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Heterodinucleoside Phosphates of 5-Fluorodeoxyuridine and Arabinofuranosylcytosine – New Drugs in Cancer Chemotherapy?

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Abstract. *The incidence of cancer is rapidly increasing and malignancies have become the number two cause of deaths in the Western world after cardiovascular diseases. In particular, colon cancer represents one of the most frequent types of malignancy. Chemotherapy is, in addition to surgery and irradiation, still one of the main treatment options against this group of diseases. Here, several chemotherapeutic treatment modalities and anticancer compounds for the treatment of colon cancer are reviewed. In particular, a newer group of heterodinucleoside phosphates (dimers), consisting of two well known antimetabolites (5-FdUrd (5-Fluorodeoxyuridine) and Ara-C (Cytarabine)), are presented. These dimers were evaluated in several studies and might offer an additional option for the treatment of various malignancies, in particular colon carcinomas. The results are summarized in detail, as these dimers might have some significant advantages when compared with conventional regimens; they might be administered orally and might constitute an alternative treatment option for resistant tumors.*

The use of anticancer drugs as part of the treatment strategy has greatly improved the overall prognosis of cancer. Over the years, continued research in the basic as well as applied sciences has led to a greater understanding of the differences between cancer cells and normal cells. Such differences provide insight into the basis for activation of growth pathways and inactivation of growth control pathways/mechanisms of genetic alteration of oncogenes and cancer suppressor genes, thus providing improved understanding of the cause and pathogenesis of many forms of cancer. However, only a few treatments have been based on this new frontier of cancer biology – to date, most anticancer drugs are nonselective in their mechanism of action and are directed at essential components or metabolic pathways that are crucial to both malignant and normal cells.

As more knowledge accumulates about the biology of tumors and the pharmacology of anticancer agents, the use of chemotherapy will hopefully become more efficacious. In clinical practice, chemotherapy for cancer often requires a combination of drugs. The selection of standard chemotherapy combination regimens to treat individual patients is based solely on tumor histology and extent of disease (1). Therefore, understanding the clinical pharmacology of anticancer drugs is imperative for achieving optimal benefits from the use of these agents.

Three principles underlie the general approach to designing specific regimens for the treatment of cancer. These principles are: (a) drugs are more effective when used in combination, (b) drugs are more effective at higher doses, and (c) drugs should be used in conjunction with local therapies such as surgery and radiation.

Anticancer drugs in general are more effective when used in combination. The usefulness of this strategy is based on early observations made in the treatment of acute lymphocytic leukemia (2). To achieve maximum therapeutic benefit, selected drug combinations should incorporate the most active single agents known to have produced complete remissions in early clinical studies in the tumor type being treated. It is desirable to avoid an overlap of major toxicities, mechanism of action, and resistance mechanism(s). In addition, it is desirable to administer most drugs at their maximum tolerated doses with minimum time intervals between such doses. The major advantages of

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combining chemotherapeutic drugs are the promotion of additive effects or possible synergism through biochemical interactions. Another important aim is to avoid the emergence of early resistance in the tumor cell. An example of the use of biochemical interactions in selecting drug combinations is demonstrated by the administration of leucovorin to increase the binding of an active intracellular metabolite of 5-fluorouracil to its target, thymidylate synthase, thus increasing its cytotoxic effects (3).

Since micrometastases frequently develop prior to diagnosis, chemotherapy is often administered before or after local therapy with radiation or surgery. Animal and clinical experiments have shown that regimens producing the most dramatic responses in metastatic or recurrent disease have the greatest likelihood of being curative in the adjuvant setting (4).

There are a lot of experimental data supporting the use of adjuvant chemotherapy (5-7). However, while this form of therapy shows definite benefit in a subset of patients with breast cancer and colorectal cancers (8-11), this does not appear to be the case for a variety of other malignancies (12). In order to improve the efficacy of adjuvant chemotherapy, it is increasingly being investigated as neoadjuvant therapy prior to primary surgery, especially in cancer of the breast, esophagus and head and neck (13-15).

