[Frontiers in Bioscience 9, 2605-2617, September 1, 2004]

BETA-LACTAMS AND THEIR POTENTIAL USE AS NOVEL ANTICANCER CHEMOTHERAPEUTICS DRUGS

Deborah Kuhn [1], Cristina Coates [2], Kenyon Daniel [1], Di Chen [1], Mohammad Bhuiyan [1], Aslamuzzaman Kazi [1], Edward Turos [2] and Q. Ping Dou [1]

[1] The Prevention Program, Barbara Ann Karmanos Cancer Institute, and Department of Pathology, School of Medicine, Wayne State University, Detroit, Michigan, USA, [2] Department of Chemistry, College of Arts and Sciences, University of South Florida, Tampa, Florida, USA

TABLE OF CONTENTS

1. ABSTRACT

The discovery of natural and synthetic antibiotics is one of the most important medical breakthroughs in human history. Many diseases, such as bacterial meningitis, pneumonia, and septicemia, are now curable with the use of antibiotics. Antibiotics are efficacious, generally well tolerated in patients, and have a low toxicity level. It is for these reasons antibiotics remain an attractive target for drug discovery. Traditional beta-lactam antibiotics (e.g. penicillins, penems, cephalosporins) have a bicyclic ring structure that is conformationally rigid and functions to inhibit bacterial cell wall synthesis. In addition to the bactericidal action of antibiotics, it has been discovered that many antibiotics are capable of inhibiting tumor cell growth. There are currently many antitumor antibiotics approved for cancer therapy, which work to inhibit tumor cell growth by DNA intercalation. The use of beta-lactams as prodrugs has also met with success by aiding delivery of the chemotherapeutic directly to tumor sites. Recently, a novel class of *N*-thiolated monobactams, so termed because they possess a monocyclic ring instead of the bicyclic ring, has been found to induce apoptosis potently and specifically in many tumor cell lines but not in normal, non-transformed cell lines. Other beta-lactams, such as the polyaromatics, have been found to slow or inhibit tumor cell growth, and the 4-alkylidene beta-lactams are capable of inhibiting matrix metalloproteinases and leukocyte elactase activity. These data indicate that synthesis and evaluation of beta-lactams are a promising area for further development in anticancer research.

2. INTRODUCTION

Cancer is a heterogeneous disease and can be characterized as the growth of a malignant cell population that eventually leads to the interference of normal physiological functions. Anticancer drug research focuses on inhibition of tumor cell growth and induction of apoptosis in the malignant cell population. Apoptosis, or programmed cellular death, first described by Kerr *et al*. in 1972, is characterized by the ability of a cell to undergo a step-by-step self suicide program without affecting neighboring or adjoining cells (1). Activation of the apoptotic program in tumorigenic cells is essential for cancer prevention and treatment. A significant focus in anticancer drug discovery is to selectively induce tumor cell apoptosis with limited toxicity to normal cells. Tumor

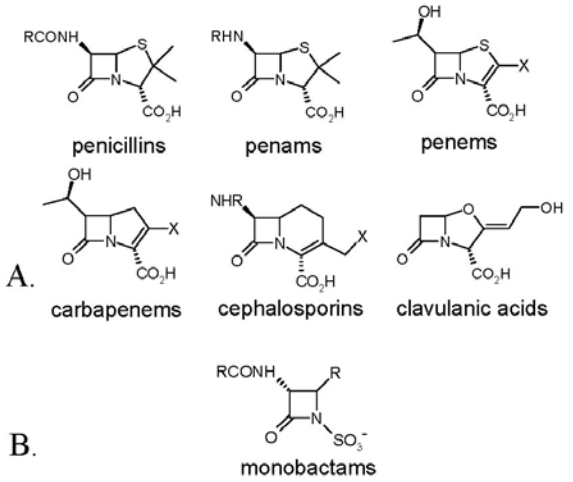

Figure 1. Structure of bicyclic (A) and monocyclic (B) beta-lactam families.

cells often have multiple alterations in their apoptotic machinery and/or signaling pathways that lead to increased levels of growth and proliferation. The absence of a tumor suppressor protein (such as p53) or the activation of an oncogenic protein (such as Bcl-2) can inhibit tumor cell apoptosis (2,3). Therefore, overriding these mutations can lead to stimulation of the apoptotic signaling pathway and cell death in tumor cells.

Currently, the beta-lactams are the most exploited family of antibiotics used for the treatment of bacterial infections (figure 1A). Beta-lactams are secreted by molds from the *Penicillum* genus and Sir Alexander Fleming first coined the name "penicillin" in 1928. Fleming observed bacteriolysis in a broth contaminated with *Penicillum* at St. Mary's Hospital in London, England (4). It would be many years until the import of this discovery was fully appreciated. Later studies at Oxford by Abraham, Florey and Chain resulted in the isolation of penicillin and subsequent drug trials (4). X-ray crystallography performed by Dorothy Hodgkin revealed that penicillin is a thiazolidine ring fused to a four membered beta-lactam ring (5; figure 1A). Later research focused on identifying several other antibiotics isolated from natural sources. Bacteria from the genus *Cephalosporium* also excrete beta-lactam containing compounds. Today the cephalosporin antibiotics, and their derivatives, comprise a large portion of the antibiotic therapies used (6,7). Several other classes of bicyclic beta-lactams were found to also possess antibacterial properties, such as penams, carbapenems and clavulanic acids (figure 1A).

The beta-lactams are powerful and potent inhibitors of bacterial growth and many different moieties of bicyclic beta-lactams have been isolated or synthesized since the discovery of penicillin (8). In 1981, two independent groups from the Squibbs and Takeda laboratories isolated the first *N*-thiolated beta-lactams from natural sources (9,10). These beta-lactams were the first to have a *N*-sulfonic acid group attached directly to the nitrogen in the lactam ring. The term "monobactam" was coined for these lactams, which have a flexible monocyclic ring, lack the carboxylic acid moiety, yet still retain a high bactericidal property (figure 1B).

Of late, a new class of *N*-thiolated beta-lactams was found to inhibit *Staphylococcal* and methicillin-resistant *S. Aureus* (MRSA) growth (11-13). The novel lactams most active against MRSA have an *N*-methylthio-substitution. These compounds are unaffected by penicillinases, such as beta-lactamase, an enzyme produced by some bacteria that degrades beta-lactams (14).

3. CURRENT ANTIBIOTIC MODALITIES IN CANCER TREATMENT

The tetracyclines are antibiotics that have been used for the treatment of infection for decades (15). This family of compounds includes: tetracycline, doxycycline, and minocycline. Although these compounds are known for their effects on mitochondria, their ability to inhibit matrix metalloproteinases, the enzymes required for angiogenesis, may prove more beneficial (15). Col-3, a modified tetracycline, is now in clinical trials. Doxycyline has been shown to reduce tumor burden in mouse models and osteolytic bone metastasis as a result from breast cancer (15). These results have enabled doxycycline to enter clinical trials.

Rapamycin (RAPA) is a microbial macrolide from *Streptomyces hygroscopicus* and is used as an immunosuppressive to prevent organ rejection (16). It can also induce anti-proliferative effects by inhibiting cyclin-dependent kinases and inhibit retinoblastoma protein phosphorylation leading to cell cycle arrest (17-19). RAPA has been shown to have growth-suppressive effects in a broad range of cancers (20). For instance, Nepomuceno *et al*., have shown that RAPA is capable of preventing the growth of Epstein Barr virus positive (EBV+) B-cell lymphomas. Severe Combined Immune Deficient (SCID) mice treated with 1.5 mg/kg/day RAPA remained tumor free for up to six weeks after injection with peripheral blood from liver transplant patients, while mice without RAPA treatment developed tumors within three weeks (21). Their results suggest that rapamycin may be able to control other EBV-related cancers and those associated with organ transplant.

The novel histone deacetylase inhibitor, FK228, has been recently isolated from *Chromobacterium violaceum* (22). This antibiotic is a bicyclic peptide with a non-cysteine disulfide bridge that has been found to reverse H-ras transformed NIH-3T3 cells (22). The activity of FK228 against tumor cells is in the ng/mL range while effects against normal cells are not seen below concentrations of 1 μg/mL (23). The action of FK228 indicates that it is more effective against large tumors with an established blood supply over small tumors that do not yet require a capillary network (23). Specifically, FK228 down-regulates mRNA levels of vasoendothelial growth factor, a principle component of the angiogenesis pathway. This evidence strongly supports FK228 as a potential candidate for cancer chemotherapy.

