Cancer Chemother Pharmacol (1994) 33: 281–285

Cancer Chemotherapy and Pharmacology

Combined oral administration of etoposide and arabinofuranosylcytosine-5′-stearylphosphate enhances the antitumor effect against P388 ascites tumors

Masamune Higashigawa[1], De-Chen Cao[1], Koji Matsui[1], Shigeyuki Yamada[1], Hideshi Kakitou[2], Yoshiyuki Kagawa[2], Hideki Inamochi[1], Masaru Ido[1], Minoru Sakurai[1]

[1] Department of Pediatrics, Mie University School of Medicine, 2-174 Edobashi, Tsu-city, Mie-ken, 514 Japan
[2] Department of Pharmacy, Mie University Hospital, 2–174 Edobashi, Tsu-city, Mie-ken, 514 Japan

Received: 20 October 1992/Accepted: 6 August 1993

Abstract. We investigated the antitumor effect of oral administration of etoposide and arabinofuranosylcytosine-5′-stearylphosphate (C18PCA) against P388 ascites tumors in B6D2F1 mice. Etoposide (25 mg/kg) and C18PCA (5 mg/kg) were given orally on days 1–5 after tumor inoculation. The median life span of the mice treated with etoposide or C18PCA alone was 19.5 and 18 days, respectively. The combination of both drugs significantly extended the median life span to 33 days. To clarify this enhancement of the increase in median life span, we examined intracellular deoxyribonucleoside triphosphate (dNTP) pools, cell-cycle distribution, DNA fragmentation, and the time course of the plasma drug concentration. Etoposide had no effect on intracellular dNTP pools in this experimental system, whereas treatment of cells with C18PCA or with the combination of both drugs resulted in a significant increase in dTTP pools to values ranging from 1.8- to 2.0-fold higher than the control levels. There was a significant increase in cells in the S+G2/M phase when cells had been treated with both etoposide and C18PCA. Agarose-gel electrophoresis of the extracted DNA revealed that C18PCA enhanced the fragmentation of DNA, with a length of about 180 bp being induced by etoposide. The plasma peak levels of etoposide (1000 n*M*) and ara-C (50 n*M*) were observed at 20 and 30 min after the simultaneous administration of both drugs, respectively. The plasma etoposide level gradually decreased to 10% of the peak level at 240 min after administration. On the other hand, the plasma concentration of ara-C was maintained at above 20 n*M* at 240 min. These observations suggest that C18PCA and etoposide act on P388 murine leukemic cells by accumulating cells in the S+G2/M phase. Even if the plasma concentration of ara-C is low, the repair of DNA damage by etoposide may be hindered in the presence of ara-C following an increase in DNA fragmentation.

Correspondence to: Dr. M. Higashigawa, Department of Pediatrics, Mie University School of Medicine, 2–174 Edobashi, Tsu-city, Mie-ken, 514 Japan

Introduction

Arabinofuranosylcytosine (ara-C) is one of the most important antitumor agents in the treatment of human acute myelogenous leukemia [1]. Since ara-C is rapidly inactivated by cytidine deaminase into 1-β-D-arabinofuranosyluracil (ara-U) [9], it is of great benefit to modify ara-C to a deaminase-resistant form. Saneyoshi et al. [29] demonstrated that a series of newly synthesized ara-C monophosphate (ara-CMP) esters having 10- to 20-carbon alkyl groups exhibited a high degree of antitumor activity against L1210 murine leukemia. Arabinofuranosylcytosine-5′-stearylphosphate (C18PCA) was selected from the various ara-CMP esters as one of the most promising orally effective antileukemic drugs with prolonged activity [5, 17]. C18PCA is gradually converted to ara-C and the concentration of ara-C is retained much longer [5, 17].