Colorectal Cancer

Colorectal cancer is a major public health problem in Western countries with the highest incidence rates reported in North America, Australia, New Zealand and Western Europe. It is the third most common cancer in men and women and the third most common cause of cancer death in both sexes. The age-specific incidence rises sharply after age 40, with 90% of cancers occurring in individuals of age 50 and older. Within the large intestine, 69% of cancers occur in the colon and 31% in the rectum. Although the specific etiology of colorectal cancer remains unknown, it is likely that the disease derives from genetic mutations in the colon epithelium, which ultimately result in the neoplastic phenotype. In some cases, genetic mutations may be inherited as germline mutations, and manifest as familial colon polyp or cancer syndromes. In other cases, somatic mutations in the colon epithelium, perhaps related to environmental or nutritional exposures, ultimately result in the formation of colon cancer. However, in most cases, adenomatous polyps are precursors to the development of invasive tumors.

Patients with inflammatory bowel disease have an increased risk of developing colorectal cancer. Carcinoma complicating ulcerative colitis is related to the duration of active disease, extent of colitis, duration of symptoms and development of mucosal dysplasia (16). The risk of developing carcinoma in those with total colitis is estimated at 10-25 times that of the general population. A similar increase in risk has been estimated for those with Crohn's disease – these patients also have an increased risk of small-bowel carcinomas. Nutritional factors have also been implicated in the development of colorectal cancer, including diets high in fat or low in fiber as well as deficiencies in vitamin D, vitamin E and selenium (17-19).

Treatment of early stage colorectal cancer. Surgery is the initial therapy of choice for localized, potentially curable colon cancer. Disease-free and overall survival following surgical resection depends primarily on the pathologic stage of the tumor. Adjuvant chemotherapy has clearly been shown to reduce the risk of recurrence and increases the likelihood of survival of patients with node-positive colon cancer. The combination of 5-fluorouracil (5-FU) and levamisole administered for 1 year postoperatively resulted in a 41% reduction in risk of recurrence and a 33% reduction in risk of death compared with no therapy (20). The results of several large randomized trials have led to the replacement of this regimen by the combination of 5-FU and leucovorin (LV) for 6 months (21-24), which should be considered the standard adjuvant regimen for patients with resected high-risk colon cancer.

Ongoing adjuvant chemotherapy trials compare standard 5-FU/LV alone to oral fluoropyrimidines, including capecitabine and the combination of tegafur and uracil (UFT). Oral fluoropyrimidines are prodrug formulations of 5-FU, which are designed to selectively achieve high concentrations of 5-FU or an active metabolite in the tumor after enzymatic conversion. Capecitabine was developed to avert the gastrointestinal toxicity associated with 5-FU. It is preferentially activated into 5-FU at the tumor site (25) and was recommended for FDA approval for the treatment of breast cancer. Clinical trials are ongoing to further define other clinical activities. Tegafur is absorbed from the small intestine and converted to 5-FU. It has demonstrated significant antitumor activity against neoplasms sensitive to 5-FU (26). UFT is a 4:1 concentration combination of uracil and tegafur. Uracil in this combination prevents the catabolism of 5-FU, by competitively inhibiting uracil dehydrogenase enzyme activity, predominantly in the tumor cells (26,27). The use of UFT also resulted in significant antitumor effects in neoplasms sensitive to 5-FU.

Treatment of metastatic colorectal cancer. 5-FU has been the cornerstone in the chemotherapeutic treatment of colorectal cancer for over 40 years. The relatively modest response rates achieved with this drug have prompted numerous evaluations of modulating agents and alternate schedules of

administration. The modulation of 5-FU by leucovorin is perhaps the most successful biochemical modulation strategy to be brought from the laboratory to the clinic. By depleting the intracellular stores of reduced folates, the addition of leucovorin results in more sustained inhibition of thymidylate synthase by fluorodeoxyuridylate and increased 5-FU cytotoxicity (28). Other attempts at improving the efficacy of 5-FU chemotherapy include the addition of cisplatin, α-Interferon and N-(phosphonacetyl)-L-aspartate (PALA) (29).

