A Brief Look at Antitumor Effects of Doxycycline in the Treatment of Colorectal Cancer and Combination Therapies

Authors Name : Miss. More Subodhini Budha 1st , Mr. Jaweed Khan 2nd
Guide Name : Mr. Dr. Tarbej Mujawar 3rd

Abstract

One of the worst diseases to affect humans is colorectal cancer (CRC), which is regarded as the second most common cancer worldwide. On the other hand, a number of therapeutic modalities, including as surgery, radiotherapy, chemotherapy, and immunotherapy, are being developed throughout time in response to the difficulties associated with cancer treatment. Research demonstrated that, as compared to monotherapy, combination treatments have produced somewhat positive clinical results in terms of slowing the growth of the tumour and improving patient survival. Doxycycline, an antibiotic, is one of the substances and medications that can be used in chemotherapy and has been shown to be effective in treating a number of cancers, including colorectal cancer. Doxycycline has been shown to have anti-tumor qualities and to be able to suppress anti-apoptotic and angiogenic proteins, among other methods, to assist regulate the growth of tumours. Furthermore, research has demonstrated the ability to impede the growth of tumours when doxycycline is used in combination with other anti-tumor medications such doxorubicin, anti-angiogenic agents, and anti-checkpoint blockers. Thus, the anti-tumor mechanisms of doxycycline in the treatment of colorectal cancer and associated combination therapies were summed up in this review.

Introduction

One of the most frequent and deadliest gastrointestinal tract malignancies, colorectal cancer (CRC) kills around two million people annually worldwide, with those over 50 having a greater risk of developing this disease (Xie et al., 2020). Research revealed that a number of factors, such as dietary antigens, microbiota pattern, genetic mutations in genes involved in anti-tumor responses and DNA repair, and chronic inflammatory gastrointestinal tract diseases like inflammatory bowel disease (IBD), significantly raise the risk of this kind of cancer (Fearon and Vogelstein, 1990; Lopez et al., 2018). Thus, dysregulated inflammatory responses in the tumour microenvironment (TME) and chronic inflammation may be important tumour progression promoters in the pathophysiology of colorectal cancer (CRC) (Park et al., 2018). Surgery and chemotherapy are regarded as the primary therapeutic strategies for patients with

colorectal cancer (CRC) and have been shown to improve patient survival, according to prior research and experience. 5-fluorouracil has been used for decades in chemotherapy for colorectal cancer patients, and studies have shown that this anti-tumor medication can prolong the patients' lives by around a year. Due to their increased efficacy and frequent use in clinical settings, combination therapies utilising several anti-tumor drugs have been studied by researchers for a number of decades (M McQuade et al., 2017). However, natural substances with lower toxicity may be a safer option in cancer therapy due to the different adverse effects of the medications used in chemotherapy. Natural substances having anti-tumor qualities include antibiotics like doxorubicin, actinomycin, mitoxantrone, mitomycin, doxycycline, and salinomycin. Recently, ivermectin and monensin have been utilised to treat a range of infectious disorders and cancers (Markowska et al., 2019). Research has demonstrated that the combination of doxycycline and other anti-tumor medications can produce synergistic anti-tumor effects by blocking anti-apoptotic proteins like Bcl-2-associated X protein (BAX) and interleukin-8 (IL-8) as well as matrix metalloproteinases (MMP-2 and MMP-9) that are involved in angiogenesis and tumour cell migration (Son et al., 2009). Research revealed that the cyclooxygenase (COX) enzymes and MMPs are crucial for the angiogenic and metastatic processes of colorectal cancer. As a result, blocking the proliferation and invasion of CRC tumour cells may be achieved therapeutically by focusing on these enzymes. The results of an in vitro investigation using two different human CRC cell lines (LS174T and HT29) showed that NS 389, a COX inhibitor, and doxycycline, an MPPs inhibitor, together could prevent tumour cell proliferation and invasion. According to recent research, doxycycline and copper nanoparticles together had a synergistic antitumor and significant cytotoxic effect on HeLa and HepG2 cell lines (Yaqub et al., 2020). Doxycycline and indomethacin have the ability to block the expression of β-catenin and transcription activity in tumour cells, which can lead to the reduction of cancer cell growth both in vitro and in vivo in cases of colon cancer (Veeramachaneni et al., 2003). On the other hand, a different study found that although doxycycline induces tumour cell death in colorectal cancer (CRC), it does not have a synergistic effect on tumour inhibition when combined with platinum drugs such oxaliplatin and cisplatin (Sagar et al., 2009). Therefore, this review aimed to summarise the anti-tumor properties of doxycycline and its effects in the treatment of colorectal cancer (CRC), as well as the significance and efficacy of combination therapies using doxycycline and other anti-tumor compounds and drugs. This was done in light of the importance of cancer chemotherapy with minimal toxicity and high efficacy.

