

Pharmacology and Therapeutic Applications of Eneidyne Antitumor Antibiotics

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Abstract: The natural compounds that interfere with cellular DNA such as enediyne antitumor antibiotics might be important chemotherapeutic agents for the treatment of cancer. In this article, the pharmacology and anticancer activity of the enediyne antitumor agents that are approved for clinical use and undergoing pre-clinical or clinical evaluation are reviewed. Most enediyne compounds have shown potent activity against the proliferation of various cancer cells, including cells that display resistance to other chemotherapeutic drugs. Eneidyne derivatives, such as an immunoconjugate composed of an enediyne compound and monoclonal antibody, reveal stronger activity and selectivity for human cancer cells. The mechanism underlying the anticancer activity of these enediyne antitumor agents may mainly lie in their generation of DNA double-strand breaks. Increasing evidence shows that the enediyne-induced DNA double-strand breaks can engage the activation of DNA damage response proteins, arresting cell cycle progression and eventually leading to apoptotic cell death. Continued investigation of the mechanisms of action and development of new enediyne derivatives and conjugates may provide more effective therapeutics for cancer treatments.

Keywords: Natural product, enediyne antibiotic, DNA cleavage, anticancer activity, cell cycle arrest, apoptosis, polymer-protein, immunoconjugates, cancer therapy.

INTRODUCTION

The enediyne antitumor antibiotics are produced by certain microorganisms. They represent a family of natural products with potent antitumor and antimicrobial activities, such as neocarzinostatin (NCS) [1, 2], calicheamicins (CAL) [3-6], esperamicins (ESP) [7, 8], dynemicins (DYN) [9, 10], lidamycin (LDM) [11-13], kedarcidin [14-16] and so on. These enediyne antibiotics are some of the most potent antitumoral agents and have unique molecular architecture, intri-

models, they also markedly inhibit the growth of transplantable cervical carcinoma HeLa, leukemia P388, L-1210 and melanoma B16 with the exception of NCS (Table 1) [21-28].

The enediyne antibiotics have been divided into two sub-families, including 9-membered cyclic enediynes such as NCS, kedarcidin, LDM, maduropeptin [29] and N1999A2 [30-32], and 10-membered cyclic enediynes such as CAL, ESP, DYN, and shishijimicins A-C [33] (Fig. 1). 9-membered cyclic enediynes are composed of the chromo-

Table 1. Producing Strains and Anticancer Activities of Eneidyne Anticancer Antibiotics

Agent	Producing Strain	<i>In Vitro</i> IC ₅₀ (nM)	<i>In Vivo</i> ID ₅₀ (µg/kg)	References
Neocarzinostatin	<i>Streptomyces carzinostaticus</i>	225~900	380	[21-23]
Lidamycin	<i>Streptomyces globisporus C-1027</i>	0.01~0.5	0.25~0.5	[20, 24]
Kedarcidin	<i>Actinomycete strain L585-6</i>	1	2~3.3	[14, 16]
Calicheamicins	<i>Micromonospora echinospora ssp</i>	6~9	0.5~1.5	[5, 25]
Esperamicins	<i>Actinomadura verrucosospora</i>	0.3~8.3	0.1~0.2	[26, 27]
Dynemicins	<i>Micromonospora chersina M956-1</i>	0.9~10	30~60	[9, 28]

IC₅₀, half-inhibiting concentration; ID₅₀, half-inhibiting dosage.

cate mechanisms of action and remarkable biological activities [17-19]. Most of the enediyne antibiotics have rapid and strong activities against cancer cells, and exhibit much higher antitumor activity relative to widely used chemotherapy drugs such as adriamycin [20]. *In vitro* experiments show that the half-inhibiting concentration of the enediyne antibiotics is approximately 1~100 µg/ml. In animal

phore and apoprotein with noncovalent binding. The enediyne core of the chromophore is located in the center of the pocket and other substituents are arranged around the core. The chromophore is the anticancer part, but the free chromophore is labile. Although the apoprotein is inactive in cleavage of DNA, it plays an important role in drug action by stabilizing the labile chromophore. It is attractive to speculate that the apoprotein is resistant to proteases, protects the chromophore from deactivation and may serve to deliver the enediyne to intracellular target DNA [34]. Only N1999A2 is a non-protein 9-membered cyclic enediyne antibiotic and is