Lavendamycin, which possesses a quinoline-5,8-dione core, is an antibiotic derived from *Streptomyces*

lavendulae and isolated in 1981 (24). While the native compound did not pass clinical trials due to poor solubility and general toxicity, newly designed analogs show promise as potential chemotherapeutic drugs (25). The analogs of this compound possess a modified ring structure of the native compound to increase solubility and selectivity for p53-deficient cells (25). When lung carcinoma A549 cells were treated with the analog MB-97, the cells accumulated and activated p53 (25). These results suggest that this compound acts as a strong DNA damaging agent, a property it shares with other antibiotic/antitumor compounds like streptonigrin and the anthracyclines (25). Parental compounds of the MB-97 lavendamycin analog showed *in vivo* toxicity in the range of 0.4 mg/kg, whereas the new analog did not display toxicity until a treatment of 400 mg/kg was reached. Administration of another lavendamycin analog, MB-51, at doses of 300 mg/kg to mice bearing tumors resulted in an 80% reduction of tumor mass (25). These results strongly suggest that further examination of lavendamycin analogs as chemotherapeutic agents is necessary.

Discovered in 1966, bleomycin (derived from *Streptomyces verticillus*) is a well-studied antibiotic/antitumor agent (26). This compound is a principle treatment for testicular cancer and demonstrates reduced myelotoxicity (27). The most well known mode of action for this compound is its oxygen-dependent degradation of DNA (27). Unfortunately, bleomycin possesses potential fatal pulmonary toxicity (27). A recent 2003 clinical trial examined the effects of bleomycin treatment with mitomycin C as a follow up treatment to postoperative irradiation for patients with advanced head and neck cancer (28). This combined therapy improved survivability and the toxic effects (primarily mucositis) were considered within acceptable limits (28).

The anthracycline class of antibiotics include doxorubicin, daunorubicin, idarubicin, and epirubicin. Doxorubicin (DOX) and daunorubicin (DNR) have been used for over 30 years to treat a variety of solid and hematological tumors. DOX and DNR work by intercalation into DNA and inhibition of Topoisomerase II via binding to the Topo II/DNA ternary complex to promote its stabilization (29,30). Unfortunately, DOX and DNR also have high toxicity due to their mechanism of action, production of reactive oxygen species (ROS), which leads to toxicity of the cardiomyocytes and subsequent chronic and acute cardiomyopathies (31-33). Improvements in DOX and DNR structures lead to the development of idarubicin and epirubicin. Although these two analogs do have decreased toxicity and improved activity, there still is a significant risk to patients using these chemotherapeutic drugs (34,35).

Antibiotics are an intriguing class of compounds, not only for their ability to control bacterial infection but also for their capability to function as chemotherapeutic agents in cancer. There are an unlimited number of compounds bacteria can create. Based on the properties of existing antibiotics, studies into active analogs or novel synthetic compounds continue. Typically, antibiotics have reduced or no toxicity though there are exceptions, e.g. high doses can result in toxicity. Furthermore, these compounds can serve the dual roles of treating cancer or fighting potential infection during chemotherapy as an adjunct treatment. One of the more recent antibiotics to enter into the class of antibiotic/antitumor compounds is the class of beta-lactams.

4. TRADITIONAL ROLES OF BETA-LACTAMS

The family of beta-lactams, so named because they all contain a beta-lactam ring, have been used for many years to treat bacterial infections. Traditional beta-lactam antibiotics, such as the penicillins and cephalosporins, contain, in addition to the beta-lactam ring, a carboxyl group in close proximity to the lactam nitrogen, which is required for antimicrobial activity. These antibiotics act as bactericidal agents by serving as a substrate for peptidogylcan transpeptidase, the enzyme responsible for crosslinking the *N*-acetylglucuronic acid (NAG) and *N*-acetylmuramic acid (NAM) moieties in the peptidogylcan layer surrounding the periplasmic space and membrane of bacteria. The transpeptidase enzyme is acylated by the beta-lactam, which results in a weakened cell wall and osmotic lysis of the bacteria. Since the commercial availability of penicillin in 1940, many other beta-lactam antibiotics with medicinal effects have been isolated and synthesized. Many thought that the war on infectious diseases was over after the discovery of penicillin as we have used antibiotics to treat and cure many diseases that were once fatal, namely tuberculosis, bacterial meningitis, and pneumonia. Unfortunately, bacteria developed resistance by producing enzymes to hydrolyze the beta-lactam moiety rendering the antibiotic inactive (36). This resistance has spawned a renewed interest in identifying and synthesizing new active antibiotics to treat resistant strains, derived mainly through resistant genes located in plasmids. Bacterial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are currently susceptible to only one current antibiotic (vancomycin).

Antibiotics are substances created by microorganisms or synthetically designed to kill or inhibit the growth of bacteria. These compounds are the staple of treatment for bacterial infection. In addition, there are numerous antitumor antibiotics that are currently used to treat cancer, such as the anthracyclines, bleomycin, mitomycin C, dactinomycin, and mithramycin. The major mechanism of action for these antitumor antibiotics is DNA intercalation or inhibition of DNA synthesis. Beta-lactam antibiotics are traditionally used only for bacterial infections, however, several novel classes of beta-lactams have been shown to possess anticancer properties as well. We have found that a class of beta-lactams, the *N*-thiolated beta-lactams, induce tumor cell apoptosis by introducing DNA damage in a potent, and more importantly, a tumor cell-specific manner with little or no effect on normal cells (37, 38). Cainelli *et al*., describe that 4-alkylidene-beta-lactams inhibit matrix metalloproteinases-2, and -9 (MMP), essential for the tumor induced neovascularization (39). Banik *et al*., also show that beta-lactams with polyaromatic

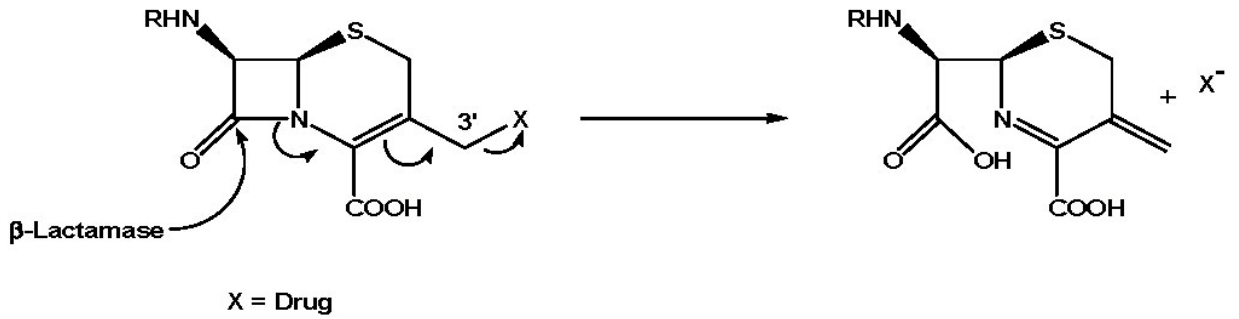

Figure 2. Diagramatic presentation of cephalosporin prodrug reaction. Beta-lactamase induces hydrolytic cleavage of the beta-lactam ring, which causes a secondary reaction leading to the release of the anticancer drug in the 3'position.

substituents induce tumor cell death in a variety of cancer cell lines, such as ovarian, prostate, breast, colon, and leukemic *in vitro* and demonstrated inhibition of tumor cell growth in mice (40) (see Section 6 for details).

5. BETA-LACTAMS AS PRODRUGS FOR ANTICANCER CHEMOTHERAPIES

5.1. Background

The most widely applied beta-lactams for prodrug based cancer chemotherapy have been the cephalosporins. The cephalosporins were chosen as prodrug candidates because of their inherent reactivity when hydrolyzed by beta-lactamase enzymes. Hydrolytic cleavage of the beta-lactam ring causes a secondary reaction that triggers the expulsion of the 3'-substituent (figure 2). The cytotoxic component can be attached to this position and then released when the cephalosporin beta-lactam ring is hydrolyzed by the enzyme.

Cephalosporins have also been used in the selective targeting of anticancer compounds to tumor cells using Antibody Directed Enzyme Prodrug Therapy (ADEPT) and this method has received much attention in recent years (41,42). ADEPT is a drug delivery strategy that employs an enzyme covalently attached to a monoclonal antibody that is specific for a tumor cell antigen. This strategy allows for the delivery of the cytotoxic agent masked as a prodrug to specifically target tumor cells. To achieve site-specific generation of the cytotoxic agent, the monoclonal antibody-enzyme immunoconjugate (mAb-enz) is given to the patient first. This allows for prelocalization of the mAb-enz on the targeted tumor cell surface. The prodrug, which is the substrate of the enzyme, is then administered leading to targeted release of the drug.

