advantage of PMAAc nanoparticles is given by pKa value (5.6-7) that was close to the pH of the tumour extracellular environment, which led to a maximum swelling ratio at pH 7.4 with a release efficacy of 5-FU by 93.2% (Mishra *et al.*, 2014).

Another example for pH-sensitive drug systems are solid lipid nanoparticles that present important advantages for 5-FU administration such as: a higher incorporation of hydrophilic drug, improved physical stability, good biocompatibility and low toxicity (Kumar, 2000). Thus, Yassin *et al.* obtained 5-FU incorporated in spherical solid lipid nanoparticles. These nanoparticles presented a biphasic drug release and an accumulation in the tumours due to EPR effect (Yassin *et al.*, 2010b). Subudhi *et al*. described a pH-dependent system for colon cancer treatment using curcumin and 5-FU co-encapsulated in thiolated chitosan nanoparticles. These spherical nanoparticles exhibited high release in acidic pH of the tumour microenvironment (Subudhi *et al.*, 2015). Moreover, antitumour efficacy studies demonstrated a notable cytotoxicity on HT-29 human colon carcinoma cells *in vitro* as well as an improved drug bioavailability and tumour growth inhibition *in vivo* (Anitha *et al.*, 2014; Subudhi *et al.*, 2015). These important antitumour effects of this formulation were related to cyclooxygenase-2 inhibition as a result of the synergic effect of curcumin and 5-FU (Subudhi et al., 2015).

Chitosan-based nanoparticles have also been studied for colon targeting, as these systems are capable of protecting the drugs through the upper gastrointestinal tract, and can release the encapsulated agents in the colon via degradation by colonic microflora (Park *et al.*, 2010). Thus, Li *et al.* developed chitosan nanoparticles for the administration of 5-FU in combination with leucovorin (Li *et al.*, 2011). A biphasic and simultaneous release phase of these drugs from the chitosan nanoparticles has been observed. Moreover, efficient drug encapsulation and increased loading capacity for both drugs were reported (Li *et al.*, 2011). To actively target colon cancer cells, Anekant Jain and Sanjay Jain developed 5-FU-loaded chitosan nanoparticles coupled with hyaluronic acid, as receptors of this ligand are overexpressed in HT-29 colon cancer cells (Jain and Jain, 2008). These nanoparticles showed a cell internalization 7.9 fold higher than in the case of nanoparticles without ligand after 4h incubation (Jain and Jain, 2008).

It is known that microflora-activated systems take the advantage of the enzymes produced by colonic bacteria that enhance drug release from the biodegradable polymeric nanoparticles (Yassin *et al.*, 2010b). An example is the Citrus pectin nanoparticles for active targeting of colorectal cancer cells since Citrus pectin is a ligand for galectin-3 receptors overexpressed in these cancer cells (Subudhi *et al.*, 2015). Moreover, Citrus pectin has also been shown to have anti-proliferative activity on the same cell line (Subudhi *et al.*, 2015). These nanoparticles were also coated with Eudragit S100 that provided an intestinal fluid pH-sensitive release system. Apart from this, drug release can also be facilitated through degradation of pectin which is mediated by the intestinal microflora (Subudhi *et al.*, 2015).

Another promising anticancer therapy that combined chemotherapy with hyperthermia was described by Clares *et al.* who used FU-loaded magnetoliposomes (Clares *et al.*, 2013). Multilamellar liposomes were used to incorporate supermagnetic magnetite nuclei and 5-FU (Clares *et al.*, 2013). The sustained release pattern of the chemotherapeutic drug from these liposomes was improved and accelerated by electromagnetic field (Clares *et al.*, 2013).

To reduce side effects of 5-FU, a formulation of chitosan poly(ε-caprolactone) nanoparticles encapsulated the hydrophobic prodrug of 5-FU, doxifluridine (Wang and Peng, 2011). This system presented a slow release profile of doxifluridine, which was only intracellularly converted to 5-FU by thymidine phosphorylase, leading to increased anticancer effects in HT-29 colon cancer cells (Wang and Peng, 2011).

Despite the multitude of the nanoparticulate formulations developed for 5-FU delivery to CRC, serious adverse effects could not be overcome. For instance, these nanoparticles could accumulate easily in different organs such as liver, kidneys and spleen (Yan *et al.*, 2010). Moreover, small quantities of nanoparticles have been detected even in the heart, brain and lung (Tsai *et al.*, 2011; Anitha *et al.*, 2014). Therefore, future studies for the optimization of the tumour targeted therapies based on nanoparticles incorporating 5-FU are needed.

Conclusions

CRC-targeted 5-FU delivery discussed within this review by using nanoparticulate delivery systems lead to achievement of intratumour high concentrations of the cytotoxic drug and a local drug release in a controlled manner, which could not be reached by conventional administration strategies of 5-FU. Nevertheless, future research is needed to improve specificity of these nanoparticulate carriers for the tumour targets.

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