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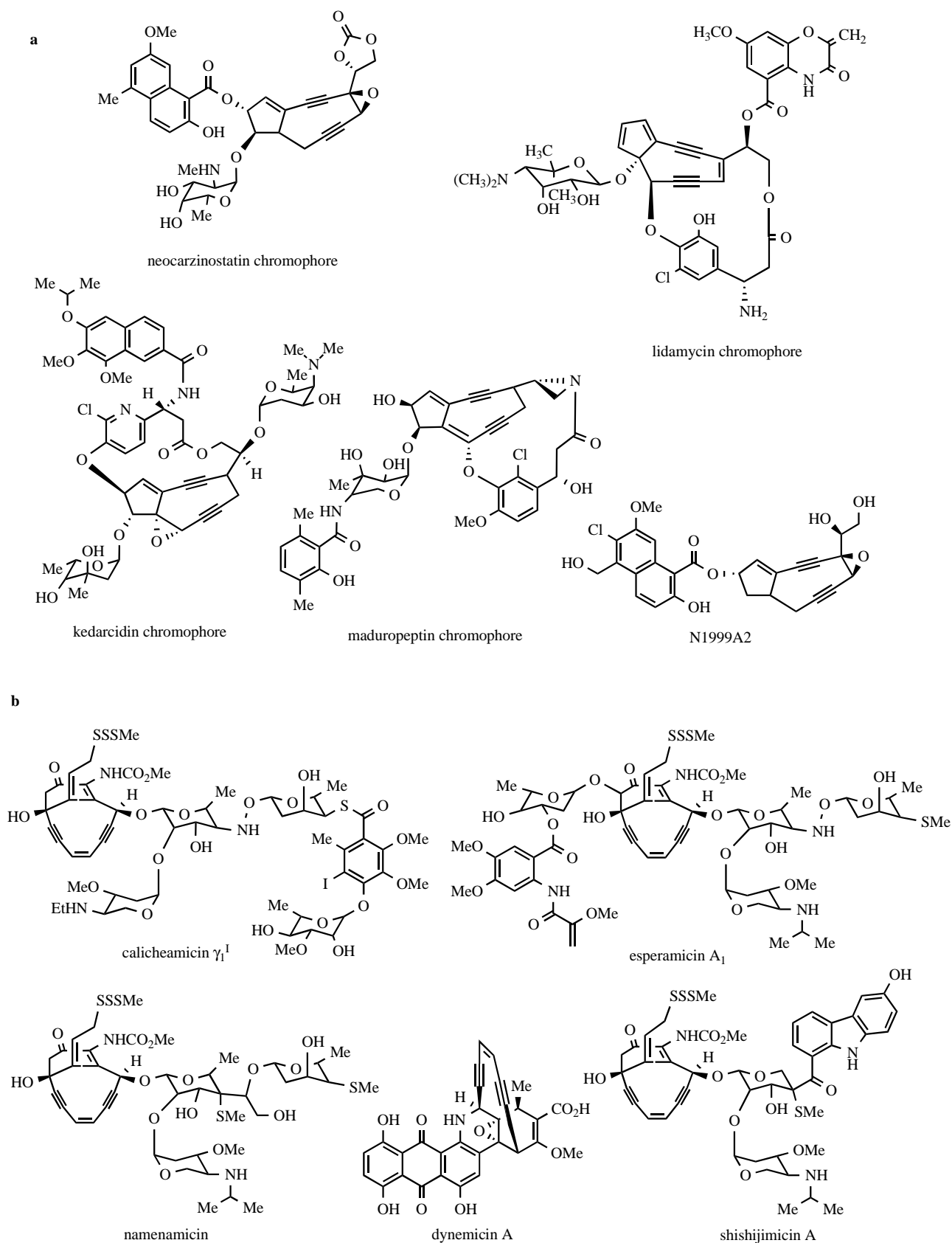


Fig. (1). The structures of the natural enediyne antitumor antibiotics. **a**, the 9-membered family; **b**, the 10-membered family.

stable in nature [35]. The structures of 10-membered cyclic enediynes do not contain an apoprotein and are more stable than those of 9-membered cyclic enediynes.

In present review, we attempt to recapitulate the pharmacology and potential applications of enediyne antitumor antibiotics.

MECHANISM OF ACTION**DNA Cleavage**

The enediyne anticancer antibiotics represent one class of DNA damaging agents expected to be exquisitely sensitive to alterations in DNA structure and dynamics. They contain

either DNA intercalating groups (such as DYN) or DNA minor groove binding function (such as CAL and NCS). The biological actions of these molecules are a result of at least three important functional domains. Each molecule comprises 1) an assemblage that consists of an enediyne moiety; 2) a delivery system that conveys the enediyne moiety to its DNA target; and 3) a triggering device that, when suitably