The benefits of this method are many compared to administering the prodrug or parent drug alone (43-46). The catalytic nature of the enzyme will allow for the conversion of a stoichiometric excess of prodrug substrate. In addition, lower doses of the antibody-enzyme conjugate and higher doses of the prodrug can be administered, which reduces the toxicity. The surrounding tumor cells not bearing the target epitope can also be effectively dosed with the cytotoxic drug. This can lead to lower side effects due to tumor-specific localization of the parent drug. Furthermore, beta-lactamase enzymes are not endogenous to humans, making them highly exploitable for ADEPT and therefore eliminating premature cleavage of the prodrug by other naturally occurring enzymes. In order for the monoclonal antibody-enzyme immunoconjugate system to be successful, there are several criteria required for therapeutic efficacy. First, the mAb-enzyme conjugate should not elicit an immune response and have a high tumor/blood ratio. Secondly, the antibody portion of the immunoconjugate must selectively target and have a high binding affinity for the tumor cell antigen. Lastly, the unbound conjugate must be readily cleared from the blood-stream before the prodrug is administered. The following section will discuss antibody-enzyme-prodrug systems that have undergone evaluation to treat various types of cancers.

5.2. Nitrogen Mustards

The alkylating agents or nitrogen mustards include cyclophosphamides, chloroambucil, and melphalan, and function by replacing an alkyl group for a hydrogen atom, leading to the formation of DNA adducts, or abnormal base pairing and cross linking of DNA. Cyclophosphamide and ifosfamide exist as prodrugs and are activated by hepatic enzymes into active species (47). While the nitrogen mustards are efficacious, they are extremely cytotoxic which results in unwanted side effects. For example, 7-(phenylacetamido) cephalosporin mustard (CM) prodrug was at least 50 times less toxic than phenylenediamine mustard (PDM) toward H2981 human adenocarcinoma cell line *in vitro* (48). The monoclonal antibody-enzyme conjugate L6-BCβL was shown to activate CM in an immunologically specific manner, which resulted in a level of cytotoxicity comparable to PDM (48). *In vivo* studies indicated that CM was less toxic to nude mice than PDM, however treatment was hampered due to severe tail necrosis following intravenous injection (49).

A modified analog of CM, 7-(4-carboxybutamido) cephalosporin mustard (CCM), showed higher activity *in vitro* when administered with L6-EClβL. CCM was found to be less cytotoxic than PDM on H2981 cells, with IC_{50} values of 25-45 μg and 1.5 μg, respectively. When the monoclonal antibody-enzyme conjugate L6-EClβL was administered 96 h prior to CCM, there was observed significant antitumor activity *in vivo* (49). The *in vivo* experiments concluded that administration of CCM in nude mice was less toxic than CM, and both prodrugs (CM

and CCM) were significantly less toxic than PDM.

The prodrug C-Mel, a cephalosporin carbamate derivative of melphalan, was also shown to have antitumor activity (50). C-Mel was activated in an immunologically specific manner by the L49-sFv-βL conjugate. *In vitro* cytotoxicity assays using 3677 human melanoma cells treated with the C-Mel prodrug in conjunction with L49-sFv-βL showed that c-Mel was 40 times less toxic than melphalan alone, IC_{50} = 53μg and 1.3 μg, respectively. *In vivo* studies demonstrated that nude mice with growing tumors treated with L49-sFv-βL and C-Mel at 150 mg/kg/injection underwent complete regressions, and 3 out of 5 mice were eventually cured (50).

5.3. Methoyxtrexate

Methotrexate (MTX) is a folic acid analog, or antimetabolite, which was first developed and used clinically in the 1940's (51). MTX interacts with dihydrofolate reductase (DHFR), an enzyme critical in folate metabolism (52). Unfortunately, drug resistance can often occur due to increased endocytosis of the antifolate by multidrug resistance protein (MDRPs) pumps and folate transporters (53-56). The prodrug of MTX is a potent cytotoxic agent and antimetabolite developed for ADEPT therapy. MTX was found to be a good substrate of beta-lactamase but showed identical cytotoxicity to that of the parent drug alone (45). No further evaluations have been conducted.

5.4. 5-Fluorouracil

Another group of antimetabolites include the pyrimidine analogs, such as 5-fluorouracil and gemcitabine, and the purine analogs, such as 6-mercaptopurine and 6-thioguanine. These analogs substitute for nucleic acid bases in both DNA and RNA synthesis, but drug resistance to these antimetabolites has been implicated due to the nucleoside transporters (NT), which mediate uptake of nucleic acids into dividing cells (57). 5-Fluorouracil is an anticancer drug often used in the treatment of colon cancer. The prodrug of 5-fluorouracil was shown to be cleaved by beta-lactamase, however, the *in vitro* cytotoxicity was found to be the same as that of the parent drug alone (58).

5.5. Vinca Alkaloids

The vinca alkaloids are potent anticancer drugs derived from natural sources that are used to treat the acute leukemias, lymphomas, and some solid tumors. Two cephalosporin-vinca alkaloid prodrugs were found to have a 5-fold less cytoxicity to LS174T colon adenocarcinoma cells than the parent drug LY233425 and LY266070 (59,60). LY233425-cephalosporin prodrug was found to be equipotent when administered with the F(ab')-beta-lactamase conjugate with the parent drug (59). *In vivo* studies with mouse models of human colorectal carcinoma tumors demonstrated long term regressions when LY266070-cephalosporin prodrug was administered with mAb-beta-lactamase (60).

5.6. Doxorubicin

Cephalosporin-doxorubicin prodrugs have been developed that show promising anticancer properties when used in conjunction with mAb-beta-lactamase conjugates. *In vitro* studies of C-Dox on H2981 lung adenocarcinoma cells revealed that the prodrug was less toxic than doxorubicin alone. The prodrug was also immunospecifically activated by the L6-ECIβ-lactamase conjugate to release doxorubicin *in vitro* (61). Additionally, another study showed that a different mAb-beta-lactamase/cephalosporin-doxorubicin prodrug system effectively delivered doxorubicin to a series of MCF7 breast carcinoma, OVCAR3 ovarian carcinoma, and T380 and LS174T colon tumor xenografts (62). The maximum tolerated dose of the prodrug was equivalent to that of the free drug when compared to the degree of tumor suppression, however tumors did not regress. A polymer prodrug of cephalosporin-doxorubicin has been developed and was shown to increase the survival rate and decrease the tumor growth rate of mice when treated in conjunction with a polymer bound beta-lactamase enzyme (63). The combination of polymer-prodrug and polymer-enzyme was non-toxic with the doses used in the study.

5.7. Mitomycin C

Two cephalosporin prodrugs of mitomycin C were evaluated against H2987 lung adenocarcinoma and clone 62 melanoma cell lines (64). *In vitro* studies showed that one of the prodrugs (prodrug 1) had comparable cytotoxicity to the parent drug, whereas another prodrug (prodrug 3) was 40- and 10- fold less toxic toward H2987 and clone 62 melanoma cells. Prodrug 3 also was immunospecifically activated by L6-F(ab')-beta-lactamase and 96.5-F(ab')-beta-lactamase conjugates that are selective toward H2987 and clone 62 cells, respectively (64).

5.8. Paclitaxel

Anticancer drugs derived from natural sources comprise a large body of the drugs currently approved for chemotherapies. Treatments with paclitaxol (Taxol), derived from *Taxus brevifolia* (Pacific yew tree) and a semisynthetic analog, docetaxol, target rapidly dividing cancer cells by increasing microtubule polymerization, thereby inhibiting anaphase during cell cycle (65,66).

A prodrug of paclitaxel has been shown to be immunospecifically activated by the fusion protein L49-sFv-beta-lactamase (67). *In vitro* cytotoxicity assays performed on 3677 melanoma cells expressing the melanotransferrin (p97) antigen revealed that the prodrug was 12 to 30 times less cytotoxic than the parent drug (67).

