Bioorganic & Medicinal Chemistry 22 (2014) 1104–1114

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Design, synthesis and ex-vivo release studies of colon-specific polyphosphazene–anticancer drug conjugates

Rajiv Sharma [a,b,*], Ravindra K. Rawal [a,c], Manav Malhotra [a], A. K. Sharma [d], T. R. Bhardwaj [a]

[a] Polymer Chemistry and Technology Research Laboratory, Department of Pharmaceutical Chemistry, Indo-Soviet Friendship (I.S.F), College of Pharmacy, Ferozepur Road, Moga 142 001, India
[b] Research Scholar, Uttarakhand Technical University, Dehradun 248 007, India
[c] The University of Georgia, College of Pharmacy, GA 30602, USA
[d] School of Pharmacy, Asmara College of Health Sciences, Asmara, Eritrea

ARTICLE INFO

Article history:
Received 9 August 2013
Revised 14 December 2013
Accepted 16 December 2013
Available online 25 December 2013

Keywords:
Azoreductase
Polyphosphazene
Colon-specific
Methotrexate
Gemcitabine

ABSTRACT

Colon-specific azo based polyphosphazene–anticancer drug conjugates (**11**–**18**) have been synthesized and evaluated by ex-vivo release studies. The prepared polyphosphazene drug conjugates (**11**–**18**) are stable in acidic (pH = 1.2) buffer which showed that these polymer drug conjugates are protected from acidic environment which is the primary requirement of colon specific targeted drug delivery. The ex-vivo release profiles of polyphosphazene drug conjugates (**11**–**18**) have been performed in the presence as well as in the absence of rat cecal content. The results showed that more than 89% of parent drugs (methotrexate and gemcitabine) are released from polymeric backbone of polyphosphazene drug conjugates (**14** and **18**) having *n*-butanol (lipophilic moiety). The in-vitro cytotoxicity assay has also been performed which clearly indicated that these polymeric drug conjugates are active against human colorectal cancer cell lines (HT-29 and COLO 320 DM). The drug release kinetic study demonstrated that Higuchi's equation is found to be best fitted equation which showed that release of drug from polymeric backbone as square root of time dependent process based on non-fickian diffusion. Therefore, the synthesized polyphosphazene azo based drug conjugates of methotrexate and gemcitabine are the potential candidates for colon targeted drug delivery system with minimal undesirable side effects.

1. Introduction

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Cancer is the second leading cause of deaths worldwide. In 2013, about 580,350 deaths are expected due to cancer; almost 1600 people died per day in USA.[1] Cancers are of many types like breast cancer, prostate cancer, lung cancer, colorectal cancer and leukemia etc. Among cancers, colorectal cancer is the third most common cause of deaths in both men and women in USA. According to the American Cancer Society, about 141,210 people were diagnosed with colorectal cancer resulted to 49,380 people death.

The colon specific targeted drug delivery has been developed as one of the most successful approach.[2] Colon has become the center of attraction for the treatment of colonic diseases such as crohn's diseases, ulcerative colitis and inflammatory bowel diseases and colon cancer.[3–5] The prodrug also has the potential for the delivery of proteins and peptides which are sensitive to the enzymes both in stomach and intestine.[6] There are mainly two types of approaches to deliver a drug to colon: (i) covalent linkage of drug with a carrier; (ii) delivery of intact drug to the colon. The covalent linkage of drug with carrier includes prodrug approach, azo bond conjugates, glycoside conjugates, glucuronide conjugates, cyclodextrin conjugates, amino acid conjugates and polymeric prodrug approach. The delivery of intact form of drug includes coating with pH sensitive polymers, biodegradable polymers, redox-sensitive polymers and embedding in biodegradable matrices and hydrogels etc.[7–9] In the prodrug approach, drug is covalently bound to the carrier that improve the physicochemical properties by increasing the drug concentration at the target site, decrease toxicity and undesirable side effects.[2] To achieve the successful delivery of drugs to the target site i.e. colon drug needs to be protected from the gastrointestinal tract (GIT) environment or absorption in the upper GIT and then releases in the target site.[10] It is well known that colonic microflora has large number of anaerobic bacteria's (about 10^8–10^9 bacterial count/g gut contents in rats and 10^{10}–10^{12} bacterial count/g gut contents in humans) which is not present in the rest part of GIT such as β-glycosidase, β-glucuronidase, nitroreductase, nitrate reductase and azoreductase.[11,12] There are large numbers of enzyme based prodrugs available but none of

* Corresponding author. Tel.: +91 9988080822.
E-mail address: drrajiv81@yahoo.in (R. Sharma).

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.12.037

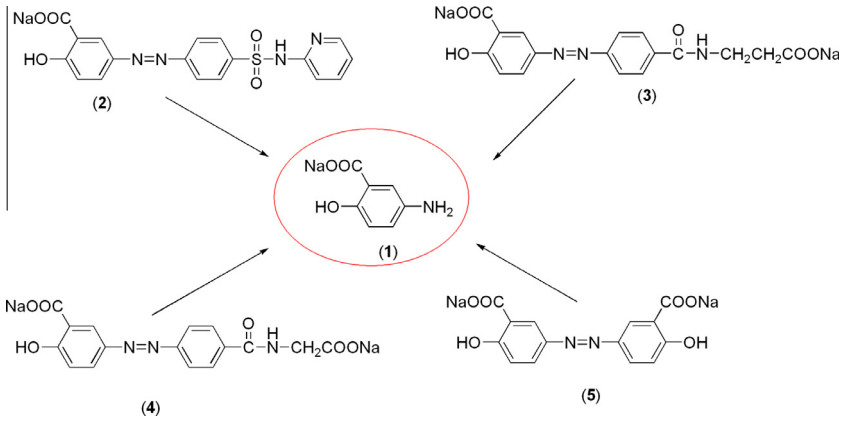

Figure 1. Structures of azo based prodrugs of 5-aminosalicylic acid (5-ASA) (**1**): sulfasalazine (**2**); balsalazide (**3**); ipsalazine (**4**); olsalazine (**5**).