The oral fluoropyrimidines were designed to facilitate protracted drug exposure without the need for indwelling catheters and infusion pumps. 5-FU cannot be administered orally due to rapid metabolism to inactive metabolites by dihydropyrimidine dehydrogenase (DPD) located in the gut wall and liver. To circumvent this, 5-FU prodrugs that are not substrates for DPD have been designed or 5-FU has been administered with specific DPD inhibitors. Both strategies have been effective in permitting delivery of pharmacologically active concentrations of 5-FU into systemic circulation.

Capecitabine is an oral fluoropyrimidine carbamate that is converted to 5-FU by a three-step process in the liver and tumor tissues. It is currently indicated for first-line treatment of colorectal cancer when fluoropyrimidine monotherapy is preferred.

UFT consists of uracil plus tegafur in a 4:1 molar ratio. UFT given with leucovorin (LV) is known as Orzel. Tegafur is a 5-FU prodrug, uracil competitively inhibits DPD, and LV modulates thymidylate synthase (TS) – this results in prolonged therapeutic drug levels similar to continuous infusion of 5-FU.

Irinotecan (CPT-11), a topoisomerase-1 inhibitor, was initially approved for patients whose tumors progress following treatment with 5-FU. The standard of care for the front-line treatment of metastatic colorectal cancer changed in March 2000 from 5-FU/LV to the three-drug combination 5-FU/LV/CPT-11, resulting in a significantly higher response rate, a longer progression-free survival and an increased overall survival compared to 5-FU/LV (30).

Among the most active drugs currently being investigated for treatment of colorectal cancer is oxaliplatin, a diaminocyclohexane (DACH) platinum, that has produced objective tumor regression in 10% of patients with 5-FU refractory disease and in 24% of previously untreated patients (31,32).

General Mechanism of Action

The mechanism of action of anticancer drugs involves the alteration of signal pathways in cancer cells. In most cases, the signals are also affected in normal dividing cells. Many of the antimetabolites (*e.g.*, 5-fluorouracil, methotrexate,

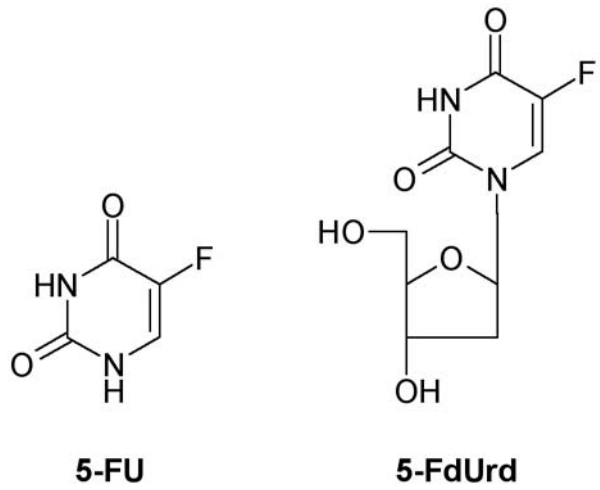

Figure 1. *Structural formulae of 5-fluorouracil (5-FU) and 5-fluoro-2'-deoxyuridine (5-FdUrd).*

and 6-thioguanine) and alkylating agents (cisplatin, melphalan) require chemical or enzymatic activation intracellularly before cytotoxicity can be achieved. Thus, the presence of the required activating enzymes in any tumor type is a prerequisite for the effectiveness of such a drug.

It is obvious that for any drug to be useful it has to be present and maintained at adequate concentrations at its site of action. Therefore, physical characteristics such as plasma protein binding, route of administration, first-pass metabolism and diffusion characteristics will influence the delivery of anticancer drugs to their site of action. To produce cytotoxicity, most anticancer drugs require uptake into the cell. Both normal and cancer cells undergo the same phases during division. A genetic predisposition or environmental factors result in the dysregulation of the normal cell division, leading to a proliferative advantage for the malignant population in cancer. This is fundamentally true of most cancers, for example, a mutation or deletion in the p53 tumor suppressor gene results in the disruption of G_1- to S-phase in the cell cycle. Cells expressing normal p53 are arrested in G_1-phase in response to DNA damage secondary to cytotoxic drugs, allowing for repair of the DNA damage (33,34).