Doxycycline

Doxycycline belongs to the tetracycline class of antibiotics and is a broad-spectrum bacteriostatic agent (antibiotic) that was synthetically derived from oxytetracycline, a naturally occurring tetracycline produced by Streptomyces species bacteria (Kundu et al., 2015; Thillainayagam and Ramaiah, 2016). Doxycycline was produced and clinically developed by Pfizer Inc. in the early 1960s, when it was marketed under the Vibramycin® brand (Tan et al., 2011). The Food and Drug Administration (FDA) has approved doxycycline for the prevention or treatment of certain conditions under each of the following categories: anthrax, acute intestinal amebiasis, traveler's diarrhoea, rickettsial infections, bacterial infections, respiratory tract infections, Lyme disease, ophthalmic infections, and severe acne (FDA, 2018a). Doxycycline has also being researched as a possible treatment for particular malignancies since some research indicates that it can prevent cancerous cells from growing and dividing, cause apoptosis, and stop the gap phase, which is when they get ready to make DNA (Kundu et al., 2015). Although doxycycline is useful for treating many different ailments, this chapter focuses on using it to prevent malaria. Pfizer Inc. filed a new medication application to the FDA in 1992, adding an indication for malaria prophylaxis to the product insert (Arguin and Magill, 2017). Tan et al. (2011) reported that the indication was added in 1994.

Adults who wish to prevent malaria should take 100 mg daily starting 1-2 days before they reach an endemic area, 100 mg daily while they are in the endemic area, and 100 mg daily for 28 days after they leave the endemic area. This regimen is approved for a maximum of four months, according to the FDA package insert (FDA, 2018a). Research investigating the prolonged (≥4 months) usage of doxycycline for the prevention of malaria has yielded inconsistent outcomes on its acceptability. Doxycycline was well tolerated by Australian military personnel stationed in Cambodia (n = 600) for 12 months or Somalia (n = 900) for 4 months. Only 7 (0.6%) and 15 (1.7%) of the personnel in Cambodia and Somalia stopped taking the medication due to concurrent adverse events involving gastrointestinal symptoms or photosensitivity (Shanks et al., 1995a). But according to a survey conducted by Korhonen et al. (2007), of 228 U.S. Peace Corps volunteers who had taken doxycycline on average for 19 months, 45 (20%) of the participants said they had to switch medications as a result of serious side effects that included gastrointestinal distress, itchy skin reactions, photosensitivity, and vaginal yeast infections.

Overall, research indicates that doxycycline adherence rates for malaria prophylaxis range from 70% to 84%, and that following the dosage instructions may be more difficult than following the instructions for weekly prophylactic drugs (e.g., mefloquine, tafenoquine). U.S. forces in Somalia showed 98% adherence to mefloquine but only 81% adherence to doxycycline in one trial (Sánchez as al., 1993). In another study,

70% of participants followed a weekly prophylactic regimen but only 50% adhered to a daily doxycycline regimen (Watanasook et al., 1989). Moreover, research indicates that there is a gradual decline in adherence to the doxycycline schedule. Doxycycline adherence dropped from 60% after two months to 44% at four months in one trial of Australian soldiers stationed in Cambodia (Shanks et al., 1995a). Doxycycline probably has poorer adherence during the post-travel period than a prophylactic medication with a shorter post-travel regimen, like atovaquone/proguanil (A/P). Studies have shown that drugs with longer post-travel requirements tend to have worse adherence than drugs with shorter post-travel regimens (Overbosch et al., 2001).

Individuals receiving once-daily prophylaxis showed higher adherence than those prescribed once-weekly prophylaxis, according to other studies. In order to investigate adherence rates for malaria prophylaxis in a war zone, Brisson and Brisson (2012) performed an online poll among 1,200 military personnel deployed to Afghanistan between 2002 and 2012. 528 out of the 530 people who started the survey finished it, representing a 44% response rate to the first dissemination of the survey. 3.6% of respondents were prescribed mefloquine, 90.1% were supplied doxycycline, 0.9% were prescribed A/P, 0.2% were prescribed primaquine, and 4.4% were not sure which preventive medication they were prescribed. These findings were reported by the authors. Sixty-one percent of those who were administered once-daily prophylaxis reported total adherence, compared to only 38 percent of those who were provided once-weekly prophylaxis (e.g., mefloquine). Although P. falciparum resistance to doxycycline is unknown, advances in prophylaxis have been linked to low dosages, potentially low serum levels, and noncompliant patients (Tan et al., 2011).