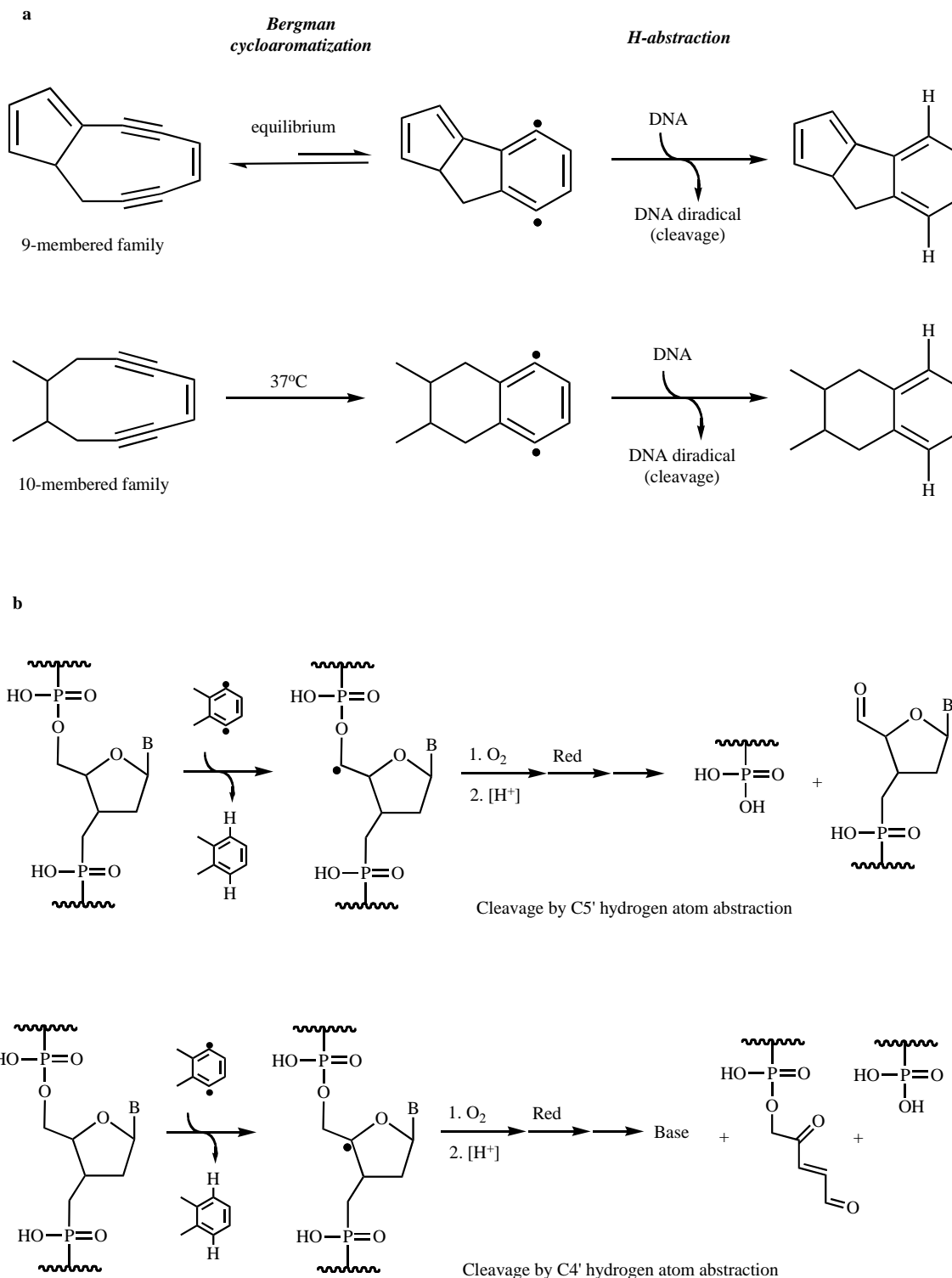


Fig. (2). Mechanism of action of enediyne anticancer antibiotics (a). DNA cleavage initiated by C4' or C5' hydrogen atom abstraction (b). B: nucleobase, Red: reduction.

activated, initiates the cascade of reactions that leads to generation of the reactive chemical species [36]. The enediynes antibiotics share a common mechanism for producing DNA lesions (Fig. 2a): First, the enediynes undergo cycloaromatization reactions resulting in formation of highly reactive diradical intermediates. Secondly, these highly reactive radicals are capable of abstracting hydrogen atoms from the DNA backbone to trigger DNA damage [37].

It has been reported that enediynes cleave double-stranded DNA, causing both single and double strand cuts. The enediynes bind to the minor groove of double helical DNA at specific sites, such as predominantly sequences at 5'-TCCT-3', 5'-TTGT-3' and 5'-ATCT-3' for CAL [38, 39], 5'-GGAGCGC-3' for NCS [40], 5'-CTACTACTGG-3', 5'-AG-3', 5'-AT-3', and 5'-GC-3' sequences for DYN [41, 42], 5'-TCCT-3' for kedarcidin [43], 5'-GGT-3' for N1999A2 [35], 5'-CTC-3', 5'-TTC-3', and 5'-TTT-3' sequences for ESP [44], and at 5'-CTTTT-3', 5'-ATAAT-3', 5'-CTTTA-3', 5'-CTCTT-3', and especially 5'-GTTAT-3' for LDM [45]. The studies seem to suggest 4' hydrogen atom abstraction along with 5' hydrogen abstraction from the targeted DNA deoxyribose sugars (Fig. 2b). In the case of CAL, the diradical abstracts hydrogen atoms from duplex DNA at the C-5' position of the cytidine and the C-4' position of the three nucleotide base pairs removed on the 3' side of the complementary strand, leading to cleavage of both strands of DNA [19]. For instance, NCS is converted into a diradical that attacks the C-5' position of the deoxyribose of mainly thymidylate residues in DNA. In the ESP-mediated DNA degradation, thymidylate and deoxycytidylate residues at the C-4' and/or C-5' position are preferred cleavage sites [44]. The LDM diradical also abstracts hydrogen atoms at the C4' and C5' position of the adenine residue on the opposite strand, inducing double-strand break [45].