There are a number of mechanisms by which anticancer drugs result in cytotoxicity. Advances in the molecular sciences continue to increase the spectrum of mechanisms of action of new anticancer agents. Broadly, cancer chemotherapeutic agents act on cancer cells largely by interacting with DNA or its precursors, inhibiting the synthesis or function of new nucleic materials, DNA and RNA, causing irreparable damage to vital nucleic acids by intercalation (anthracyclines), alkylation (cyclophosphamide, chloroethylnitrosoureas), or enzymatic inhibition mechanisms. Other mechanisms of

cytotoxicity include targeting the proliferative process by disrupting membranes, microtubules (vinca alkaloids) and hormone receptors (antiestrogens).

Antimetabolites

Antimetabolites are generally structural analogues of naturally occurring intracellular metabolic intermediates essential for the normal function of a cell (pyrimidines or purines). Such similarities allow these drugs or their metabolites to serve as substrates for key intracellular enzymes. The substrate substitution ultimately results in the inhibition of key enzymes necessary for synthesis of folic acid, pyrimidines or purines for DNA or RNA formation in neoplastic cells. Since DNA synthesis occurs in the S-phase of cell division, most antimetabolites are termed S-phase-specific in their action. In many cases, they are best administered by prolonged infusion. The antimetabolites that are of established use and significance in oncology include folic acid analogues, *e.g.*, methotrexate (amethopterin), pyrimidine analogues (5-fluorouracil), cytarabine, purine analogs and gemcitabine, a nucleoside analog. While some of these drugs have broad applications in oncology, others are used mainly for the treatment of hematological malignancies, *e.g.*, cytarabine (Ara-C).

5-Fluoropyrimidines. The impetus for synthesis of fluorinated pyrimidines came from the observation that rat hepatomas use radiolabelled uracil more avidly than nonmalignant tissues (35). This implied that the enzymatic pathways for use of uracil, and possibly analogues of uracil, differ between malignant and normal cells and represent a possible target for antimetabolite chemotherapy. These drugs have shown the predicted biochemical action and have become very useful in the treatment of human solid tumors, including breast cancer, gastrointestinal adenocarcinomas and squamous cell carcinomas arising in the head and neck. They have invoked interest not only because of their inherent antitumor activity, but also because of their synergistic interaction with other antitumor agents, irradiation, physiologic nucleosides, and leucovorin. The chemical structures of the initial two 5-fluoropyrimidines to enter clinical trials are shown in Figure 1.

The simplest derivative, 5-fluorouracil (5-FU), is an analog of uracil with a fluorine atom substituted at the carbon-5 position of the pyrimidine ring in place of hydrogen. The fluorine atom is slightly bulkier than hydrogen but does not impede the anabolism of 5-FU. Activation to the nucleotide level is essential to the antitumor activity of this class of compounds. Following rapid transport into the cell, a significant amount of the drug is converted by ribosylation and phosphorylation reactions to three metabolites, two of which, fluorouridine triphosphate (5-FUTP) and fluorodeoxyuridine monophosphate (5-FdUMP), are known to be active. Cytotoxicity occurs subsequently to the incorporation of 5-FUTP directly into RNA, and/or the inhibition of thymidylate synthase activity by 5-FdUMP, which is enhanced by reduced folates. The latter reaction depletes the cell of thymidine triphosphate (TTP), a necessary precursor of DNA synthesis (36,37). A third mechanism of action for this drug has been proposed: the inhibition of pre-rRNA processing (an essential step for protein synthesis) by 5-FU (38). Nevertheless, since the relative contribution of each of the mentioned mechanisms is not clear, it is conceivable that specific mechanisms will be tumor-specific based on the intratumoral metabolic pattern of 5-FU. 5-FU is used in combination with other compounds in the treatment strategies of a variety of carcinomas, including colorectal cancer with or without metastases, breast cancer, hepatic tumors, head and neck cancers, carcinoma of the ovary, cervix, urinary bladder, vulva and pancreatic cancer (39).