Anti-tumor effects of doxycycline

One of the leading causes of death in the globe is cancer. It is typified by unchecked cell growth and multiplication. Ehrlich carcinoma is a type of mouse breast cancer cell line that has significant sensitivity to several anticancer drugs and bears a striking resemblance to human tumours. Ehrlich carcinoma is distinguished by a rapid tumour development rate, the potential for transplantation, and a broad range of applications in identifying the anticancer activity of recently developed compounds. In the 1960s, the FDA initially approved doxycycline (DOXY) as an antibacterial agent. DOXY has a lengthy half-life of 18 to 22 hours and 100% oral bioavailability. By attaching to active aminoacyl-tRNAs that have the A-site at the 30S subunit of bacterial ribosomes, DOXY inhibits the synthesis of proteins by bacteria. The mitochondrial ribosome's 28S subunit in mammals is similar to the bacterial ribosome's 30S subunit. Because bacterial and mitochondrial ribosomes are similar, one of DOXY's adverse effects is the inhibition of mitochondrial biogenesis. This side effect has been repurposed as a therapeutic approach in oncology, where the goal is

to prevent cancer cells from growing mitochondria. The rare side effects of DOXY during clinical use include neutropenia, anaemia, and thrombocytopenia.

Nowadays, nanotechnology is widely used in numerous medical treatments. By increasing the solubility, bioavailability, and retention duration of a wide range of hydrophilic and hydrophobic drugs, polymeric nanoparticles (PNPs) are frequently utilised to improve their therapeutic efficacy. PNPs offer a number of benefits over conventional medications, including reduced toxicity, improved targeting to the targeted tissue, higher efficacy, defence against biological fluids degrading the medication, controlled release, and improved absorption and bioavailability. PNPs can be made from a variety of medications to treat a range of illnesses, including cancer, diabetes, fungal infections, and cardiovascular conditions. Prior research has emphasised the significance of DOXY preparation in nanoformulations. For instance, DOXY-PNPs were made to lessen their toxicity or to increase their antibacterial efficacy against specific types of bacteria. However, DOXY-PNPs were not previously designed. In the meanwhile, various forms of DOXY nanoparticles were prepared as an antitumor agent to improve medication delivery and efficacy, increase its penetration into cancer cells, or boost its anticancer activity.

Doxycycline in the treatment of CRC

For more than 50 years, doxycycline has been used as an antibiotic in clinical settings to treat a variety of bacterial, mycoplasma, chlamydiae, rickettsiae, and protozoan illnesses. Their primary mode of action is to suppress the synthesis of proteins by limiting the binding of aminoacyl t-RNA to 30S ribosomes. In addition to its antibacterial properties, doxycycline is thought to obstruct the formation of mitochondrial proteins, which has led to the identification of additional consequences. The capacity of doxycycline to inhibit matrix metalloproteinase (MMPs) in a variety of malignancies, including prostate, melanoma, osteosarcoma, breast, leukaemia, and colorectal cancers, has recently led to a resurgence of interest in the drug's research. It has been demonstrated that some TCNs function as apoptotic inducers. Although TCNs are becoming more and more recognised as anti-invasive agents, their exact mechanisms and role in conjunction with other agents are yet unknown.

The process by which chemotherapeutic medicines cause cancer cells to die is known as apoptosis. The intrinsic and extrinsic routes are the two primary mechanisms/pathways of apoptosis. The proteolytic enzymes known as caspases, or cysteine proteases, are crucial to the process of apoptosis. One of the executioner caspases engaged in both the intrinsic and extrinsic apoptotic pathways is caspase 3. The second most frequent cancer in women and the third most prevalent in men is colorectal cancer. In the UK, it is the cause of roughly 16,000 deaths annually. In addition to radiotherapy and surgery, chemotherapy is a vital component of colorectal cancer treatment. Two of the most often utilised chemotherapeutic drugs in the chemotherapy of different cancers are oxaliplatin and cisplatin. However, side effects including as

metastasis and illness recurrence are typical when using regimens containing platinum compounds. Doxycycline's lengthy half-life and ease of oral administration are among its benefits. It has been demonstrated to cause apoptosis in colorectal cancer cell lines and to impede cell invasion and proliferation. In this study, using the HT 29 colorectal cancer cell line, we examined whether or not doxycycline functions in synergy with cisplatin and oxaliplatin.