Etoposide, a semisynthetic derivative of podophyllotoxin, is effective against a spectrum of human tumors, such as small-cell lung cancer, testicular carcinoma, acute myelogenous leukemia, and lymphomas [24, 31]. We and other investigators have previously reported a synergistic interaction between ara-C and etoposide [25, 28]. Recently, low-dose ara-C and/or etoposide have been reported to be effective in the treatment of some cases of refractory leukemia [11, 18]. We describe herein an enhanced antitumor effect of oral administration of C18PCA and etoposide on P388 murine leukemia.

Materials and methods

Chemicals. Etoposide and C18PCA were supplied by Nippon Kayaku Co. Ltd., Japan.

Mice and tumor cell line. Male (C57BL/6 × DBA/2)F1 (hereafter called B6D2F1) mice weighing from 31 to 32 g each were kept at Mie University Animal Center under constant conditions (12-h light:dark regimen; Oriental Chew pellet food and water ad libitum). The mice used for these experiments were 10–11 weeks of age. The strain of P388 leukemia was maintained in B6D2F1 mice by weekly intraperitoneal (i.p.) inoculation

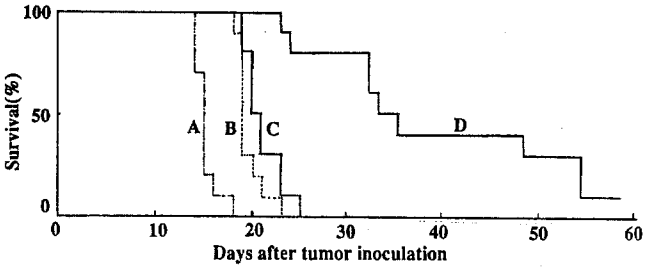

Fig. 1. Survival curves plotted by the Kaplan-Meier life-table method. Ten mice in each group were inoculated i.p. with 10^6 P388 cells 24 h before treatment. On days 1–5 after tumor inoculation, (*A*) normal saline containing 25% (v/v) DMSO, (*B*) 5 mg/kg C18PCA, (*C*) 25 mg/kg etoposide, and (*D*) both 5 mg/kg C18PCA and 25 mg/kg etoposide were given. The combination of both drugs shows the best therapeutic effect ($P<0.001$, generalized Wilcoxon test)

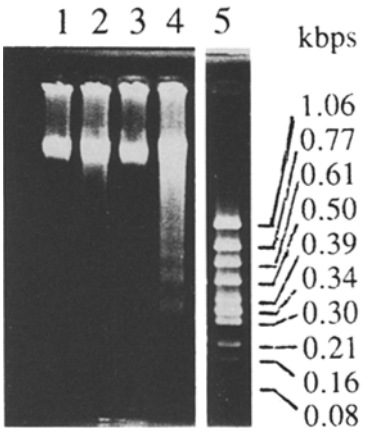

Fig. 2. Effect of etoposide and C18PCA on DNA fragmentation. DNA was extracted as described in Materials and methods, and 5 μg of each sample was loaded onto agarose gel. Electrophoresis was performed for 40 min at 58 V. The DNA was stained with ethidium bromide, and the gel was photographed under UV light. *Lane 1*, Control; lane 2, 25 mg/kg etoposide for 5 days; *lane 3*, 5 mg/kg C18PCA for 5 days; lane 4, both etoposide and C18PCA; *lane 5*, Hinc II-digested products of øX174 DNA

of 1×10^6 cells. Mice that were used in experiments received 1×10^6 ascites tumor cells i.p. in 0.2 ml normal saline.

Drug administration. Ten mice in each group were treated with C18PCA, etoposide, and both C18PCA and etoposide from day 1 to day 5 after tumor inoculation. The doses of C18PCA and etoposide were 5 and 25 mg/kg per day orally, respectively. We selected the etoposide dose that produces a 1-μ*M* plasma concentration as based on previous experiments [5, 17, 20, 26], because oral administration of etoposide at 25 mg/day achieves a 1-μ*M* plasma concentration in clinical use. Just before their use, C18PCA and etoposide were dissolved in water and normal saline containing 25% (v/v) dimethylsulfoxide (DMSO), respectively. The drugs were force-fed by a stomach tube. For the combination of C18PCA and etoposide, the drugs were simultaneously given in the same vehicle, a solution of normal saline containing 25% DMSO. Normal saline containing 25% DMSO was given to the mice in the control group on the same schedule.