5.9. Radioimmunoconjugates

Radioimmunotherapy is a method to deliver a radioisotope to a specific target area namely tumor cells. One disadvantage of method is that it can lead to dose-limiting toxicities through radiation exposure to non-targeted organs. A recent study has shown that a radioimmunoconjugate containing a cleavable linker can release the radioisotope upon administering an enzyme thus lowering systemic radiation exposure (68). This approach utilized a ^{131}I-labled cephalosporin conjugated to Tositumomab, a mAb specific for the CD20 antigen via a synthetic linker. Upon administration of the beta-lactamase

enzyme, the radiolabel would be released causing rapid clearance from the blood and normal organs. *In vivo* studies of mouse models with human Ramos B lymphoma tumor xenografts revealed no decrease of the injected dose after 1 h of beta-lactamase treatment (68). However, after 4 h there was a noticeable decrease in the radioactive content from the tumor as well as blood, liver, lung and marrow demonstrating that there was rapid clearance of the radiolabel after injection of the radioimmunoconjugate and beta-lactamase enzyme (68). In addition, there was an enhanced tumor to blood % injected dose ratio at the beginning time points of the study.

The ADEPT system allows for the use of agents that, when given systemically, are too toxic for use in the clinic. The diversity of cancer drugs that are utilized is well demonstrated. Additionally, many studies have shown that the active drug is generated at the tumor site and at concentrations that could not be used in normal systemic administration of the parent drug. Further advancements to improve the efficacy of mAb-enzyme/prodrug therapies have resulted in modifying the mAb-beta-lactamase conjugate. Recombinant formed monoclonal antibody-beta-lactamase conjugates showed improved anticancer therapeutic activities compared to the synthetically formed conjugates (69).

6. POTENTIAL USE OF BETA-LACTAMS AS ANTICANCER DRUGS

6.1. *N*-Thiolated beta-Lactams

6.1.1. Structure-Activity Relationships

Currently, there are many types of antibiotics (e.g. anthracyclines, bleomycin) that have been used to treat cancer. However, research into the possibility of utilizing beta-lactam antibiotics as potential anticancer medications has been relatively non-existent. Our most recent studies suggest that beta-lactams could play a role as anticancer drugs (37,38). In the following sections, we will discuss the different *N*-methylthio beta-lactams and their effects on cancer cells as well as some insights into possible mechanisms of action. We have demonstrated that *N*-thiolated beta-lactams have tumor cell-killing ability through induction of DNA-damage and subsequent apoptosis (37). Synthesis of other beta-lactams have aided in identifying important structure-activity-relationships (SARs). Additionally, we have also shown that *N*-thiolated beta-lactams have the ability to preferentially induce apoptosis in tumor cells, but not in normal or non-transformed cell (38).

Perhaps one of the most important findings with beta-lactams was that they did not need to possess a bicyclic ring with its rigid conformity to be bactericidal (9,10). This allows for a broader range of synthetic analogs to be made that possess antibacterial or antitumor activity. We have screened a large number of synthetic beta-lactams for their ability to promote tumor cell death (37,38). Of the compounds screened, we chose a lead compound, Lactam 1, to be the basis for additional synthesis of analogs (figure 3). Our findings yielded several important SARs. Lactam 1 was shown to induce apoptosis in a variety of tumor cell lines, namely, breast (MDA-MB-231, MCF-7), prostate (PC-3, DU-145), head-and-neck (PCI-13), SV-40 transformed lung cells (VA-13) and leukemic (Jurkat T) cell lines (37, 38).

Other important SARs were observed also when cells were treated with the beta-lactams in cellular toxicity assays. Foremost, is the necessity of the *N*-methyl-thio group that when absent abolishes the apoptosis-inducing activity. Also observed was the inverse relationship between the number of carbon atoms off the *N*-thio group. Increasing the number of carbons from one to two decreased the amount of apoptosis observed by ~50% (Lactam 1 vs. Lactam 3). A four carbon chain off the *N*-thio group further decreased apoptosis-inducing activity by ~65%, and substitution of the *N*-methylthio group with a *N*-benzylthio group lead to ~70% decrease in apoptosis. Observations about the position of the chloro group off the phenyl ring also provided key SAR information. Isomers with the –Cl group in the *meta* or *para* position revealed that Lactams-5 and –6, while still capable of inducing apoptosis, were less potent than Lactam 1 (37). To determine if deletion or substitution of the *ortho* –Cl on the phenyl ring would increase or decrease activity, several analogs of Lactam 1 were synthesized with substituted halogen or non-halogen groups for the –Cl (figure 3). It was found that increasing the size of the group in the *ortho* position correlated with increased cell death (38). In fact, elimination of the *ortho* substituted group resulted in the least amount of activity, while substitution with a nitro group lead to the greatest amount of activity. The $–NO_2$ substituted analog, Lactam 12, exhibited the strongest effect and consistently induced apoptosis comparable with Lactam 1, but at half the concentration (38).

According to these results, several key features should be retained for future design and synthesis of beta-lactams with antitumor properties: 1) the *N*-methyl-thio group must remain intact without additional carbon chains, 2) although *meta* and *para* substitutions on the phenyl ring still preserve their apoptotic producing abilities, substitutions in the *ortho* position are most potent, and lastly 3) the larger groups in the *ortho* position correlates with enhanced apoptotic-inducing activity (37,38).

6.1.2. Apoptosis Induction

A number of synthetic *N*-methylthio-beta-lactam compounds have been found to induce apoptosis in a number of tumor cell lines, such as the human leukemia Jurkat T cells, breast cancer (MCF-7, MDA-MB-231), prostate (PC-3, DU-145), and head-and-neck (PCI-13) cells (37,38). Several of these compounds (figure 3) caused induction of caspase-3/-7 activity, effector caspases whose activation is indicative of the apoptosis (70). The potency of these beta-lactams is as follows, Lactam 12 >10 > 11 > 1 > 6 ≥ 5 > 3 > 4 > 9 > 8 > 7 > 2 (37,38). A nuclear stain to determine the morphological changes of apoptotic nuclei showed that Lactam 12 induced cellular detachment of 50-60 % of total cell population (38). Additionally, beta-lactam treatment instigated cleavage of poly(ADP-ribose) polymerase (PARP) from its 116 kDa full length form to the 85 kDa fragment (37) which occurred in conjunction

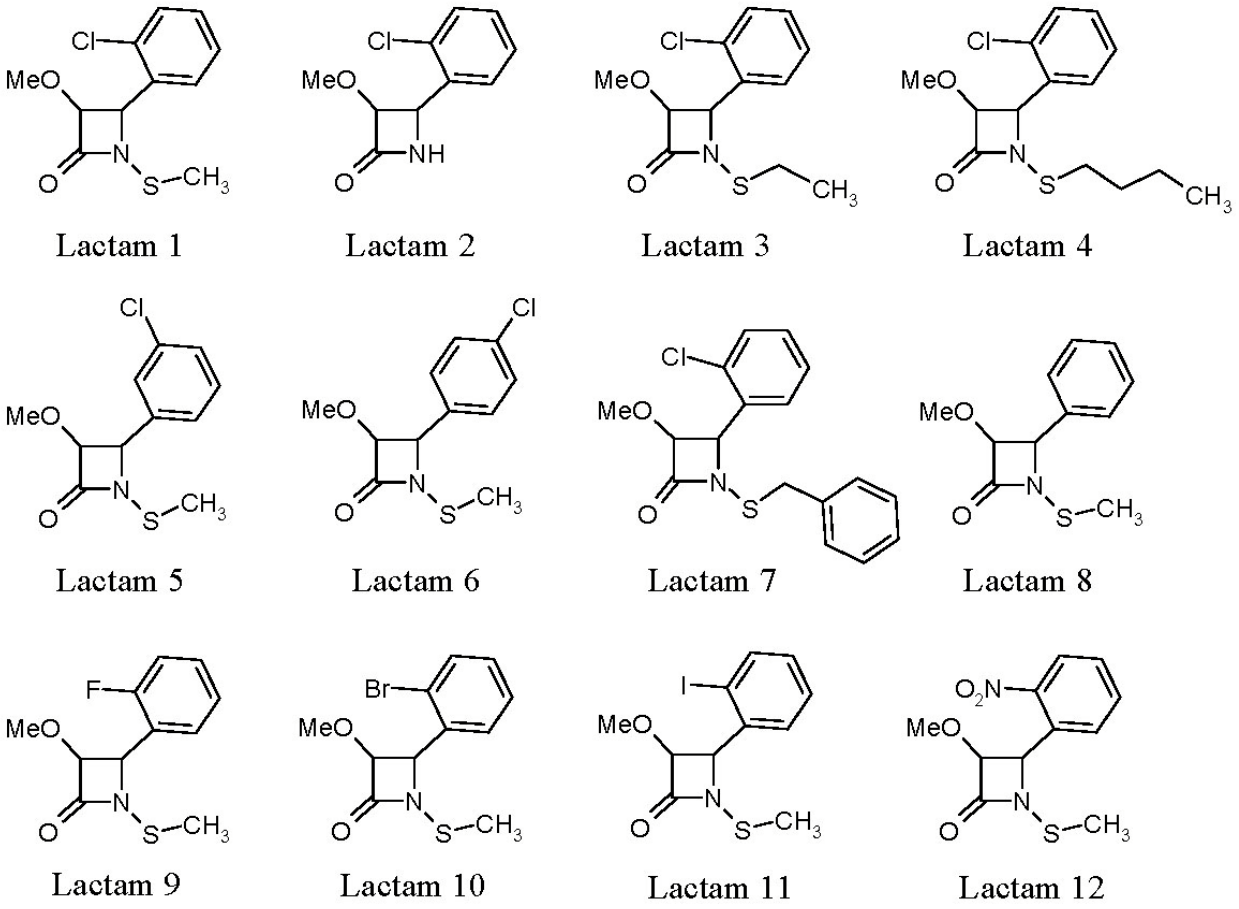

Figure 3. Structure of *N*-thiolated monobactams.