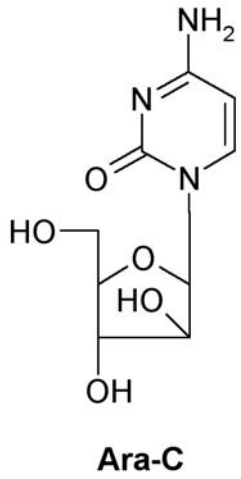

Figure 2. *Structural formula of Arabinofuranosylcytosine (Cytarabine, Ara-C).*

Several mechanisms have been hypothesized for the observed resistance to 5-FU by tumor cells. These include loss or decreased activity of the key enzyme required for its activation, increased clearance, overproduction of thymidylate synthase (acquired resistance) through gene amplification, overexpression, or mutation (40). Other mechanisms involve the use of so-called salvage pathways of purine or pyrimidine synthesis (41), which circumvent pathways of the *de novo* synthesis and as a function of DNA damage response due to the loss of p53 function in tumor cells (40,42). In attempts to circumvent resistance to 5-FU by tumor cells, a number of modulators have been used to increase the cytotoxicity of the drug including folinic acid

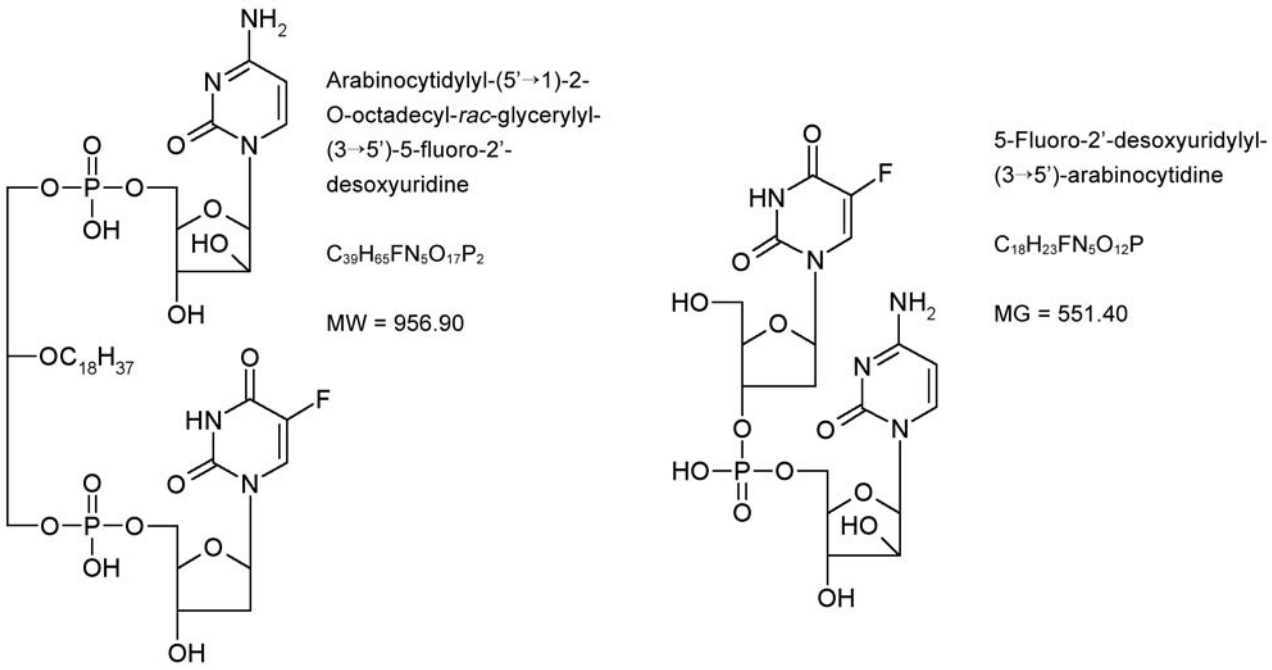

Figure 3. *5-FdUrd-Ara-C dimers.*

and eniluracil (43). 5-FU is mainly metabolized in the liver, approximately 5% of a given dose is excreted unchanged in urine up to 6 hours and a large amount is excreted as CO_2 from the lung. Toxicity includes myelosuppression, severe mucositis and diarrhea, especially in combination with leucovorin. The gastrointestinal toxicity is more severe with continuous infusion regimens. Other toxicities include alopecia, nail changes, dermatitis, acute cerebellar syndrome, cardiac toxicity and hand-foot syndrome.