Drug effect. The antitumor effect of the drugs was evaluated by comparing the prolongation of the postinoculation life span. The period of observation was 60 days. The probability of survival rate was estimated by the life-table analysis method of Kaplan and Meier. Statistical analysis was made by the generalized Wilcoxon method. Enhancement was considered to be present when the percentage of increase in the median life span (%ILS) of treated over control animals for the combination of etoposide and C18PCA was greater than the sum of the %ILS values for the two drugs given as single agents at the same dose levels [22].

Sample preparation for high-performance liquid chromatography and cell-cycle analysis. After tumor inoculation, six mice in each group were treated without antitumor drug or with C18PCA alone, etoposide alone, or the combination of both drugs. The mice were treated with the doses described above on days 7–11 after tumor implantation. On day 12, the mice were killed by cervical dislocation. Cells were collected, washed three times with normal saline, and processed for the determination of cell progression, the extraction of nucleotides, and the evaluation of DNA fragmentation. For the determination of cell-cycle distribution, 1×10^3 cells were run in FACScan and analyzed for their DNA contents using the Cell FIT program. The deoxyribonucleoside triphosphate (dNTP) pools were extracted from 3×10^7 cells by a modified version of the method of Khym [15] and Garrett and Santi [3] as described elsewhere [7]. The samples were stored at –20°C until subjected to high-performance liquid chromatography (HPLC).

Determination of dNTP pools. The separation of the dNTP pools was performed using a Waters ALC/GPG 204 HPLC equipped with a 6000 A pump, a 710 B sample processor, a 730 data module and a TSK gel DEAE 2SW column (Tosoh Ltd., Japan). The elution was done with 0.06 *M* disodium hydrogen phosphate with 20% acetonitrile at pH 6.6 as described previously [7]. Isocratic separation was carried out at a flow rate of 0.7 ml/min. The quantitation was done by means of peak-height measurement. Statistical determination was made using Student's *t*-test, depending on the experimental data analyzed.

Purification and analysis of DNA. In all, 2×10^6 cells were incubated in 10 m*M* TRIS-10 m*M* ethylenediaminetetraacetic acid (EDTA; pH 7.5) containing 100 μg proteinase K/ml and 0.5% sodium dodecyl sulfate at 37°C for several hours. After the addition of NaCl at a final concentration of 150 m*M*, the DNA was extracted twice with phenol/chloroform and once with chloroform as described elsewhere [8], treated with RNase A (100 μg/ml), recovered by ethanol precipitation, and loaded onto 2.0% (w/v) agarose gels. Next, 5 μg of DNA in each lane was electrophoresed for 40 min at 58 V. The DNA present on the gel was stained with ethidium bromide (0.5 μg/ml) and photographed under UV light.

Determination of plasma ara-C and etoposide concentrations. Etoposide (25 mg/kg per day) and C18PCA (5 mg/kg per day) were simultaneously given once to 30 mice. Five mice in each group were killed by ether treatment at 10, 20, 30, 60, 120, and 240 min after administration of the drugs. The blood was drawn directly from the heart and collected in heparinized tubes containing tetrahydrouridine at a final concentration of 500 μ*M*. The plasma concentration of ara-C was determined by radioimmunoassay using the method of Sato et al. [30]. The plasma concentration of etoposide was measured by HPLC with fluorescence detection as described elsewhere [10, 16].

