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ANTILEUKEMIC ACTIVITY OF SULFONAMIDE CONJUGATES OF ARABINOSYLCYTOSINE

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Aim: **Cytosine arabinoside is routinely used for treatment of leukemias and lymphomas. However, because of its extensive metabolic inactivation and limited activity in chemotherapy, new analogues of araC are being tested. The aim of this work was to synthetize two araC conjugates and evaluates their cytotoxic/antileukemic activity.** ***Methods:*** **Synthesis of araC-sulfonamide conjugates A and B was performed in anhydrous conditions using cyclostyling and 5'-chlorocyclocytidine as starting material. Elemental analysis and NMR, IR and UV spectrometry were used for structure confirmation. The synthesized araC conjugates were tested for their cytotoxicity in L1210 leukemia cells** ***in vitro*** **and for therapeutic activity and toxicity** ***in vivo*** **in leukemia L1210-bearing mice.** ***Results:*** **The cytotoxic activities of araC and two synthesized conjugates A and B were expressed as IC_{50} (μmol/l) and were compared respectively. The conjugate A is 303-times less active and the conjugate B is 757-times less active than araC. Consequently, the antileukemic activity and the acute toxicity of these compounds were examined in experiments involving leukemia L1210-bearing mice. Statistically significant therapeutic outcome was observed when the dosage of both araC conjugates was increased 10-times compared to araC. Next, the ration of cytotoxicity** ***vs*** **therapeutic activity for araC and both conjugates was performed. It was recorded that the ration between cytotoxicity and therapeutic activity for araC is 3333, for the conjugate A and B, the ration is significantly lower (110 and 44). This indicates that the inactivation of araC conjugate A is 30-times slower and the inactivation of conjugate B is 75-times slower as araC inactivation.** ***Conclusions:*** **The differences in cytotoxic and therapeutic activity registered in araC treatment and between two araC-analogues are most probably caused by slow liberation of araC from both conjugates. We are considered that prolonged araC liberation protected them from inactivation and extended the time period of therapeutic action both araC conjugates. The obtained results can serve as stimuli for further investigation of new araC-analogues.**
Key Words: **arabinosylcytosine, cytarabine, sulfanilamide, conjugates, antileukemic activity, leukemia L1210.**

AraC belongs among biologically active antimetabolites that are structural analogues of naturally occurring intracellular metabolic intermediates essential for the normal function of a cell. AraC as such is an antimetabolite of deoxycytidine and cytidine and serves as a substrate for some intracellular enzymes utilizing deoxycytidine and cytidine as a substrate. This ultimately results in the inhibition of key enzymes necessary for synthesis of biological macromolecules, mainly of DNA. Ara-C is being used in the treatment of hematological malignancies, usually in combined chemotherapy [1–5]. It acts through inhibition of DNA and RNA synthesis as well as reparative DNA synthesis. Intracellular activation of araC is required for its cytotoxic and therapeutic effects. Ara-C is being activated to a therapeutically active form, ara-C-triphosphate (ara-CTP) which inhibits DNA synthesis and induces apoptosis [6–8]. The extensive inactivation of ara-C in clinical situations represents a significant limit for the therapeutic outcome. Ara-C is deaminated to its inactive metabolite arabinofuranosyluracil (ara-U), which is caused by cytidine deaminase [9–11]. Because of this problem, synthesis of many ara-C analogs that are more stable towards deamination was performed [for example 12, 13].

In order to enhance the cytotoxic effects of ara-C, a strategy of masking ara-C by the synthesis of a conjugate with another molecule [14–17] was employed in this work. This is expected to increase the efficacy ara-C and might also be able to overcome drug resistance which still remains the major problem in cancer chemotherapy [18–20]. Additionally, pharmacokinetic and pharmacodynamic properties of ara-C are also altered [13, 21, 22]. The molecule selected for the preparation of conjugates with ara-C was sulfanilamide [23].

The main goal of this work was to compare the cytotoxic/therapeutic activity of ara-C with these of sulfanilamide conjugates. In growth inhibition assay, the *in vitro* activity of the conjugates in L1210 leukemia cell line was compared with the effect of single ara-C administration. Employing *in vivo* experiments, therapeutic potential and acute toxicity of these compounds were determined in L1210-leukemia bearing mice. Again, these parameters were compared to the data obtained with araC treatment.

MATERIALS AND METHODS

Synthesis of araC conjugates. Melting points were determined on a Stuart Scientific SMP1 melting point apparatus and are uncorrected. The Science Analytical Facilities (SAF), Faculty of Science, Kuwait

University, performed all the instrumental analyses. Elemental analyses were determined on LECO elemental analyzer CHNS 932 apparatus, and were within ± 0.4% of the calculated values. 1H NMR spectra were recorded on Bruker DPX 400 NMR spectrometer using DMSO-d6 as solvent and tetramethylsilane (TMS) as an internal reference. The chemical shifts were reported in ppm. Infrared (IR) spectra were recorded on Perkin Elmer System 2000 FT-IR spectrometer. Column chromatography was carried out with silica gel (Kieselgel 60, 70–230 mesh; Aldrich). TLC was conducted on 0.25 mm precoated silica gel plates (60F254, Merck).