The deoxyribonucleoside derivative 5-fluoro-2′-deoxyuridine (5-FdUrd) is used primarily for hepatic administration. The activity of 5-FdUrd is comparable to that of 5-FU and, due to its exceptionally high hepatic extraction, it has a marked pharmacological advantage when administered *via* the hepatic artery (44). The delivery of chemotherapy into the hepatic artery has been facilitated by the development of implantable infusion pumps. The liver is the most common site of metastases from colorectal cancer and liver metastases derive most of their blood supply from the hepatic artery. Thus, 5-FdUrd is a useful drug for hepatic arterial chemotherapy of liver metastases. Several prospective randomized trials comparing systemic fluoropyrimidine therapy with hepatic arterial infusion (HAI) have now been completed (45-50). In all studies, the response rate to HAI therapy was significantly higher than to systemic treatment. A meta-analysis of these studies has confirmed the significantly higher response rates for HAI therapy and revealed a survival advantage (51). The toxicity of HAI, once considerable, has been ameliorated with the introduction of new drug combinations and new schedules of drug administration. The most significant toxicity is jaundicing secondary to sclerosing cholangitis induced by chemotherapy. Ulceration of the gastric and/or duodenal mucosa has also been reported due to inadvertent perfusion of the mucosa of the stomach or duodenum *via* collateral branches of the hepatic artery. Approaches that appear to reduce the toxicity of HAI therapy include addition of dexamethasone to the infusate and alternating intraarterial administration of 5-FdUrd with intraarterial infusion of 5-FU.

Arabinofuranosylcytosine. Arabinofuranosylcytosine (Cytarabin, Ara-C) is an analog of 2'-deoxycytidine and a well-established anticancer agent with high activity in acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL). Ara-C acts as an analog of the physiologic nucleoside deoxycytidine and has multiple effects on DNA synthesis through inhibition of DNA polymerase-α, incorporation into DNA, or termination of DNA chain elongation (52). To be effective, the drug must be sequentially phosphorylated intracellularly to Ara-CTP by the action of deoxycytidine kinase (dC kinase) and other appropriate nucleotide kinases, such as deoxycytidine

monophosphate kinase (dCMP kinase) and nucleoside diphosphate kinase (NDP kinase). Ara-C is metabolized mainly in the liver to Ara-CTP and has a terminal elimination half-life of 0.5 to 2.5 hours. Close to 85% of the dose is excreted in the urine as metabolites. Dose-limiting toxicity is myelosuppression. Other toxicities include seizures with intrathecal administration, dermatitis, conjunctivitis, megaloblastic anemia, hepatic dysfunction, fever and pneumonitis. Ara-C is included in a large number of standard combination therapy protocols resulting in cure rates of 5-15% in AML and 30-70% in ALL, respectively. Ara-C is also used for prophylaxis and treatment of CNS leukemia and has shown activity in patients with chronic lymphocytic leukemia, non-Hodgkin's lymphoma and myelodysplastic syndrome. Furthermore, Ara-C shows activity in Hodgkin's disease and non-Hodgkin's lymphomas and, therefore, is one of the most powerful antitumor agents in the treatment of hematological malignancies. However, its major disadvantages are the short plasma half-life and rapid degradation to its inactive metabolite arabinofuranosyluracil (Ara-U), which prevents the administration of Ara-C against solid tumors and also impedes its oral application. Thus, in order to reach the tumor site of solid tumors as an active compound, Ara-C needs to be protected from early inactivation to Ara-U, which is caused by cytidine deaminase. The chemical structure of Ara-C is shown in Figure 2.