CAL, ESP and DYN have potent DNA breakage activity in the presence of thiol compounds and the characteristic of DNA cleavage is induction by NADPH [46, 47]. NCS requires sulfhydryl activation for the activity which results in lower selectivity and cytotoxic activity. LDM has higher DNA cleaving ability compared with other enediyne compounds such as NCS, ESP, CAL and kedarcidin. Even in the absence of thiols or reductants, LDM still induces high DNA breakage. LDM induces novel DNA interstrand cross-links and drug monoadducts under anaerobic conditions [48], which is similar to that in the center regions of large tumors.

Cell Cycle Arrest

The enediyne-caused DNA damage initiates cellular recovery mechanisms, which involve activation of DNA damage response pathways, cell cycle arrest and apoptosis. CAL-induced double-stranded breaks were repaired slowly but completely and resulted in high levels of H2AX phosphorylation and efficient cell cycle arrest [49]. In addition, the double strand break-repair deficient Mo59J cell line was hyper-sensitive to CAL. To investigate the mode of action of CAL in living cells, oligonucleotide microarrays were used to monitor its effects on gene expression across the entire yeast genome [50]. With longer CAL exposure, genes involved in chromatin arrangement, DNA repair and/or oxidative damage, DNA synthesis and cell cycle checkpoint con-

trol as well as other nuclear proteins were all differentially expressed [50]. Additionally, ribosomal proteins and a variety of metabolic, biosynthetic, and stress response genes were also altered in their expression.

LDM-induced cell cycle arrests were associated with the status of the tumor suppressor gene *p53*. LDM induced G1 arrest in *p53* wild-type MCF-7 cells at low concentrations, and caused both G1 and G2/M arrests at higher concentrations with an increase of *p53* and *p21*, and a decrease of phosphorylated retinoblastoma protein, *Cdk1* and *cyclin B1* protein levels [51]. The downregulation of *cyclin B1* by LDM in MCF-7 cells was associated with decreasing *cyclin B1* mRNA levels and promoting protein degradation. In contrast, LDM induced only G2/M arrest in *p53*-mutant MCF-7/ADR cells. LDM-induced G2/M arrest in MCF-7/ADR cells was correlated with the reduction of *cyclin B1* expression due to inducing *cyclin B1* protein degradation [51]. LDM also induced G2 arrest in *p53*-mutant human colon carcinoma HT-29 cells, which was associated with increasing phosphorylated levels of *Chk1*, *Chk1*, *Cdc25C*, *Cdc2* and expression of *Cdc2* and *cyclin B1* [52]. In addition, cytoplasmic localization of *cyclin B1* was also involved in LDM-induced G2 arrest.

NCS treatment inhibits cellular proliferation through G2 cell cycle arrest and apoptosis induction in cervical cancer HeLa, INBL, CaSki and C33A cell lines [53]. This effect was related to nuclear accumulation of *p53* protein and an increase of *p21*. The mechanism of NCS-induced G2 block is closely related to X-ray-induced G2 block [54]. Caffeine was found to act as a stimulator of the recovery of HeLa-S3 cells from the G2 phase arrest induced by NCS. When caffeine was added together with NCS, NCS-induced inhibition of DNA synthesis and mitosis was markedly reduced [55].

Cell Death

After DNA damage, cells can be arrested at some phase of the cell cycle to facilitate DNA repair or induced apoptosis. DNA damage is a critical event preceding cellular apoptosis and appears to signal cell death through the mitochondria. Prokop *et al.* [56] reported that CAL-induced apoptosis was independent of the death-receptor/ FADD-mediated signals. Moreover, CAL triggered apoptosis in a *p53*-independent manner. The cell death proceeded *via* activation of mitochondria, release of cytochrome *c* and activation of caspase-9 and -3. The execution of cell death occurred through a fully Bax-dependent mechanism. Interestingly, caspase inhibition by the pancaspase-inhibitor zVAD-fmk interfered with CAL-induced mitochondrial activation [57]. The simple enediyne mimics were less potent than CAL, presumably because they lack the oligosaccharide DNA-binding domain. The ability of the enediynes to induce apoptosis was related to their ability to make double-stranded cuts in DNA.