Results

Effect of individual drugs

As compared with the control group (median survival, 14 days), the median life span was slightly extended when mice were treated with C18PCA or etoposide alone, to 18 days (%ILS, 28.6%) and 19.5 days (%ILS, 39.5%), respectively. However, the administration of both drugs significantly increased the survival of mice (33 days, %ILS, 135.7%). The survival of this group as evaluated by the Kaplan-Meier life-table method was significantly better than that of the other groups ($P<0.001$; Fig. 1). The %ILS

Table 1. Intracellular dNTP pools in P388 murine leukemic cells treated with etoposide and C18PCA

	dNTP pools (pmol/10^6 cells)		
	dTTP	dCTP	dATP
Control (n = 6)	19.1+ 8.7	7.3+3.0	3.5+2.9
Etoposide (n = 6)	22.0+ 5.0	8.4+1.9	4.7+1.6
C18PCA (n = 6)	42.8+19.2*	13.0+6.4	7.1+3.2
Etoposide + C18PCA (n = 6)	34.6+10.3*	9.8+4.0	6.1+1.2

Data represent mean values + SD
* P = 0.02 (Student's t-test)

Table 2. Effect of oral administration of C18PCA and etoposide on cell-cycle distribution in P388 murine leukemia cells in vivo

Drug	Cell-cycle distribution (%)			
	G0/G1	S	G2/M	S+G2/M
Control (n = 4)	59.0+2.8	25.9+3.0	15.1+2.8	41.0+2.8
Etoposide (n = 3)	55.1+8.3	28.2+4.8	16.7+3.5	44.9+8.3
C18PCA (n = 4)	55.6+2.4	33.8+2.3*	10.6+0.8*	44.4+2.3
Etoposide + C18PCA (n = 4)	50.5+1.5*	32.9+1.4*	16.5+2.7	49.5+1.6*

Data represent mean values + SD
* P <0.01 (Student's t-test)

of the mice treated with the combination of both drugs was greater than the sum of the ILS values obtained for those treated with the two drugs given as single agents, which would suggest the presence of an enhanced antitumor effect of etoposide and C18PCA.

DNA fragmentation

Treatment of cells with both etoposide and C18PCA induced DNA fragmentation to a much greater extent as compared with treatment with etoposide alone (Fig. 2). The lengths of DNA fragments were multiples of about 180 bp on examination of a DNA sample using agarose-gel electrophoresis. The increased DNA fragmentation induced by the combination of etoposide and C18PCA may have been responsible for the extended survival of the mice treated with these drugs.

Intracellular dNTP pools and ara-C triphosphate levels and cell-cycle distribution

To clarify the mechanism by which the combination of etoposide and C18PCA extended the life span of mice and increased DNA fragmentation, we examined intracellular levels of ara-C triphosphate (ara-CTP) and dNTP pools as well as changes in DNA histograms among the four different treatment groups. The results are shown in Table 1.

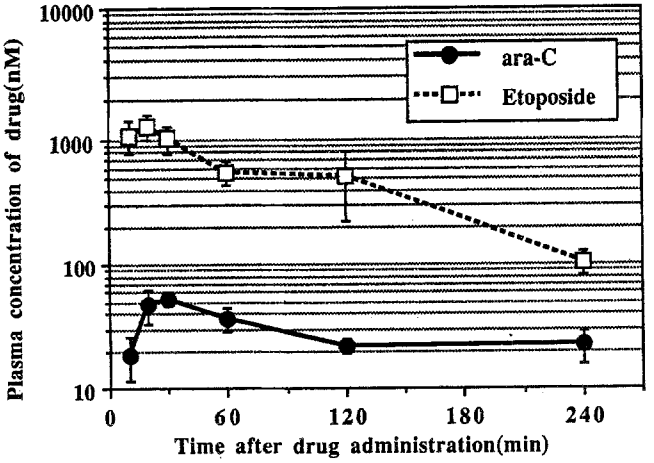

Fig. 3. Plasma concentration-time curves generated for etoposide and ara-C after the oral administration of etoposide and C18PCA. Each data point indicates the mean value for 5 determinations. Bars indicate the SE