Heterodinucleoside Phosphates Containing 5-Fluorodeoxy-uridine and Arabinofuranosylcytosine

In order to enhance the cytotoxic effects of 5-FdUrd and Ara-C, a new strategy of masking nucleoside phosphates by the synthesis of amphiphilic heterodinucleoside phosphates has been developed. These dimers act as duplex drugs as they contain two active moieties with different mechanisms of action, which are activated after uptake in the target cell. This combination of powerful antitumor agents should increase the efficacy of 5-FdUrd and Ara-C and might also be able to overcome drug resistance, which still remains the major problem in cancer chemotherapy. Due to their amphiphilic structure, an enhanced cellular uptake and a different drug distribution can be expected since monophosphorylated nucleosides could be formed directly in the tumor cell after enzymatic cleavage of the dimer. Thus, the molecule would not have to pass the first phosphorylation step, which is known to be rate-limiting. Consequently, low activities of nucleoside-5'-monophosphate kinases might be circumvented by the dimers, resulting in increased antitumor effects. The amphiphilic nature of these agents provides them with pharmacokinetic and pharmacological properties that are different from the parent drugs 5-FdUrd and Ara-C. Therefore, the dimers are expected to exert improved antitumor effects even on neoplasms not sensitive to 5-FdUrd and Ara-C. Structural formulae of the most promising dimers are shown in Figure 3.

It is conceivable that, due to their strong affinity to cell membranes, the amphiphilic dimers could influence and/or perturb various signal transduction pathways, alter cell surface receptor confirmations, or influence the lateral diffusion of proteins in cell membranes. Therefore, these dimers might retain their marked cytotoxic activity in malignant cells with low numbers of nucleoside-transporting molecules (*e.g.*, CML, CLL, or lymphomas), with low kinase activities (*e.g.*, Ara-C-resistant leukemia), or, due to different cellular uptake and specific lipophilic properties, also in multidrug-resistant (MDR) tumor cells.

In 1982, Grant and Cadman examined the effect of pretreatment with 5-FdUrd on the intracellular metabolism and *in vitro* cytotoxicity of Ara-C in L1210 murine leukemia cells. 5-FdUrd treatment enhanced Ara-C accumulation and 1-beta-D-arabinofuranosylcytosine-5'-triphosphate formation and produced synergistic cytotoxicity (53). More recently, our group observed enhanced effects of a combination of 5-FdUrd and Ara-C in leukemia cells and in leukemia-bearing mice (54).

Schott and coworkers synthesized several novel lipophilic analogues of 5-FdUrd and Schwendener and coworkers incorporated them into liposomes. They found that the prodrugs incorporated into liposomes were about 10 to 30 times more active against murine colon 38 carcinoma, compared with the free drug (55). Later, Schott and coworkers decided to conjugate 5-FdUrd and Ara-C. They synthesized various amphiphilic 5-FdUrd-Ara-C heterodinucleoside phosphates in an attempt to provide a new drug combination, to circumvent resistance, and to introduce antitumor agents, which might also be administered orally. In addition, they developed a number of lipophilic conjugates of Ara-C and 5-FdUrd with long-chain fatty acids and incorporated them into liposomes to examine their cytostatic effect and possibilities of tumor cell-specific therapy (56-70).

The cytotoxic properties of the first new heterodinucleoside phosphate 5-FdUrd-NOAC, which was composed of 5-FdUrd and N(4)-octadecyl-1-beta-D-arabinofuranosylcytosine (NOAC), were recently tested in DU-145 and PC-3 human prostate cancer cells. The authors found that the compound inhibited thymidylate synthase and cell cycle progression, causing proliferation arrest and apoptosis in both cell lines (71). In addition, the dimer could circumvent drug resistance, which might be due to the release of the respective monophosphates of Ara-C and/or 5-FdUrd.

Apoptosis induction has emerged as a significant therapeutic principle for the effective elimination of cancer

cells (72,73). Thus, the intrinsic propensity to undergo apoptosis is a general determinant for chemotherapeutic sensitivity and, therefore, represents a target for pharmacological modulation (74,75). This led us to examine the apoptosis-inducing activities of these novel heterodinucleoside phosphates in a number of human tumor cell lines, resulting in significant proportions of apoptotic cells.