LDM induced human promyelocytic leukemia HL-60 cells to undergo apoptosis with morphological changes, condensation of nuclear chromatin, and a typical ladder pattern of DNA fragments [58]. The characterization of cell death induced by LDM in human hepatoma BEL-7402 cells differs from typical apoptosis [59]. LDM could markedly enhance

the expression of *c-myc* and *c-fos* and inhibit the expression of *N-ras*, and affect the distribution of the cytoskeleton in BEL-7402 cells [60]. LDM-induced apoptosis was more effective in human colorectal cancer cells with wild type p53 than those with mutant or deleted p53 [61]. Activation of the p53 signaling pathway led to caspase activation and its inhibitor VAD-fmk blocked apoptosis induced by low dose LDM through the inhibition of the mitochondrial pathway [61]. In contrast, high dose LDM caused rapid apoptosis through a more direct DNA damaging mechanism that was independent of activation of p53 and caspase and could not be blocked by a caspase inhibitor.

NCS induces apoptosis in MCF-7 cells as characterized by decreased Bcl-2 and increased Bax levels, which induce the release of cytochrome c from the mitochondria, and then activates caspase 9. Activation of caspase 9 follows and induces sequential activation of caspase 7 and caspase 6, respectively, leading to apoptosis [62]. Overexpression of Bcl-2 is shown to be protective against apoptosis induced by a variety of mechanistically diverse chemotherapeutic drugs. However, induction of Bcl-2 expression potentiated apoptosis and differentiation induced by NCS [63]. This effect was related to an increase in cellular glutathione, suggesting that reduction-activated cytotoxic drugs might be useful chemotherapeutic agents in the therapy of tumors with Bcl-2-related chemotherapeutic resistance.

THE ENEDIYNE AGENTS APPROVED FOR USE AS ANTICANCER DRUGS

NCS

NCS is the first enediyne antitumor antibiotic in clinical use for the treatments of leukemia, gastric carcinoma and pancreatic adenocarcinoma [21, 64, 65]. In clinical studies NCS is shown to be active against acute leukemia. With NCS alone 9 out of 51 patients obtained a complete remission with 9 more achieving a partial remission. To enhance antitumor activity, NCS has been tried in combination with Ara-C, daunorubicin and prednisolone [66]. Antitumor activity of NCS also has been seen in hepatoma and hematologic malignancies [67]. NCS was administered by both continuous and intermittent intravenous infusion to patients with a variety of malignant diseases [68]. Leukemic patients on intermittent therapy evidenced greater changes in bone marrow cellularity than those treated by continuous infusion.

Anorexia, nausea, and vomiting were the most frequent side effects. In phase I and phase II evaluations, dose-limiting toxicity was myelosuppression, which occurred late [69]. Myelosuppression was more pronounced in patients who had received previous chemotherapy. Acute administration of the drug was associated with rigors in approximately half the patients. Gastrointestinal side effects

were mild. Three patients had a severe acute reaction resembling anaphylaxis. Allergic reactions were more frequent with intermittent than with continuous infusions.

SMANCS

The clinical trials of NCS were hindered by anaphylactic responses due to the apoprotein. To prevent this allergic reaction, NCS has been rendered immunologically inert by coupling to styrene maleic acid-based polymer (SMANCS). A principal advantage in the use of SMANCS is the tumor-targeting mechanism through an enhanced permeability and retention effect, and the potential for a reduction or elimination of toxicity, such as a marked reduction in bone marrow toxicity normally associated with the use of NCS [70]. SMANCS was used for the treatment of hepatoma, gastric carcinoma and lung cancer [71-73], which was approved in Japan (Table 2) [74]. Since then, hepatic arterial infusion of a SMANCS/Lipiodol emulsion has been used as one of the practical treatments for advanced or recurrent hepatocellular carcinoma [72, 75].

In the first clinical evaluation of SMANCS, 86% of patients showed decreased concentrations of α -fetoprotein and 95% showed a decrease in tumour size. A subsequent multi-centre phase II study with primary hepatoma also showed a relatively high response rate (36~40%) [76]. The most successful use of SMANCS has been seen when it was administered as a patient-individualized treatment, which was dose per tumour size, and follow-up treatments were given on a need basis [77].

Statistical analysis showed that arterial infusion therapy with SMANCS/Lipiodol appears to be effective for large renal cell carcinoma without metastases in conjunction with surgery [78]. In surgical patients without metastases who underwent SMANCS/Lipiodol infusion, the 5- and 10-year survival rates were 83.0% and 75.2%, respectively. In patients with larger tumors, the survival rate at 13 years was 75% in the SMANCS/Lipiodol infusion group and 0% in the surgery group. Konno T *et al.* reported [79] a clinical evaluation of arterial infusion of SMANCS in patients with mostly unresectable hepatoma. Marked antitumor effects were shown in the decreased serum alpha-fetoprotein levels (86% of cases) and tumor size (95% of cases).

