Development of Anticancer Prodrugs and Tumor Specific Adjuvant Prodrugs for Chemotherapy

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Abstract

Despite the advances made in the past few decades in cancer chemotherapy, many conventional anticancer drugs display relatively poor selectivity for cancer cells. The nonselectivity of anticancer drugs and the development of anticancer drug resistance have been recognized as serious limitations in their clinical usefulness. Therefore, a major challenge in cancer chemotherapy is the development of new anticancer agents with improved selectivity for tumor cells as well as the prevention of the host cell resistance, both of which result in the improvement of therapeutic effect against cancer cells.

Cyclophosphamide (CP), a widely used anticancer agent, is a prodrug that is activated by hepatic microsomal mixed-function oxidase (MFO) catalyzed C_4-hydroxylation. The resulting 4-hydroxycyclophosphamide (4-OH-CP) is converted to the ring-opened tautomer to aldophosphamide (Aldo) which subsequently undergoes a base-catalyzed β-elimination to generate cytotoxic phosphoramide mustard (PDA) and acrolein. The cytotoxic activity of CP is attributed to the aziridinium ion species derived from PDA that cross-links interstrand DNA.

Although CP has shown a relatively high oncotoxic selectivity and therapeutic efficacy, a number of side effects are accompanied largely due to the liberated acrolein, a byproduct of the β-elimination. Among the side effects, the most serious problem is the urotoxic effect, i.e., hemorrhagic cystitis which is open dose-limiting in CP treatment.

Development of resistance to anticancer agents is currently one of the major problems in cancer chemotherapy. The use of CP is also associated with this kind of problem which results in the lack of its efficiency. One of the primary deactivation pathways of CP is the oxidation of Aldo to carboxyphosphamide, which is catalyzed by aldehyde dehydrogenase (AlDH). The induction of AlDH activity has been demonstrated

to be an acquired resistance mechanism of CP in certain murine tumor cells.

Benzyl phosphoramide mustard (Benzyl PDA), 2,4-difluorobenzyl phosphoramide mustard (2,4-Difluorobenzyl PDA) and methyl phosphoramide mustard (Methyl PDA) were examined as lipophilic, chemically stable prodrugs of PDA. These phosphorodiamidic esters were designed to undergo biotransformation by hepatic microsomal enzymes to produce PDA without generation of acrolein and to be active against CP-resistant tumor cells. The rate of formation of alkylating species, viz., PDA, from these prodrugs and their *in vitro* cytotoxicity against mouse embryo Balb/c 3T3 cells were comparable to or better than that of CP. Preliminary antitumor screening against L1210 leukemia in mice, however, suggested that these prodrugs are devoid of any significant antitumor activity [Increase of life span (ILS) < 25 %] *in vivo*.

Several N^3-methyl-4-(alkylthio)cyclophosphamide derivatives were developed as chemically stable, biooxidative prodrugs of 4-OH-CP, the activated species of CP. All the prodrugs underwent N-demethylation in a time dependent manner when incubated with rat hepatic microsomes, which resulted in the formation of formaldehyde as well as alkylating species. Among the prodrugs, N^3-methyl-4-(diethyldithiocarbamoyl)CP showed exceptional *in vitro* cytotoxicity against 3T3 cells as well as against a panel of human tumor cell lines, with a paticular sensitivity to leukemia and small cell lung cancer cell lines. Preliminary *in vivo* antitumor evaluation against L1210 leukemia in mice showed that all of the prodrugs were active (ILS >25 %). N^3-Me-4-DDTC-CP was the most active compound with the ILS of 100 % and one out of four long term survivor (30 days). Further studies exploring the clinical usefulness of N^3-methyl-4-(alkylthio)CPs are required to evaluate whether these prodrugs can be useful anticancer agents with uroprotective effect.

O^6-benzylguanine is a very effective inactivator of the human DNA repair protein O^6-alkylguanine-DNA alkyltransferase (AGT). This protein can significantly reduce the therapeutic effectiveness of chloroethylating anticancer drugs. Although O^6-benzylguanine shows considerable promise as an alkyltransferase inhibitor and is currently being evaluated in clinical trials, its usefulness may be limited by the lack of specificity of its uptake in tumor cells versus normal cells. Therefore, this research was designed to develop prodrugs of O^6-benzylguanine as chemotherapeutic adjuvants for inactivation of AGT in antibody-directed enzyme prodrug therapy (ADEPT) in order to achieve improved therapeutic efficacy against tumor cells.