Ara-CTP was not detectable in the cells from all treatment groups. The increases in deoxythymidine triphosphate (dTTP) pools observed after dosing with both C18PCA alone and the combination were of similar magnitude. Next we examined the effect of oral administration of C18PCA and etoposide on cell-cycle distribution in P388 leukemia cells in vivo. Treatment of cells with C18PCA significantly increased the number of cells in the S phase but decreased the quantitiy of cells in the G2/M phase, resulting in no significant difference in the percentages of S+G2/M phases (%S+G2/M phases). However, a significant increase in the %S phase and a lack of change in the %G2/M phase resulted in a significant increase in the %S+G2/M phase in the group treated with the combination of etoposide and C18PCA (Table 2).

Concentration-time curve for etoposide and ara-C

Figure 3 shows the plasma concentration-time curve for etoposide and ara-C after simultaneous single oral administrations of etoposide (25 mg/kg per day) and C18PCA (5 mg/kg per day). Etoposide and ara-C levels in plasma reached a peak at 20 and 30 min, respectively. The plasma etoposide level gradually decreased to 10% of the peak level at 240 min after administration. On the other hand, C18PCA was gradually converted to ara-C such that an ara-C level of 20–50 nM was maintained for a prolonged period after oral administration.

Discussion

Our study demonstrates that the combination of etoposide and C18PCA produces an impressive increase in life span as compared with either C18PCA or etoposide alone. Although the 5-mg/kg daily dose of C18PCA is lower than the minimum effective dose reported by other investigators [17], the combination of both drugs produced an ILS of 135.7%. Our data do not show that the imbalance in intra-

cellular dNTP pools observed after treatment with both C18PCA alone and the combination is capable of explaining the differences in the antitumor effect. Our data, however, show that the enhancement of cytotoxicity by C18PCA and etoposide may be due to modulation of the cell-cycle distribution. Recently, oral administration of etoposide has been demonstrated to be effective in the treatment of human cancers [4, 12, 34, 36]. Therefore, C18PCA might be a potent biochemical modulator of etoposide not only in hematological diseases but also in nonhematological diseases such as lung cancer.

Ara-C is a cell-cycle phase-specific drug, and its cytotoxicity is dependent on the percentage of cells in the S phase and on the amount of ara-CTP incorporated into DNA [32]. However, ara-C is rapidly inactivated by the enzyme cytidine deaminase into ara-U [9]. C18PCA, a newly synthesized deaminase-resistant derivative of ara-C [29], is given orally and is gradually converted to ara-C [5, 17]. Ara-C undergoes phosphorylation to form ara-CTP, which competitively inhibits DNA polymerase in opposition to the normal substrate deoxycytidine triphosphate (dCTP) [2]. We previously reported that intracellular dNTP pools were elevated by ara-CTP, which inhibited DNA polymerase in P388 leukemia cells [5, 6]. Therefore, we estimated the elevation of dTTP pools in the cells treated with C18PCA as reflected in the elevation of ara-CTP, which was, however, undetectable in this HPLC system (less than 5 pmol) [14].

A previous report has shown that the accumulation of cells in the G2/M phase occurs after etoposide treatment [23]. We cannot clearly explain the reason why an accumulation of cells in the G2/M phase after treatment with etoposide alone was not observed. However, we speculate that the plasma concentration of etoposide reached in this experiment might have been too low to induce the accumulation of cells in the G2/M phase. Etoposide produces topoisomerase II-mediated DNA strand breaks, which may be responsible for its cytotoxicity [21, 35]. Some authors have reported that etoposide induces a ladder pattern of DNA fragmentation, the lengths of which are multiples of about 180 bp [13, 33]. Our results demonstrated that there was a significant increase in the number of cells in the S+G2/M phase.