Combination cancer therapy with doxycycline

It is believed that cancer stem cells, or CSCs, are the "root cause" of therapy-resistant patients, distant metastases, and tumour recurrence in advanced cancer patients. Consequently, in order to locate and eliminate CSCs, new therapeutic approaches are required. This aim so continues to be an unmet medical necessity. Based on high-resolution proteomics analysis, we have recently determined that increased mitochondrial mass represents a novel common and defining trait of CSCs. Significantly, enhanced mitochondrial mass can be used as a proxy for higher levels of mitochondrial protein translation and/or increased mitochondrial biogenesis. As a result, this straightforward metabolic finding offers a novel method for i) identifying CSCs and ii) eliminating them. More specifically, we demonstrated that CSC activity from a diverse population of live cells could be efficiently enriched and purified using a mitochondrial fluorescent marker (MitoTracker). In this case, the cancer cells with the largest mitochondrial mass also possessed the strongest functional capacity for anchorage-independent growth, which is typically linked to the possibility of metastasis. Pre-clinical experiments demonstrated that the 'Mito-high' cell sub-population exhibited the strongest tumor-initiating capacity in vivo. The ability to undergo cell proliferation, which was sensitive to CDK4/6 inhibitors like palbociclib, was strictly correlated with high mitochondrial mass. Complementary results were obtained with other fluorescent mitochondrial probes for ROS and hydrogen peroxide, as well as NADH auto-fluorescence, an established marker of mitochondrial "power"/high OXPHOS activity.

Furthermore, we showed that the propagation of CSCs might be stopped by using a number of kinds of non-toxic antibiotics. A side effect of some antibiotic classes is the inhibition of mitochondrial protein translation due to the conserved evolutionary parallels between aerobic bacteria and mitochondria. Tetracyclines are one such class of antibiotics, with Doxycycline serving as the archetypal member of the family. This investigation revealed that tetracycline antibiotics, like Doxycycline, might be repurposed to kill CSCs in a variety of cancer types. These eight different cancer types comprised melanoma and glioblastoma, as well as DCIS, breast (ER(+) and ER(−)), ovarian, prostate, lung, and pancreatic

carcinomas. The growth of primary cultures of CSCs from patients with advanced metastatic breast cancer (isolated from ascites fluid and/or pleural effusions) was likewise effectively stopped by doxycycline. Doxycycline, astonishingly, acts as a potent radio-sensitizer, effectively breaking through radio-resistance in breast cancer stem cells. Given that most patients with ER(+) breast cancer are now treated with radiation therapy, hormonal therapy containing an anti-estrogen, and breast-conserving surgery (lumpectomy), this has significant therapeutic ramifications. Approximately fifty years ago, in 1967, Doxycycline was initially made available as a medication with FDA approval. With a half-life of 18 to 24 hours and an absorption of over 100%, it possesses exceptional pharmacokinetic characteristics. But as is typically the case with novel therapeutic approaches, there is always a risk of drug resistance developing. This demonstrates that cancer cells can, in fact, resist the effects of doxycycline by switching back to a phenotype that is exclusively glycolytic. Thankfully, this escape mechanism's metabolic inflexibility makes it possible to target Doxycycline-resistant (DoxyR) CSCs more successfully with a variety of different metabolic inhibitors, such as vitamin C, which stops aerobic glycolysis functionally.

Conclusion :

It is now clear from a number of functional investigations that mitochondria in cancer cells represent a significant new therapeutic target. The FDA-approved antibiotic doxycycline functions as an inhibitor of mitochondrial protein translation, suggesting that it could be useful in treating cancer cells by specifically targeting their mitochondria. In this article, however, we have discovered a unique metabolic pathway that allows CSCs to successfully evade Doxycycline's anti-mitochondrial effects by adopting a phenotype that is exclusively glycolytic. Because of their metabolic rigidity, DoxyR CSCs are therefore more vulnerable to other metabolic disruptions, making it possible to eradicate them with natural remedies and other FDA-approved medications. Therefore, by comprehending the metabolic underpinnings of doxycycline resistance, we have been able to create a new synthetic lethal method that targets CSCs more precisely.

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