Mylotarg

The direct use of the enediyne antibiotics as antitumor drugs is generally limited due to the lack of tumor-cell specificity and extreme cytotoxicity. It is highly desirable to generate modified enediyne compounds with improved specificity and pharmacological properties. Mylotarg (CMA-676, gemtuzumab ozogamicin), the combination of CAL with an antibody that binds to CD33 antigen, was approved by the FDA in 2000 (Table 2) [80, 81]. It is the first in a new class

Table 2. Enediyne Derivative and Conjugate in Clinical Use

Enediyne Derivative	Approved	Tumor Type	References
SMANCS	Japanese Government in 1993	hepatocellular carcinoma	[74]
Mylotarg (gemtuzumab ozogamicin)	FDA in 2000	acute myeloid leukemia	[80, 81]

of antibody-targeted chemotherapy for the treatment of patients 60 years and older in first relapse with CD33-positive acute myeloid leukemia (AML) [82-84]. The mechanism of action of Mylotarg involves the drug binding to CD33 antigen on the AML cell membrane, then being internalized. At this point, ozogamicin is released from the antibody inside the lysosomes of cells which then migrates to the cell nucleus and binds to DNA, causing breaks in the double helix resulting in cell death [85].

Mylotarg effects were analyzed in the patients with relapsed AML in a phase II study [83]. After infusion, near complete saturation of CD33 antigenic sites by Mylotarg was reached for AML blasts, monocytes, and granulocytes, whereas Mylotarg did not bind to lymphocytes. Saturation levels prior to the start of the second Mylotarg treatment cycle were significantly increased compared with background levels before the start of the first cycle. After binding to the CD33 antigen, Mylotarg was rapidly internalized. A continuous renewed membrane expression of CD33 antigens can significantly increase the internalization process and thereby the intracellular accumulation of the drug. Mylotarg-induced apoptosis in myeloid cells indicated that Mylotarg is rapidly and specifically targeted to CD33 positive cells, followed by internalization and subsequent induction of cell death.

The patients treated with Mylotarg exhibited far less toxicity than patients who received standard chemotherapy. In contrast to standard chemotherapy, Mylotarg therapy did not result in hair loss, severe oral mucositis, or damage to the intestinal mucosa [86]. The incidence of all severe or NCI grade 3 or 4 treatment-emergent adverse events was at least 5% of patients. An acute infusion-related symptom complex of fever and chills, and less commonly hypotension and dyspnea, might occur within 24 h of treatment with Mylotarg [87]. Grade 3 or 4 symptoms of hypertension, hyperglycemia, and hypoxia also have been reported. Fewer infusion-related events were observed after the second dose.

INVESTIGATIONAL ENEDIYNE AGENTS IN DEVELOPMENT

LDM

LDM strongly inhibited DNA and RNA synthesis and showed extremely potent cytotoxicity toward various cancer

cells [88-90]. The half-inhibiting concentration of LDM was about 1000-fold more potent than that of mitomycin C and adriamycin in human cancer cells. LDM exhibited marked inhibition of transplantable tumors in mice, which included leukemia L1210, P388, ascites hepatoma H22, sarcoma 180 and melanoma Harding-Passey [20]. Moreover, in human hepatoma BEL-7402 xenografts, the growth inhibition rate by LDM was 68.7%. The inhibition rate of tumor growth by LDM was higher than that of mitomycin C at the tolerated dose [90].

LDM was highly potent in suppressing angiogenesis at a minimum effective dose. LDM blocked bFGF binding to its receptor, and inhibited the formation of the bFGF-receptor immune complex [91]. LDM markedly inhibited pulmonary metastasis of Lewis carcinoma in mice. At equitoxic dosage, LDM was more effective than mitomycin for the inhibition of metastatic foci [92]. LDM decreased the expression of MMP-9 and increased the expression of its inhibitor TIMP-1. LDM showed anti-invasive activity by inhibiting the production of type IV collagenase whilst enhancing the production of tissue inhibitor metalloproteinase [93]. LDM inhibited growths of subcutaneous, orthotopic, and intra-hepatic transplanted tumors and hepatic metastases of murine colon carcinoma 26 [94]. LDM may be a new potent anti-angiogenesis agent with markedly anti-metastatic activity.

LDM in combination with cisplatin had synergetic effects on the proliferation of BEL-7402 cells, and produced internucleosomal DNA fragmentation with a decrease of Bcl-2 expression [95]. Distamycin A enhanced LDM-induced apoptosis and cytotoxicity with amplification of DNA cleavage [96, 97]. Moreover, a quinacrine-netropsin hybrid molecule potentiated the cytotoxicity and apoptosis induced by LDM with caspase-3 activation, and DNA ladder formation in HL-60 cells [98]. Furthermore, inhibition of p38 MAPK by SB203580 not only attenuated LDM-induced G2 arrest in HT-29 cells, but also potentiated LDM-induced apoptosis [99]. Combination of LDM with an anticancer agent, DNA-binding ligand and MAPK inhibitor may represent a new strategy for cancer therapy.