Furthermore, the amount of DNA fragmentation was greater when cells had been treated with etoposide and C18PCA as compared with etoposide alone. Our data also demonstrated that there was a time lag between the peak level of etoposide and that of ara-C and that C18PCA was gradually converted to ara-C such that ara-C levels were maintained for a prolonged period. Although etoposide and C18PCA were given simultaneously in this experiment, the plasma concentration curve resembled that resulting from the sequential administration of etoposide and low-dose ara-C at 3–4 h after administration of the drugs. One possible explanation for the enhanced effect of etoposide and C18PCA is that the repair of DNA damage by etoposide may be hindered in the presence of ara-C. Kufe et al. [19] have reported that the amount of ara-C incorporated into DNA closely correlates with the cytotoxic effect of ara-C in L1210 cells in vitro. We have previously reported that treatment of L1210 cells with etoposide 3 and 6 h prior to their exposure to [^{3}H]-ara-C increases the amount of [^{3}H]-ara-C incorporated into DNA as compared with that observed without etoposide treatment [27].

These observations support our tentative conclusion that etoposide and C18PCA act on P388 murine leukemic cells by accumulating the cells in the S+G2/M phase. Even if the plasma concentration of ara-C is low, the repair of DNA damage by etoposide may be hindered in the presence of ara-C following an increase in DNA fragmentation. The enhancement of both DNA fragmentation and the amount of ara-C incorporated into DNA might explain the enhanced cytotoxicity of etoposide and C18PCA.

Acknowledgements. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture and by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare of Japan. The authors thank Nippon Kayaku Co. Ltd., for the generous gift of C18PCA and etoposide.

References

1. Ellison RR, Holland JF, Weil M, Jacquillat C, Boiron M, Bernard J, Sawitsky A, Rosner F, Gussoff B, Silver RT, Karanas A, Cuttner J, Spurr CL, Hayes DM, Blom J, Leone LA, Haurani F, Kyle R, Hutchison JL, Forcier RJ, Moon JH (1968) Arabinosyl cytosine: a useful agent in the treatment of acute leukemia in adults. Blood 32: 507–523
2. Furth JJ, Cohen SS (1968) Inhibition of mammalian DNA polymerase by the 5′-triphosphate of 1-β-D-arabinofuranosylcytosine and the 5′-triphosphate of 9-β-D-arabinofuranosyladenosine. Cancer Res 28: 2061–2067
3. Garrett C, Santi DV (1979) A rapid and sensitive high pressure liquid chromatography assay for deoxyribonucleoside triphosphates in cell extracts. Anal Biochem 99: 268–273
4. Hainsworth JD, Johnson DH, Frazier SR, Greco FA (1990) Chronic daily administration of oral etoposide in refractory lymphoma. Eur J Cancer 26: 818–821
5. Higashigawa M, Hori H, Ohkubo T, Kawasaki H, Yoshizumi T, Sakurai M (1990) Deoxyribonucleoside triphosphate pools and ara-CTP levels in P388 murine leukemic cells treated with 1-β-D-arabinofuranosylcytosine-5′-stearylphosphate, which is a newly synthesized derivative of 1-β-D-arabinofuranosylcytosine. Med Oncol Tumor Pharmacother 7: 223–226
6. Higashigawa M, Ido M, Nagao Y, Kuwabara H, Hori H, Ohkubo T, Kawasaki H, Sakurai M (1991) Decreased DNA polymerase sensitivity to 1-β-D-arabinofuranosylcytosine-5′-triphosphate in P388 murine leukemic cells resistant to vincristine. Leuk Res 15: 675–681
7. Higashigawa M, Ido M, Ohkubo T, Kawasaki H, Kamiya H, Sakurai M, Taniguchi K, Hamazaki M (1989) Increased sensitivity to 1-β-D-arabinofuranosylcytosine in P388 murine leukemic cells resistant to etoposide. Leuk Res 13: 39–42
8. Higashigawa M, Ochiai H, Ohkubo T, Kawasaki H, Nobori T, Kamiya H, Sakurai M (1988) Incorporation of N^4-behenoyl-1-β-D-arabinofuranosylcytosine into DNA as 1-β-D-arabinofuranosycytosine. Med Oncol Tumor Pharmacother 5: 265–271
9. Ho DHW, Frei E III (1971) Clinical pharmacology of 1-β-D-arabinofuranosylcytosine. Clin Pharmacol Ther 12: 944–954
10. Idzu G, Yazawa Y, Tachibana M, Terada T, Hashimoto Y, Shimazu T (1989) Determination of etoposide in plasma by high performance liquid chromatography with fluorescence detector. Clin Rep 23: 4809–4813
11. Jensen MK, Stentoft J (1986) Low-dose cytosine arabinoside in the treatment of relapsed and refractory acute non-lymphocytic leukemia. Acta Haematol (Basel) 76: 127–129
12. Johnson DH, Greco A, Strupp J, Hande KR, Hainsworth JD (1990) Prolonged administration of oral etoposide in patients with relapsed