5-FdUrd inhibits cell proliferation by S-phase arrest, which is caused by TS inhibition (36), single-strand breaks, and DNA fragmentation (76). Cell cycle-dependent cytotoxicity of Ara-C is due to incorporation of Ara-CTP into DNA and its interaction with DNA polymerase α, resulting in a block of G1 cells at the G1-S transition (77).

Recently, Bergmann and coworkers examined the activity of fatty acid ester derivatives of Ara-C in leukemic and solid tumor cells and found a clear structure-activity relationship. The cytotoxicity of these compounds correlated with the length and – to a lesser extent – with the number of double bonds of the acyl group. The derivatives with a C18-alkyl side-chain showed the highest antiproliferative activity (78).

Our group reported on the cytotoxic effects of three novel 5-FdUrd-Ara-C dimers obtained in several drug-sensitive (CCL228, CCL227 and HT-29) as well as in highly 5-FU-resistant CCL227 human colon carcinoma cells and L1210 murine leukemia cells. The IC_{50} values were almost identical for both sensitive and resistant CCL227 cells, indicating that the heterodimers are able to overcome 5-FU resistance. We also showed that the dimers are effective in inducing apoptosis in a dose-dependent manner in both HT-29 human colon tumor and L1210 leukemia cells. In addition, we examined the *in vivo* effects in L1210 leukemia-bearing mice and found significant increases of the life span of treated animals as compared to untreated controls (79). More recently, we demonstrated that one of these dimers is able to overcome 5-FdUrd/Ara-C cross-resistance in H9 human lymphoma cells and induces dose-dependent apoptosis. Besides, this agent exerted its cytotoxicity without causing remarkable cell cycle perturbations in both sensitive and cross-resistant H9 cells (unpublished data). It can be assumed that the dimer is cleaved into the monophosphorylated monomers (5-FdUMP and Ara-CMP), resulting in sustained intracellular drug concentrations over an extended period that consequently increase the duration and magnitude of the cytotoxic effects.

Conclusion

Antineoplastic chemotherapy is not uniformly successful because many types of cancer in humans are either intrinsically resistant to treatment, acquire resistance during therapy, or because the chemotherapy cannot be successfully delivered in cytotoxic concentrations to the tumor. Mechanisms by which drug resistance can develop are, at least in part, specific to the mechanism of action of the class of agents being utilized. However, a wide variety of biochemical and physiologic phenomena has been observed that can modulate the efficacy of antineoplastic drugs. These include reduced drug uptake by the tumor cell, enhanced drug efflux, enhanced intracellular metabolism or detoxification of the chemotherapeutic agent that limits tumor cell toxicity, and many more. Increased cell proliferation and decreased cell death (by means of apoptosis) are two major processes that contribute to the progression of tumor cell growth. Consequently, new agents that can inhibit cell proliferation and/or induce apoptosis are of great therapeutic value.

We evaluated the effects of several new amphiphilic heterodinucleoside phosphates containing 5-FdUrd and Ara-C on various sensitive and resistant human tumor cell lines in comparison to the monomeric anticancer drugs 5-FdUrd and Ara-C. The findings presented here demonstrate that some of these novel agents exert even stronger antitumor effects when compared to the clinically used monomers.

The break of 5-FU, 5-FdUrd and/or Ara-C resistance observed in the growth inhibition assays may possibly be explained by the prodrug nature of the dimers, resulting in persisting intracellular drug concentrations of the monophosphorylated cleavage product and other active metabolites over longer periods compared to 5-FdUrd and/or Ara-C.

The improved effect can further be explained by the fact that the first phosphorylation step from 5-FdUrd to 5-FdUMP, catalyzed by the enzyme thymidine kinase, is the rate-limiting step. A loss in function of this enzyme leads to the development of resistance to 5-FdUrd. Therefore, the introduction of a monophosphorylated 5-FdUrd molecule could have the advantage of circumventing this first rate-limiting and resistance-causing step. Another conceivable reason for the improved potency of 5-FdUrd-Ara-C dimers over 5-FdUrd and Ara-C could be that the molecule contains two toxic moieties that may develop synergistic activities in the tumor cell.

Their unique properties might render these novel agents very promising candidates as anticancer drugs with significant cytostatic effects in both solid tumors and hematological malignancies.

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