Immunoconjugates

Antibody-targeted chemotherapy involves the use of a cytotoxic agent chemically linked to a monoclonal antibody (MAb) that specifically recognizes a tumor-associated anti-

Table 3. Eneidiyne Derivative and Conjugate in Development

Eneidiyne Derivative	Molecular Target	Tumor Type	References
3G11-LDM	type IV collagenase	hepatoma, colorectal carcinoma	[100, 101]
Fab-LDM	tumor-specific antigen	hepatoma BEL-7402	[102]
VH-LDP-AE	type IV collagenase	sarcoma HT-1080	[103]
CMC-544 (inotuzumab ozogamicin)	CD22	B-cell lymphoma	[104-106]
hCTM01-calicheamicin γ	MUC1 antigen	breast, ovarian carcinoma	[107, 108]
hu3S193-CalichDMH	Lewis(y) antigen	gastric, colon, prostate carcinomas	[109]
138H11-Camtheta	γ -glutamyltransferase	metastasized renal cell carcinoma	[110]
A7-NCS	tumor-specific antigen	human gastric, colorectal, pancreatic carcinoma	[111]
chA7Fab-NCS	tumor-specific antigen	human pancreatic carcinoma	[112, 113]

gen. MAb specifically delivers the cytotoxic agent to tumor cells, and maximizes its antitumor effect and minimizes its normal tissue exposure, resulting in an improved therapeutic index. Although the enediyne antitumor antibiotics have seen limited use as clinical drugs, various MAB-enediyne conjugates have shown clinical promise and success in targeted cancer chemotherapy (Table 3) [100-113].

MAB-LDM

MAB-LDM conjugates have high potency against tumors with low toxicity and show exceptional promise as “warhead” drug candidates. The conjugate 3G11-LDM against type IV collagenase displays extremely potent cytotoxicity compared with free LDM [100]. 3G11 showed positive immunoreactivity in most cases of colorectal carcinoma, and negative immunoreactivity in the adjacent non-malignant tissues. 3G11-LDM remarkably suppresses the growth of hepatoma 22 and increases the survival time of tumor bearing mice. Moreover, the antitumor efficacy and the survival time of the conjugate were higher than those of free LDM or 3G11 [114].

Shao *et al.* [88] reported that two immunoconjugates were prepared using various methods for linking LDM to MAb: (1) a direct conjugate was made by linking LDM to MAb; (2) an assembled conjugate was made by linking and reconstitution steps, in which the apoprotein was conjugated to mAb and the chromophore was added to the MAB-apoprotein conjugate. The cytotoxicity of the assembled conjugate is much stronger than that of the direct conjugate. *In vivo* the assembled conjugate selectively and highly inhibited tumor growth [115]. The Fab-LDM conjugate also showed selective cytotoxicity against cancer cells. Fab-LDM displayed higher specificity in biodistribution and exerted stronger inhibitory effects on the growth of established tumor xenografts [100, 116]. The survival time of tumor-bearing mice was increased by Fab-LDM treatment compared with free LDM [117, 118].

In order to reduce the molecular size of the immunoconjugate, a recombinant fusion protein of single-chain Fv and LDM apoprotein (scFv-LDP) was prepared through genetic engineering, and an assembled fusion protein scFv-LDM was obtained by adding the chromophore to scFv-LDP [119]. scFv-LDM significantly reduced the activity of type IV collagenase and inhibited cell invasion in highly metastatic human lung carcinoma PG cells. scFv-LDM also showed extremely potent cytotoxicity in PG cells. The engineered and assembled fusion protein scFv-LDM was a highly effective anticancer immunoconjugate with a much smaller molecular size than those ever reported previously [120].

MAB-CAL

Mylotarg is the first clinically validated cytotoxic immunoconjugate for the treatment of elderly patients with relapsed acute myeloid leukaemia. Hereafter, a number of tumour-targeted conjugates of CAL linked to MAb against various tumor targets are being explored for their therapeutic applications [121]. A similar conjugate CMC-544 was evaluated in clinical trials in patients with B-cell non-Hodgkin's lymphoma [104]. CMC-544 is a CD22-specific immunoconjugate of CAL and MAB that binds human CD22

with high affinity and causes potent cytotoxic activity against malignant CD22⁺ B cells [122, 105]. CMC-544 prevented the establishment of subcutaneous human B-cell lymphoma xenografts and also caused regression of established small and large B-cell lymphoma xenografts in nude mice [122, 105]. CMC-544 allowed long-term survival of mice with systemically disseminated B-cell lymphoma. These results support the clinical application of CMC-544 as a targeted chemotherapy in the treatment of CD22⁺ B-lymphoid malignancies.