or refractory small-cell lung cancer: a phase II trial. J Clin Oncol 8: 1613–1617
13. Kaufmann SH (1989) Induction of endonucleolytic DNA cleavage in human acute myelogenous leukemia cells by etoposide, camptothecin, and other cytotoxic anticancer drugs: a cautionary note. Cancer Res 49: 5870–5878
14. Kawasaki H, Ochiai H, Ohkubo T, Nobori T, Kamiya H, Sakurai M (1986) Analysis of intracellular deoxyribonucleic acid pool using HPLC equipped with DEAE-2SW column. Igakunoayumi 137: 1019–1020
15. Khym JX (1975) An analytical system for rapid separation of tissue nucleotides at low pressures of conventional anion exchangers. Clin Chem 21: 1245–1252
16. Kiya K, Uozumi T, Ogasawara H, Sugiyama K, Hotta T, Mikami T, Kurisu K (1992) Penetration of etoposide into human malignant brain tumors after intravenous and oral administration. Cancer Chemother Pharmacol 29: 339–342
17. Kodama K, Morozumi M, Saitoh K, Kuninaka A, Yoshino H, Saneyoshi M (1989) Antitumor activity and pharmacology of 1-β-D-arabinofuranosylcytosine-5'-stearylphosphate: an orally active derivative of 1-β-D-arabinofuranosylcytosine. Jpn J Cancer Res 80: 679–685
18. Koyama S, Itou S, Shibata A (1990) Low dose continuous infusion therapy with etoposide (VP-16) and cytosine arabinoside (ara-C) for a patient with refractory acute myelogenous leukemia. Jpn J Clin Hematol 31: 1891–1892
19. Kufe DW, Major PP, Egan EM, Beardsley GP (1980) Correlation of cytotoxicity with incorporation of ara-C into DNA. J Biol Chem 255: 8997–9000
20. Kusama K, Ekimoto H, Ishii T, Okamoto K, Takahashi K (1991) Antitumor activity by long-term administration of low-dose etoposide. Jpn J Cancer Chemother 18: 959–963
21. Long BH, Musial ST, Brattain MG (1984) Comparison of cytotoxicity and DNA breakage activity of congeners of podophyllotoxin including VP16-213 and VM-26: a quantitative structure-activity relationship. Biochemistry 23: 1183–1188
22. Mabel JA, Merker PC, Sturgeon ML, Wodinsky I, Geran RI (1978) Combination chemotherapy against B16 melanoma: bleomycin/vinblastine, bleomycin/cis-diamminedichloroplatinum, 5-fluorouracil/BCNU and 5-fluorouracil/methyl-CCNU. Cancer 42: 1711–1719
23. Maeda T, Ueda M, Yamada T, Kubo H, Okamura S, Sano T (1986) Sensitivity to etoposide of cultured cells from cervical squamous cell carcinoma. Acta Obstet Gynaecol Jpn 38: 2050–2056
24. O'Dwyer PJ, Leyland-Jones B, Alonso MT, Marsoni S, Wittes RE (1985) Etoposide (VP-16-213): current status of an anticancer drug. N Engl J Med 312: 692–700