with caspase-3 activity, the caspase shown to directly cleave PARP (71). Caspase-8, an initiator caspase capable of mitochondria-dependent and –independent apoptosis initiation (72), was also found to become active after beta-lactam treatment (37). Cytochrome *c* is a mitochondrial protein that is released during apoptosis when the membrane potential of the mitochondria is compromised and combines with several other proteins (dATP, Apaf-1, caspase-9) to form the apoptosome, which is capable of activating caspase-3 (73). The *N*-methylthio beta-lactams are also able to cause cytochrome *c* release from the mitochondria, prior to activation of caspase-3, in time- and concentration-dependent manners (37). These data confirm that beta-lactams can indeed cause apoptosis in tumor cells.

There are other lactam compounds that can also induce apoptosis. Watabe *et al*. found that gamma-lactams, which contain a five-membered ring, are capable of inducing apoptosis in HL-60 cells (74). MT-21, a synthetic gamma-lactam, activates caspase-9 followed by the subsequent activatation of caspase-3. Unlike our findings with beta-lactams, caspase-8 was not found to participate in the apoptosis signaling cascade after gamma-lactam treatment (74). Lactacystin, a gamma-lactam possessing a thio ester moiety and originally isolated from actinomycetes (75), has been found to be a potent inhibitor of chymotryptic- and tryptic-like catalytic activities of the proteasome through covalent bonding to the N-terminal threonine of the beta-subunits (76). Proteasome inhibition leads to an accumulation of p27 (77), IκB-α (78), and Bax (79), which can cause G_1 cell cycle arrest and apoptosis (80, 81). It is for these reasons many believe that proteasome inhibitors are good candidates for anticancer chemotherapeutic drugs (82-84).

6.1.3. DNA-Damage and Signal Transduction Pathways

To further investigate the cause of apoptosis after *N*-methlythio beta-lactam treatment, analysis of cell cycle changes were performed. Lactam 1 was found to increase S-phase DNA content and initiate a concomitant decrease in G_1 phase DNA. This S-phase cell cycle arrest was found to be due to an inability for treated cells to undergo DNA replication as was found from a [^{3}H] thymidine incorporation assay. DNA replication was inhibited in a time- and concentration-dependent manner with a half-maximal inhibition (IC_{50}) of [^{3}H] thymidine in Jurkat cells at 32 μM with Lactam 1 treatment (37). A terminal deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL) assay, which detects DNA strand breaks, then provided additional data to support to the hypothesis that the DNA replication inhibition was due to damage of the

genomic DNA. After just 1 h of treatment with, over half of the cell population contained DNA strand breaks and after 4 h 98 % of the cells showed DNA strand breakage (37). In a similar experiment it was found that Lactam 12 induced greater DNA damage that Lactam 1 (by 27%) after a 24 h period (37).

It was also determined that p38 MAP kinase activation is required for beta-lactam induced apoptosis (37). Activation of p38 MAP kinase can trigger apoptosis following multiple stimuli, such as DNA damage (85, 86). Protein levels of phosphorylated p38 increased significantly with Lactam treatment and cotreatment with the p38 inhibitor (PD169316) inhibited PARP cleavage and activation of caspase-3, -8, and –9 (37). Additional experiments revealed that p38 activation occurs upstream of caspase activation and that p38 activity was necessary for caspase-mediated cell death in beta-lactam treatment. Conversely, DNA strand breaks were still observed after cotreatment with PD169316 and Lactam 1, indicating that *N*-thiolated beta-lactams induced DNA damage leading to p38 activation, followed by caspase activation and subsequent apoptotic cell death.

6.1.4. Preferential Tumor Cell Killing

Many currently used chemotherapeutic drugs for cancer intercalate with cellular DNA, thus making it impossible for the cell to function which leads to subsequent apoptotic death. Unfortunately, these drugs are not "tumor-specific" and they will intercalate with any rapidly dividing cell, such as the epithelial cells lining the gastrointestinal tract, which can lead to nausea and vomiting. Tumor cell specific therapies are those that solely target tumor cell characteristics exclusively. For instance, Gleevec (STI571) is an ATP inhibitor that targets growth and proliferative signaling pathways stimulated by the Bcr-Abl oncoprotein in chronic myelogenous leukemia (87).

To determine if Lactam 1 possessed a tumor cell-specific activity human leukemic Jurkat T cells and immortalized, non-transformed natural killer cells (YT cells) were treated with Lactam 1 and the effects were determined. It was found that only the Jurkat, but not YT, cells showed apoptosis-specific PARP cleavage and decreased cell viability in both time- and concentration-dependent manner (38). Additionally, treatment with Lactam 12, which substitutes the –Cl moiety for a $–NO_2$ on the benzene ring, was found to potently and specifically induce apoptosis in only the Jurkat T cells while not affecting the non-transformed YT cells. Both Lactam 1 and Lactam 12 inhibited colony formation, indicative of cellular transformation, of prostate cancer LNCaP cells as observed in a soft agar assay. These lactams were also able to induce TUNEL-positive cells as well as caspase-3/-7 activity and apoptotic nuclei in a number of transformed tumor cell line types, but not in non-transformed cell lines (38). For example, Lactam 12 treatment induced apoptotic morphological changes and caspase-3 activity exclusively in SV-40 transformed human fibroblasts (VA-13) but not in normal non-transformed fibroblasts (WI-38) (38). This is consistent with the idea that beta-lactams could be developed into tumor-specific drugs.

6.2. 4-Alkylidene-beta-Lactams

The matrix metalloproteinases (MMPs) are a class of mammalian proteases that can, among other functions, degrade the extracellular matrix (88). Angiogenesis, the formation of new blood vessels, requires the activity of the MMPs to digest the basement membrane. The MMPs play a pivotal role in cancer progression by allowing neovascularization, which is essential for tumor growth, invasion, and metastasis (89). MMPs can be constitutively activated in cancer cells, but not in normal cells (90). Thus, targeting MMP expression and activity is a unique approach in the field of cancer research.

A class of beta-lactams, the 4-alkylidene-azetidin-2-ones, has been identified that exhibit inhibitory activity to both MMP-2 and MMP-9 as well as leukocyte elastase (LE) (39). LE can activate MMP-2 and MMP-9, and inactivate their tissue inhibitor (91). Compounds with protected hydroxy groups (Compounds 1 and 8) were found to inhibit LE. Compound 8 in particular showed an IC_{50} of 9 μM to LE activity. The green tea polyphenol (-)-epigallocatechin-3-gallate (EGCG), a known LE inhibitor, was used as a comparison and was found to be ~22-fold more active (IC_{50}=0.4 μM) than Compound 8 (39). When the hydroxyl group was unprotected, or removed all together, the beta-lactam lost its potent activity against LE, but gained considerable activity against MMP-2 and MMP-9. Two compounds in particular (Compounds 2 and 18) showed the greatest inhibitory activity on MMP-2 with IC_{50}s of 85 μM and 60 μM, respectively (39). This is a promising area of drug research because inhibition of angiogenesis not only inhibits tumor growth, but also prevents invasion and metastasis *via* the circulatory system.

6.3. Polyaromatic Beta-Lactams

In 2001, Banik *et al.* described polyaromatic imine beta-lactams with biological activity against cancer cells (40). Several synthesized compounds were tested *in vitro* for their cytotoxicity on nine cancer cell lines (40). Compounds with phenanthrene and chrysene substituents had the most activity *in vitro*, as measured by MTT assay. The maximal activity concentrations ranged from 2.5 to 40.6 μM, some well within the therapeutic range. Conversely, beta-lactams with naphthalene, anthracene, and pyrene substituents showed virtually no cytotoxicity (40).