The conjugate CMB-401 consisted of an engineered MAB hCTM01 against the MUC1 antigen, expressed on many solid tumors of epithelial origin, covalently bound to CAL [108]. CMB-401 showed targeted killing of MUC1-expressing cells *in vitro* and produced pronounced dose-related antitumor effects over an 8-fold dose range against a MUC1-expressing, ovarian xenograft tumor (OvCar-3). CMB-401 was highly active in the models in single and multiple dose regimens and gave complete regressions at the highest doses examined with good overall therapeutic ratios. CMB-401 also gave good antitumor effects at similar doses with a cisplatin-resistant MUC1-expressing cell line.

The conjugate hu3S193-CalichDMH of CAL and humanized antibody hu3S193 recognizing Lewis(y) (Le(y)) antigen could eliminate Le(y) expressing carcinomas [109]. hu3S193-CalichDMH caused selective tumor growth inhibition. *In vitro* the efficacy of hu3S193-CalichDMH was qualitatively dependent on the expression of Le(y) and on the sensitivity of the tumor cells to CAL. *In vivo* hu3S193-CalichDMH inhibited tumor growth in three separate models, causing regression and growth arrest of gastric carcinoma N87, prostate carcinoma LNCaP and colon carcinoma LOVO xenografts. Thus, the selectivity and efficacy of hu3S193-CalichDMH against Le(y)⁺ tumors supported additional evaluation of this conjugate for clinical application.

The effective treatment of metastasized renal cell carcinoma (RCC) remains one of the major challenges in urological oncology, because RCC has been resistant to all conventional experimental therapeutics. The conjugate of MAb 138H11 and CAL (Cam θ) was highly toxic to the Caki-1 RCC cells. *In vivo* 138H11-Cam θ was very effective in reducing tumor size and preventing or significantly delaying the regrowth of residual tumor cells, in contrast to the controls. This tumor-inhibitory effect was due to specific targeting by 138H11 to the RCC. In addition, the MAb itself also stimulated the local immune response [110]. 138H11-Cam θ holds promise for treatment of RCC in small metastases and residual tumor cells.

MAB-NCS

A7-NCS is a conjugate of NCS and MAb A7 by a disulfide linkage and is against human colonic and gastric cancers. The anticancer effect of A7-NCS was stronger than that of free NCS. In clinical trials, A7-NCS decreased liver metastasis in size and survival rate for the A7-NCS treated patients was higher than that of patients treated with conventional chemotherapy [123]. Furthermore, A7-NCS appeared to be superior to conventional chemotherapy and allowed for a longer survival time for patients with liver metastasis from colorectal cancer. However, Human anti-mouse antibody

(HAMA) was detected in all the A7-NCS-treated patients [124].

In order to decrease HAMA, A7-NCS was replaced by Fab fragments of chimeric Mab A7 conjugated to NCS (chA7Fab-NCS). chA7Fab-NCS was administered to seven patients with colonic cancer [125], and the results showed that chA7Fab-NCS was more rapidly cleared than A7-NCS. chA7Fab-NCS did not elicit HAMA in two of seven evaluated patients. chA7Fab-NCS elicited low levels of HAMA in the other five patients. In contrast, A7-NCS elicited high levels of HAMA in all patients tested. Anti-isotype HAMA was not seen in seven evaluated patients tested with chA7Fab-NCS, while A7-NCS elicited high levels in all patients tested.

CONCLUSIONS

The enediyne antitumor antibiotics are the masterpieces of "natural" ingenuity and share a common mechanism for producing DNA lesions in form a diradical species which abstracts deoxyribose hydrogen atoms when situated in the minor groove. They also block cell cycle progression and induce apoptotic cell death and show extreme cytotoxicity to various human cancer cell lines. The potent anticancer activity, the attractive mechanism of action, and the sequence specificity of the enediyne antibiotics make these compounds of special interest for the development of antitumor agents. Several of the naturally occurring members of the enediyne family of antibiotics have entered clinical trials, LDM specifically is currently undergoing clinical trials in China.

In practice, with the enediyne antitumor antibiotics it was difficult to prevent tumor cell growth without causing non-specific side effects, particularly for the common solid tumors. Thus, innovative drug-delivery systems are being designed to guide enediyne drugs more precisely to tumor cells and away from sites of toxicity, and/or to maintain drugs at a therapeutic concentration over long periods of time. The polymer-conjugated and antibody-linked enediyne drugs (such as SMANCS and Mylotarg) exhibited to improve an *in vivo* half-life of biological activity that was longer than those of the parental enediyne drugs. It is interesting that such drugs accumulated in the tumor much more than did the parent enediyne compounds, and thus exhibited greatly improved antitumor properties in animals and humans. Recently, polymer-protein conjugates and immunoconjugates are being used routinely as anticancer therapeutics, and are being developed as components of combination therapies.

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ABBREVIATIONS

CAL = Calicheamicin
DYN = Dynemicin

ESP = Esperamicin
HAMA = Human anti-mouse antibody
LDM = Lidamycin
MAB = Monoclonal antibody
NCS = Neocarzinostatin
SMANCS = Styrene maleic acid neocarzinostatin

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