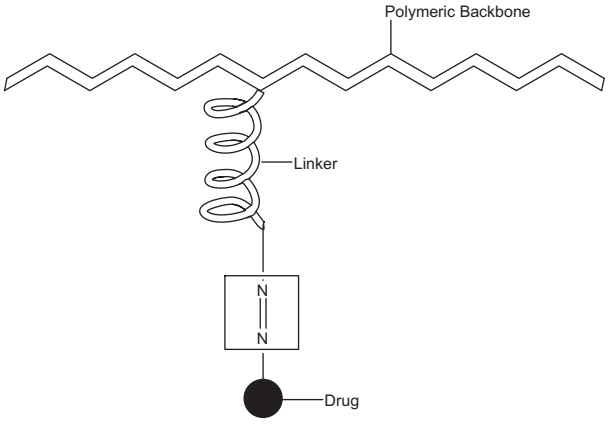

Figure 2. Technical approach for the design of polymer linked azo based prodrugs of anticancer agents.

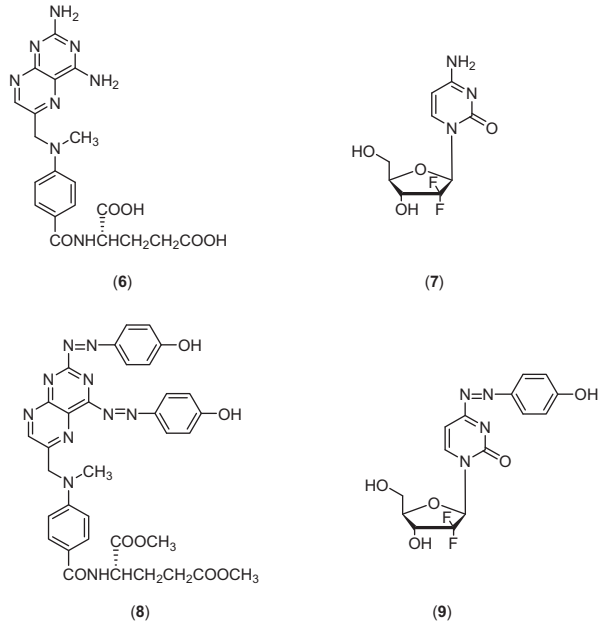

Figure 3. Chemical structures of methotrexate (**6**), gemcitabine (**7**), azo prodrugs of methotrexate (**8**) and gemcitabine (**9**).

them reached to clinically useful as azo prodrug of 5-aminosalicyclic acid (5-ASA) (**1**) such as sulphasalazine (**2**)[13] and its analogues balsalazide (**3**),[14] ipsalazine (**4**)[14] and olsalazine (**5**)[15] which were used for the treatment of inflammatory bowel disease (Fig. 1). The reason behind the success of 5-ASA derivatives is due to the presence of azo bond in these molecules and it has been found that the azo bond was reduced specifically to amine by the colonic microflora which is present in cecum and feces of human and rats.[16]

Biodegradable polymers have been widely used in the area of biomedical applications such as polylactides (PLA), polyglycolides (PGA), poly (lactide-co-glycolides) (PLGA), polyanhydrides, polyorthoesters, poly (2-hydroxy ethyl methacrylate), poly (methyl methacrylate), poly (vinyl alcohol), polyacrylamide, poly (ethylene glycol), poly (methacrylic acid),[17] chitosan, guargum, dextran, cyclodextrins[8] and polyphosphazene[18] etc. Among them, polyphosphazene (**10**) is a class of inorganic biocompatible and biodegradable polymer which has alternative nitrogen and phosphorus atom attached by single and double bond. Polyphosphazene polymer has versatile nature of polymer because of its two chlorine atoms attached on both side of phosphorus atom which can be easily replaced by nucleophilic substitution reactions. The substitution attached on both side of phosphorus in place of chlorine plays an important role in the physicochemical properties of the polymer. A plenty of research work has been done to explore the biomedical applications of polyphosphazene, for example, in vaccine delivery and immunomodulation,[19–21] tissue engineering,[22] polyphosphazene drug conjugates in anticancer chemotherapy like polyphosphazene–paclitaxel conjugate,[23] polyphosphazene–doxorubucin conjugate,[24] polyphosphazene–platinum(II) conjugates[25–29] and polyphosphazene–camptothecin conjugates[30] etc. Polymeric azo based prodrugs approach is one of the most important approach for the targeted delivery of anticancer drugs to the colon. In this approach, drug molecules are covalently bounded with polymeric backbone with an azo based drug carrier (Fig. 2). The targeted delivery of drug to the colon depends upon various factors like nature of polymeric backbone and drug carrier which protect the drug from upper GIT environment and also maintain the physicochemical properties of the polymer drug conjugate.[31]

In this present work, methotrexate (**6**) and gemcitabine (**7**) (Fig. 3) were used as model drugs. Methotrexate and gemcitabine are potent anticancer agents which have been used in the treatment of colorectal cancer alone or in combination with other drugs like 5-Flurouracil (5-FU)[32–34] but due to their absorption in the upper GIT and less oral bioavailability, these drugs cannot be considered as safer drugs for patients suffering from colorectal cancer. Therefore, colon-specific azo based prodrugs of anticancer agents like methotrexate, gemcitabine have been synthesized and