A series of i*n vivo* assays using athymic nude (nu/nu) mice was performed with the active beta-lactams mentioned above. Mice were injected with K-562 leukemia, HT-29 colon, or SKOV-3 ovarian cancer cells. A variety of treatment times and regimens were tested (40). Mice given polyaromatic beta-lactam treatments (Compound 17a) at 60 mg/kg showed negligible toxicity compared to the control mice that were given cisplatin and adriamycin. The polyaromatic induced only a slight weight loss (3.52 g), which was quickly recovered after discontinuation of treatment (40). It was also found that treatment with the beta-lactam compound delayed the onset of tumor formation by 7 ± 2 days in mice injected with HT-29 cells. Additionally, many of the mice injected with

SKOV-3 cells did not form any tumors at all (40).

7. CONCLUSIONS AND PERSPECTIVES

Cancer is lethal to 42% of those diagnosed. With millions of new patients each year, the initiative to develop suitable chemotherapeutic agents is a driving focus of medical research. However, the currently available chemotherapeutic agents are incapable of selectively targeting cancer cells from normal cells leading to treatments that are almost as hazardous as the disease itself. Beta-lactams are compounds that have been used for many years to combat microbial infections. Therefore, it is already known that these compounds possess minimal effects on non-bacterial cells; this trait is desirable of chemotherapeutic agents. Recently, the potential of beta-lactams as anticancer agents has come to light. Beta-lactams can be used as pro-drugs that are capable of specifically targeting tumor cells. Likewise, the *N*-methylthiolated beta-lactams are capable of inducing apoptosis in a wide array of tumor cells types, with little effect on normal cells. Thus making these compounds, and other beta-lactams (eg. 4-alkylidene and polyaromatics), attractive targets for structure-activity relationship studies and analog synthesis. While further study on this class of compounds in animal models should be performed to completely assess their toxicity, selectivity, and efficacy *in vivo,* the profiles reported here show an optimistic future for expanding the role of these compounds from simple antibiotics to anticancer therapeutics.

8. ACKNOWLEDGMENTS

We thank Timothy Long, Randy Hill, and David M. Smith for their contribution to the research of beta-lactams. We thank the United States Army Medical Research and Material Command (DAMD17-03-1-0175), the National Cancer Institute-National Institutes of Health, and Barbara Ann Karmanos Cancer Institute for research grants to Professor Q. Ping Dou, and the National Institutes of Health for a research grant (NIH RO1 A151351) to Professor Edward Turos.

9. REFERENCES

1. Kerr, J.F., A.H. Wyllie and A.R. Currie: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26, 239-257 (1972)

2. Hanahan, D. and R.A. Weinberg: The hallmarks of cancer. *Cell* 100, 57-70 (2000)

3. Zimmermann, K.C., C. Bonzon and D.R. Green: The machinery of programmed cell death. *Pharmacol Ther* 92, 57-70 (2001)

4. Abraham, E.P: The beta-lactam antibiotics: *Sci Am* 244, 64-74 (1981)

5. Crowfoot, D., C.W. Bunn, B. Rogers-Low and A. Turner-Jones: The X-ray crystallographic investigation of the structure of penicillin. In: The Chemistry of Penicillin. Eds: Clarke HT, Johnson JR, Robinson R. Princeton University Press, NJ. 310-366 (1949)

6. Brotzu, G: Richerche su di un nuovo antibiotico. Lavori dell'Istituto d'Igiene di Cagliari, 1-11 (1948)

7. Burton, H.S. and E.P. Abraham: Isolation of antibiotics from a species of Cephalosporium: cephalosporins P1, P2, P3, P4 and P5. *Biochem J* 50, 168-170 (1951)

8. Nathwani, D. and M.J. Wood: Penicillins. A current review of their clinical pharmacology and therapeutic use. *Drugs* 45, 866-894 (1993)

9. Imada, A., K. Kitano, K. Kintaka, M. Muroi and M. Asai: Sulfazecin and isosulfazecin, novel beta-lactam antibiotics of bacterial origin. *Nature* 12, 289 590-1 (1981)

10. Sykes, R.B., C.M. Cimarusti, D.P. Bonner, K. Bush, D.M. Floyd, N.H. Georgopapadakou, W.M. Koster, W.C. Liu, W.L. Parker, P.A. Principe, M.L. Rathnum, W.A. Slusarchyk, W.H. Trejo and J.S. Wells: Monocyclic beta-lactam antibiotics produced by bacteria. *Nature* 11, 489-491 (1981)

11. Ren, X.F., M.I. Konaklieva, H. Shi, S. Dickey, D.V. Lim, J. Gonzalez and E. Turos: Studies on Nonconventionally Fused Bicyclic beta-Lactams. *J Org Chem* 63, 8898-8917 (1998)

12. Turos, E., M.I. Konaklieva, X.F. Ren, H. Shi, J. Gonzalez, S. Dickey and D. Lim: N-thiolated bicyclic and moncyclic beta-lactams. *Tetrahedron* 56, 5571-5578 (2000)

13. Turos, E., T.E. Long, M.I. Konaklieva, C. Coates, J.Y. Shim, S. Dickey, D.V. Lim and A. Cannons: N-thiolated beta-lactams: novel antibacterial agents for methicillin-resistant Staphylococcus aureus. *Bioorg Med Chem Lett* 12, 2229-2231 (2002)

14. Holt, R.J. and G.T. Stewart: Production of Amidase and beta-Lactamase by Bacteria. *J Gen Microbiol* 36, 203-213 (1964)

15. Saikali, Z. and G. Singh: Doxycycline and other tetracyclines in the treatment of bone metastasis. *Anticancer Drugs* 14, 3-8 (2003)

16. Kahan, B.D. and J.S. Camardo: Rapamycin: clinical results and future opportunities. *Transplantation* 72, 1181-1193 (2001)

17. Kato, J.Y., M. Matsuoka, K. Polyak, J. Massague and C.J. Sherr: Cyclic AMP-induced G1 phase arrest mediated by an inhibitor (p27Kip1) of cyclin-dependent kinase 4 activation. *Cell* 79, 487-496 (1994)

18. Jayaraman, T. and A.R. Marks: Rapamycin-FKBP12 blocks proliferation, induces differentiation, and inhibits cdc2 kinase activity in a myogenic cell line. *J Biol Chem*

268, 25385-25388 (1993)

19. Albers, M.K., R.T. Williams, E.J. Brown, A. Tanaka, F.L. Hall and S.L. Schreiber: FKBP-rapamycin inhibits a cyclin-dependent kinase activity and a cyclin D1-Cdk association in early G1 of an osteosarcoma cell line. *J Biol Chem* 268, 22825-22829 (1993)

20. Huang, S. and P.J. Houghton: Targeting mTOR signaling for cancer therapy. *Curr Opin Pharmacol* 3, 371-377 (2003)

21. Nepomuceno, R.R., C.E. Balatoni, Y. Natkunam, A.L. Snow, S.M. Krams and O.M. Martinez: Rapamycin inhibits the interleukin 10 signal transduction pathway and the growth of Epstein Barr virus B-cell lymphomas. *Cancer Res* 63, 4472-4480 (2003)

22. Ueda, H., H. Nakajima, Y. Hori, T. Goto and M. Okuhara: Action of FR901228, a novel antitumor bicyclic depsipeptide produced by Chromobacterium violaceum no. 968, on Ha-ras transformed NIH3T3 cells. *Biosci Biotechnol Biochem* 58, 1579-1583 (1994)

23. Sasakawa, Y., Y. Naoe, T. Noto, T. Inoue, T. Sasakawa, M. Matsuo, T. Manda and S. Mutoh: Antitumor efficacy of FK228, a novel histone deacetylase inhibitor, depends on the effect on expression of angiogenesis factors. *Biochem Pharmacol* 66, 897-906 (2003)

24. Doyle, T.W., D.M. Balitz, R.E. Grulich, D.E. Nettleton, S.J. Gould, C. Tann and A.E. Moews: Structure determination of lavendamycin, a new antitumor antibiotic from Streptomyces lavendulae. *Tetrahedron Lett* 22, 4595–4598 (1981)

25. Fang, Y., C.M. Linardic, D.A. Richardson, W. Cai, M. Behforouz, and R.T. Abraham: Characterization of the cytotoxic activities of novel analogues of the antitumor agent, lavendamycin. *Mol Cancer Ther* 2, 517-526 (2003)

26. Umezawa, H., K. Maeda, T. Takeuchi and Y. Okami: New antibiotics, bleomycin A and B. *J Antibiot* (Tokyo) 19, 200-209 (1966)