25. Ohkubo T, Higashigawa M, Kawasaki H, Kamiya H, Sakurai M, Kagawa Y, Kakito E, Sumida K, Ooi K (1988) Sequence-dependent antitumor effect of VP-16 and 1-β-D-arabinofuranosylcytosine in L1210 ascites tumor. Eur J Cancer Clin Oncol 24: 1823–1828
26. Okamoto K, Nishikawa K, Seki T, Shibasaki C, Uchida T, Takahashi K (1985) The antitumor activity of intraperitoneally or orally administered etoposide in animals and its administration schedule dependency. Jpn J Cancer Chemother 12: 2331–2337
27. Ooi K, Ohkubo T, Kuwabara H, Higashigawa M, Kawasaki H, Kakitoh H, Kagawa Y, Inagaki S, Sumida K, Sakurai M (1993) Enhanced incorporation of 1-β-D-arabinofuranosylcytosine by pretreatment with etoposide. Cancer Invest (in press)
28. Rivera G, Avery T, Roberts D (1975) Response of L1210 to combinations of cytosine arabinoside and VM-26 or VP16-213. Eur J Cancer 11: 639–647
29. Saneyoshi M, Morozumi M, Kodama K, Machida H, Kuninaka A, Yoshino H (1980) Synthetic nucleosides and nucleotides. XVI. Synthesis and biological evaluations of a series of 1-β-D-arabinofuranosylcytosine 5'-alkyl or aryl phosphates. Chem Pharm Bull (Tokyo) 28: 2915–2923
30. Sato T, Morozumi M, Kodama K, Kuninaka A, Yoshino H (1984) Sensitive radioimmunoassay for cytarabine and uracil arabinoside in plasma. Cancer Treat Rep 68: 1357–1366
31. Sauter C, Fehr J, Frick P, Gmuer J, Honegger H, Martz G (1982) Acute myelogenous leukemia: successful treatment of relapse with cytosine arabinoside, VP 16–213, vincristine and vinblastine (A-triple-V). Eur J Cancer Clin Oncol 18: 733–737
32. Shackney SE, Ford SS, Occhipinti SJ, Ritch PS, Riccardi RR, Erickson BW Jr (1982) Schedule optimization of hydroxyurea and 1-β-D-arabinofuranosylcytosine in sarcoma 180 in vitro. Cancer Res 42: 4339–4347
33. Shimizu T, Kubota M, Tanizawa A, Sano H, Kasai Y, Hashimoto H, Akiyama Y, Mikawa H (1990) Inhibition of both etoposide-induced DNA fragmentation and activation of poly (ADP-ribose) synthesis by zinc ion. Biochem Biophys Res Commun 169:1172–1177
34. Takeda S, Takada S, Kojima T, Kinoshita K, Sakamoto S (1990) Oral etoposide therapy in stage III–IV ovarian carcinoma. J Jpn Soc Cancer Ther 25: 2562–2566
35. Wozniak AJ, Ross WE (1983) DNA damage as a basis for 4'-demethylepipodophyllotoxin-9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (etoposide) cytotoxicity. Cancer Res 43: 120–124
36. Yuki K, Kodama Y, Emoto K, Yukawa O, Onda J, Uozumi T (1989) A case report of advanced malignant mixed germ cell tumor of parasellar origin indicating marked efficacy of a salvage combined chemotherapy of CDDP and etoposide and subsequent chemotherapy using oral etoposide. Jpn J Cancer Chemother 16: 2651–2654