27. Kawai, K. and H. Akaza: Bleomycin-induced pulmonary toxicity in chemotherapy for testicular cancer. *Expert Opin Drug Saf* 2, 587-596 (2003)

28. Smid, L., M. Budihna, B. Zakotnik, E. Soba, P. Strojan, I. Fajdiga, M. Zargi, I. Oblak, M. Dremelj and H. LeSnicar: Postoperative concomitant irradiation and chemotherapy with mitomycin C and bleomycin for advanced head-and-neck carcinoma. *Int J Radiat Oncol Biol Phys* 56, 1055-1062 (2003)

29. Froelich-Ammon, S.J. and N. Osheroff: Topoisomerase Poisons: Harnessing the Dark Side of Enzyme Mechanism. *J Biol Chem* 270, 21429–21432 (1995)

30. Wang, H., Y. Mao, N. Zhou, T. Hu, T.S. Hsieh and L.F. Liu: ATP-bound topoisomerase II as a target for anti-tumor drugs. *J Biol Chem* 276, 15990-15995 (2001)

31. Frishman, W.H., H.M. Sung, H.C.M. Yee, L.L. Liu, A.I. Einzig, J. Dutcher and D. Keefe: Cardiovascular toxicity with cancer chemotherapy. *Curr Probl Cardiol* 21, 225–288 (1996)

32. Olson, R.D. and S. Mushlin: Doxorubicin cardiotoxicity: analysis of prevailing hypotheses. *FASEB J* 4, 3076–3086 (1990)

33. Minotti, G., S. Licata, A. Saponiero, P. Menna, A.M. Calafiore, G. Di Giammarco, G. Liberi, F. Animati, A. Cipollone, S. Manzini and C.A. Maggi: Anthracycline metabolism and toxicity in human myocardium: comparisons between doxorubicin, epirubicin, and a novel disaccharide analogue with a reduced level of formation and [4Fe-4S] reactivity of its secondary alcohol metabolite. *Chem Res Toxicol* 13, 1336–1341 (2000)

34. Platel, D., P. Pouna, S. Bonoron-Adele and J. Robert: Comparative cardiotoxicity of idarubicin and doxorubicin using the isolated perfused rat heart model. *Anticancer Drugs* 10, 671–676 (1999)

35. Booser, D.J. and G.N. Hortobagyi: Anthracycline antiobiotics in cancer therapy. *Drugs* 47, 223–258 (1994)

36. Bush, K., G.A. Jacoby, and A.A. Medeiros: A functional classification scheme for b-lactamases and its correlation to molecular structure. *Antimicrob Agents Chemother* 39, 1211-1233 (1995)

37. Smith, D.M., A. Kazi, L. Smith, T.E. Long, B. Heldreth, E. Turos and Q.P. Dou: A novel beta-lactam antibiotic activates tumor cell apoptotic program by inducing DNA damage. *Mol Pharmacol* 61, 1348-1358 (2002)

38. Kazi, A., R. Hill, T.E. Long, D.J. Kuhn, E. Turos and Q.P. Dou: Novel N-thiolated beta-lactam antibiotics selectively induce apoptosis in human tumor and transformed, but not normal or nontransformed, cells. *Biochem Pharmacol* 67, 365-374 (2004)

39. Cainelli, G., P. Galletti, S. Garbisa, D. Giacomini, L. Sartor and A. Quintavalla: 4-alkylidene-azetidin-2-ones: novel inhibitors of leukocyte elastase and gelatinase. *Bioorg Med Chem* 11, 5391-5399 (2003)

40. Banik, I., F.F. Becker and B.K. Banik: Stereoselective synthesis of beta-lactams with polyaromatic imines: entry to new and novel anticancer agents. *J Med Chem* 46, 12-15 (2003)

41. Bagshawe, K.D: Antibody directed enzymes revive anticancer prodrug concepts. *Br J Cancer* 56, 531-532 (1987)

42. Bagshawe, K.D., C.J. Springer, F. Searle, P. Antoniw, S.K. Sharma, R.G. Melton and R.F. Sherwood: A cytotoxic agent can be generated selectively at cancer sites. *Br J*

Cancer 58, 700-703 (1988)

43. Koppel, G.A: Recent advances with monoclonal antibody drug targeting for the treatment of human cancer. *Bioconjugate Chem* 1, 13-23 (1990)

44. Senter, P.D., P.M. Wallace, H.P. Svensson, V.M. Vrudhula, D.E. Kerr, I. Hellstrom and K.E. Hellstrom: Generation of cytotoxic agents by targeted enzymes. *Bioconjugate Chem* 4, 3-9 (1993)

45. Jungheim, L.N. and T.A. Shepherd: Design of antitumor prodrugs: Substrates for antibody targeted enzymes. *Chem Rev* 94, 1553-1566 (1994)

46. Senter, P.D. and C.J. Springer: Selevtive activation of anticancer prodrugs by monoclonal antibody-enzyme conjugates. *Adv Drug Deliv Rev* 53, 247-264 (2001)

47. Fleming, R.A: An overview of cyclophosphamide and ifosfamide pharmacology. Pharmacotherapy 17, 146S-154S (1997) Antman K.H.: Introduction: the history of arsenic trioxide in cancer therapy. *Oncologist* 6 Suppl 2, 1-2 (2001)

48. Svensson, H.P., J.F. Kadow, V.M. Vrudhula, P.M. Wallace and P.D. Senter: Monoclonal antibody-beta-lactamase conjugates for the activation of a cephalosporin mustard prodrug. *Bioconjugate Chem* 3, 176-181 (1992)

49. Vrudhula, V.M., H.P. Svensson, K.A. Kennedy, P.D. Senter and P.M. Wallace: Antitumor activities of cephalosporin prodrug in combination with monoclonal antibody-beta-lactamase conjugates. *Bioconjugate Chem* 4, 334-340 (1993)

50. Kerr, D.E., Z. Li, N.O. Siemers, P.D. Senter and V.M. Vrudhula: Development and activities of a new melphalan prodrug designed for tumor-selective activation. *Bioconjugate Chem* 9, 255-259 (1998)

51. Farber, S., L.K. Diamond, R.D. Mercer, R.F. Sylvester and V.A. Wolff: Temporary remission in acute leukemia in children produced by folic acid antagonist 4-amethopteroylglutamicacid (aminopterin). *N Engl J Med* 238, 787–793 (1948)

52. ELR, Stokstad: Folic Acid Metabolism in Health and Disease. Eds: Picciano MF, Stokstad ELR. Wiley-Liss, NY, 1–21 (1990)

53. Sirotnak, F.M., S. Kurita and D.J. Hutchison: On the nature of a transport alteration determining resistance to amethopterin in the L1210 leukemia. *Cancer Res* 28, 75–80 (1968)

54. Niethammer, D. and R.C. Jackson: Changes of molecular properties associated with the development of resistance against methotrexate in human lymphoblastoid cells. *Eur J Cancer*, 11, 845–854 (1975)

55. Masuda, M., Y. I'izuka, M. Yamazaki, R. Nishigaki, Y. Kato, K. Ni'inuma, H. Suzuki and Y. Sugiyama: Methotrexate is excreted into the bile by canalicular multispecific organic anion transporter in rats. *Cancer Res*, 57, 3506–3510 (1997)

56. Kusuhara, H., Y.H. Han, M. Shimoda, E. Kokue, H. Suzuki H and Y. Sugiyama: Reduced folate derivatives are endogenous substrates for cMOAT in rats. *Am J Physiol* 275, G789–G796 (1998)

57. Damaraju, V.L., S. Damaraju, J.D. Young, S.A. Baldwin, J. Mackey, M.B. Sawyer and C.E. Cass: Nucleoside anticancer drugs: the role of nucleoside transporters in resistance to cancer chemotherapy. *Oncogene* 22, 7524-7536 (2003)

58. Eli, Lilly and Co.: Hybritech Inc., European Patent Application 0382 411 A2 (1990)

59. Shepherd, T.A., L.N. Jungheim, D.L. Meyer and J.J. Starling: A novel targeted delivery system utilizing a cephalosporin-oncolytic prodrug activated by an antibody beta-lactamase conjugate for the treatment of cancer. *Bioorg Med Chem Lett* 1, 21-26 (1991)

60. Meyer, D.L., L.N. Jungheim, K.L. Law, S.D. Mikolajczyk, T.A. Shepherd, D.G. Mackensen and J.J. Starling: Site-specific prodrug activation by antibody-beta-lactamase conjugates: regression and long-term growth inhibition of human colon carcinoma xenograft models. *Cancer Res* 53, 3956-3963 (1993)

61. Vrudhula, V.M., H.P. Svensson and P.D. Senter: Cephalosporin derivatives of doxorubicin as prodrugs for activation by monoclonal antibody-beta-lactamase conjugates. *J Med Chem* 38, 1380-1385 (1995)

62. Meyer, D.L., K L. Law, J.K. Payne, S.D. Mikolajczyk, H. Zarrinmayeh, L.N. Jungheim, J.K. Kling, T.A. Shepherd and J.J. Starling: Site-specific prodrug activation by antibody-beta-lactamase conjugates: Preclinical investigation of the efficacy and toxicity of doxorubicin delivered by antibody directed catalysis. *Bioconjugate Chem* 6, 440-446 (1995)

63. Satchi-Fainaro, R., H. Hailu, J.W. Davis, C. Summerford and R. Duncan: PDEPT: Polymer-Directed Enzyme Prodrug Therapy. 2. HMPA copolymer-beta-lactamase and HMPA copolymer-C-Dox as a model combination. *Bioconjugate Chem* 14, 797-804 (2003)

64. Vrudhula, V.M., H.P. Svensson and P.D. Senter: Immunologically specific activation of a cephalosporin derivative of mitomycin C by monoclonal antibody b-lactamase conjugates. *J Med Chem* 40, 2788-2792 (1997)

65. Wani, M.C., H.L. Taylor, M.E. Wall, P. Coggon and A.T, McPhail: Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. *J Am Chem Soc* 93, 2325-2327 (1971)

66. Ringel, I. and S.B. Horwitz SB: Studies with RP 56976

(taxotere): a semisynthetic analogue of taxol. *J Natl Cancer Inst* 83, 288-291 (1991)

67. Vrudhula, V.M., D.E. Kerr, N.O. Siemers, G.M. Dubowchik and P.D. Senter: Cephalosporin prodrugs of paclitaxel for immunologically specific activation by L-49-sFv-beta-lactamase fusion protein. *Bioorg Med Chem Lett* 13, 539-542 (2003)

68. Beeson, C., J.E. Butrynski, M.J. Hart, C. Nourigat, D.C. Matthews, O.W. Press, P.D. Senter and I.W. Bernstein: Conditionally cleavable radioimmunoconjugates: A novel approach for the release of radioisotopes from radioimmunoconjugates. *Bioconjugate Chem* 14, 927-933 (2003)

69. Kerr, D.E., V.M. Vrudhula, H.P. Svensson, N.O. Siemers and P.D. Senter: Comparison of recombinant and synthetically formed monoclonal antibody-beta-lactamase conjugates for anticancer prodrug activation. *Bioconjugate Chem* 10, 1084-1089 (1999)

70. Nicholson, D.W., A. Ali, N.A. Thornberry, J.P. Vaillancourt, C.K. Ding, M.Gallant, Y. Gareau, P.R. Griffin, M. Labelle, Y.A. Lazebnik, N.A. Munday, S.M. Raju, M.E. Smulson, T.Yamin, V.L. Yu and D.K. Miller: Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. *Nature* 376, 37-43 (1995)

71. Lazebnik, Y.A., S.H. Kaufmann , S. Desnoyers, G.G. Poirier and W.C. Earnshaw: Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE. *Nature* 371, 346-347 (1994)

72. Sun, X.M., M. MacFarlane, J. Zhuang, B.B. Wolf, D.R. Green and G.M. Cohen: Distinct caspase cascades are initiated in receptor-mediated and chemical-induced apoptosis. *J Biol Chem* 27, 5053-5060 (1999)

73. Zou, H., Y. Li, X. Liu and X. Wang: An APAF-1.cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. *J Biol Chem* 274, 11549-11556 (1999)

74. Watabe, M., K. Machida and H. Osada: MT-21 is a synthetic apoptosis inducer that directly induces cytochrome c release from mitochondria. *Cancer Res* 60, 5214-5222 (2000)

75. Omura, S., K. Matsuzaki, T. Fujimoto, K. Kosuge, T. Furuya, S. Fujita and A. Nakagawa: Structure of lactacystin, a new microbial metabolite which induces differentiation of neuroblastoma cells. *J Antibiot* (Tokyo) 44, 117-118 (1991)

76. Fenteany, G., R.F. Standaert, W.S. Lane, S. Choi, E.J. Corey and S.L. Schreiber: Inhibition of proteasome activities and subunit-specific amino-terminal threonine modification by lactacystin. *Science* 268, 726-731 (1995)

77. Pagano, M., S.W. Tam, A.M. Theodoras, P. Beer-Romero, G. Del Sal, V. Chau, P.R. Yew, G.F. Draetta and M. Rolfe: Role of the ubiquitin-proteasome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27. *Science* 269, 682-685 (1995)

78. Verma, I.M., J.K. Stevenson, E.M. Schwarz, D. Van Antwerp and S. Miyamoto: Rel/NF-kappa B/I kappa B family: intimate tales of association and dissociation. *Genes Dev* 9, 2723-2735 (1995)

79. Li, B. and Q.P. Dou: Bax degradation by the ubiquitin/proteasome-dependent pathway: involvement in tumor survival and progression. *Proc Natl Acad Sci USA* 97, 3850-3855 (2000)

80. Dou, Q.P. and B. Li: Proteasome inhibitors as potential novel anticancer agents. *Drug Resist Updat* 2, 215-223 (1999)

81. Almond, J.B. and G.M. Cohen: The proteasome: a novel target for cancer chemotherapy. Leukemia 16, 433-443 (2002)

82. Dou, Q.P. and R.H. Goldfarb: Bortezomib (millennium pharmaceuticals). IDrugs 5, 828-834 (2002)

83. Adams, J.: Potential for proteasome inhibition in the treatment of cancer. *Drug Discov Today* 8, 307-315 (2003)

84. Kane, R.C., P.F. Bross, A.T. Farrell and R. Pazdur: Velcade: U.S. FDA approval for the treatment of multiple myeloma progressing on prior therapy. *Oncologist* 8, 508-513 (2003)

85. Kummer, J.L., P.K. Rao and K.A. Heidenreich Apoptosis induced by withdrawal of trophic factors is mediated by p38 mitogen-activated protein kinase. *J Biol Chem* 272, 20490-20494 (1997)

86. Sanchez-Prieto, R., J.M. Rojas, Y. Taya and J.S. Gutkind: A role for the p38 mitogen-acitvated protein kinase pathway in the transcriptional activation of p53 on genotoxic stress by chemotherapeutic agents. *Cancer Res* 60, 2464-2472 (2000)

87. Buchdunger, E., J. Zimmermann, H. Mett, T. Meyer, M. Muller, B.J. Druker and N.B. Lydon: Inhibition of the Abl protein-tyrosine kinase in vitro and *in vivo* by a 2-phenylaminopyrimidine derivative. *Cancer Res* 56, 100-104 (1996)

88. Chin, J.R., G. Murphy and Z. Werb: Stromelysin, a connective tissue-degrading metalloendopeptidase secreted by stimulated rabbit synovial fibroblasts in parallel with collagenase. Biosynthesis, isolation, characterization, and substrates. *J Biol Chem* 260, 12367-12376 (1985)

89. Hojilla, C.V., F.F. Mohammed and R. Khokha: Matrix metalloproteinases and their tissue inhibitors direct cell fate during cancer development. *Br J Cancer* 89, 1817-1821 (2003)

90. Wilhelm, S.M., I.E. Collier, A. Kronberger, A.Z. Eisen,

B.L. Marmer, G.A. Gran, E.A. Bauer and G.I. Goldberg: Human skin fibroblast stromelysin: structure, glycosylation, substrate specificity, and differential expression in normal and tumorigenic cells. *Proc Natl Acad Sci USA* 84, 6725-6729 (1987)

91. Sternlicht, M.D. and Z Werb: Neutrophil elastase and cathepsin G. In: Extracellular matrix, anchor, and adhesion proteins. Eds: Kreis T, Vale R. Oxford University Press, Oxford, England. 543–545 (1999)

Key Words: Beta-lactam, Antibiotics, Apoptosis, Chemotherapy, DNA damage, Cancer prevention, Drug discovery, Review

Send correspondence to: Q. Ping Dou, Ph.D., The Prevention Program, Barbara Ann Karmanos Cancer Institute, and Department of Pathology, School of Medicine, Wayne State University, 4100 John R Road, Detroit, MI 48201, Tel: 313-966-0641, Fax: 313-993-0193, E-mail: doup@karmanos.org