Synthesis of the conjugate A — 4-Amino-N-[1-(3,4-dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-4-imino-1,4-dihydro-pyrimidin-2-yl]-benzenesulfonamide. To a solution of 4-aminobenzenesulfonamide (1.03 g, 6.0 mmol) in dimethyl formamide (14 ml), sodium hydride (144 mg, 6.0 mmol) and cyclocytidine hydrochloride (784 mg, 3.0 mmol) was added with stirring under nitrogen. The reaction mixture was stirred at room temperature for 60 min, evaporated and the residue was chromatographed on a column of silica gel in the system ethyl acetate: acetone: ethanol: water 3 : 1 : 1: 1 (250 ml). Fractions of 10 ml were collected and pooled together and concentrated on a rotovap. Evaporation of the fractions 17–21 afforded the compound 1.07 g as foam. Analytical sample was obtained by crystallization from ethanol, to give 840 mg (71 % yield) of the title compound. mp 305–306 ˚C. ^{1}H NMR (DMSO-d$_6$, 400 MHz.) δ 7.71 (d, 1H, J = 7.5 Hz), 7.65 (broad s, 1H), 7.62 (d, 2H, J = 8.4 Hz), 7.52 (broad s, 1H), 6.48 (d, 2H, J = 8.62 Hz), 6.22 (d, 1H, J = 3.63 Hz), 5.87 (d, 1H, J = 7.6 Hz), 5.57 (s, 2H), 5.55 (broad d, 2H, J = 5.2 Hz), 5.03 (broad t, 1H, J = 5.5 Hz), 4.02 (broad t, 1H), 3.90 (broad, 1H), 3.79 (broad, 1H), 3.58 (broad t, 2H, J = 5.5 Hz). IR (KBr pellet, cm^{-1}): ν 3463-3242, 1644, 1597, 1551, 1467, 1308, 1247, 1126, 1077. Anal cal: $C_{15}H_{19}N_5O_6S$. This synthesis is based on the reported procedure by Novotny et al. [24].

Synthesis of the conjugate B — 8-(4-Amino-benzenesulfonyl)-5-imino-13-oxa-2,6,8-triaza-tricyclo[8.2.1.02,7]trideca-3,6-diene-11,12-diol. To a solution of 4-aminobenzenesulfonamide in dimethyl formamide, was added 60% sodium hydride in mineral (963 mg, 5.6 mmol) and 5-chlorocyclocytidine hydrochloride (785 mg, 2.8 mmol) is added with stirring under nitrogen gas. The mixture was stirred at room temperature for 60 min, evaporated and the residue was chromatographed on a column of silica gel eluting with ethyl acetate and ethyl acetate — ethanol — acetone — water 4 : 1 : 1 : 1. Fractions of 10 ml were collected and the appropriate fractions were pooled and concentrated. Evaporation of the fractions 19–23 afforded the compound in the as foam, which was triturated in cold ether and dried to give 830 mg (78% yield) of the title compound. mp 198–201 ˚C. ^{1}H NMR (DMSO-d$_6$, 400Mhz.) δ 7.71–7.59 (m, 4H), 6.48 (d, 2H, J = 8.4 Hz), 6.30 (d, 1H J = 3.0 Hz), 5.89 (d, 1H, J = 7.6 Hz), 5.77 (broad t, 2H, J = 4.9 Hz), 5.59 (broad s, 2H), 4.02–4.06 (m, 1H), 3.94–3.96 (m, 2H), 3.77–3.87 (m, 2H). IR (KBr pellet, cm^{-1}): ν 3354–3230, 1646, 1595, 1549, 1467, 1303, 1250, 1128, 1078. Anal cal: $C_{15}H_{17}N_5O_5S$. This synthesis is based on the reported procedure by Novotny et al. [24].

Cell culture. The L1210 murine leukemia cell line was purchased from ATCC (American Type Culture Collection, Manassaa, VA, USA). Cells were maintained at 37 ˚C in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (GIBCO, Grand Island Biological Co., Grand Island, NY, USA), 100 U/ml Penicillin G, 100 µg/ml streptomycin, and 2 mM L-glutamine (Sebac, Germany) in a humidified atmosphere containing 5% CO_2. Cells in the logarithmic phase of growth were used for all studies described.

Growth inhibition assay. Exponentially growing L1210 cells (0.2 x 10^6 per ml) were incubated with increasing concentrations of araC conjugates under cell culture conditions. Cytotoxicity was compared to the cytotoxicity of araC on an equimolar base. Cell counts and IC$_{50}$ values were determined after 24 h. Viability of cells was determined by staining with Trypan blue. Results are presented as IC$_{50}$ values.

Animals. Inbred DBA/2J mice of both sexes (weighing 18–22 g) were obtained from the breeding facility of the Cancer Research Institute of SAS (Bratislava, Slovak Republic). The animals were reared under standard conditions; six mice were housed in a cage. Food and water were provided *ad libitum*. The facility is certificated to perform scientific research on animals. The Ethics Committee of the Cancer Research Institute approved the *in vivo* experiments. These experiments were performed in full adherence with the European Community Guidelines principles for the care and use of laboratory animals.

Acute toxicity of drugs. Acute toxicity of the conjugates was monitored through registration of body weight of the experimental animals on daily basis during the whole experiment.

In vivo antileukemic activity. L1210-leukemia bearing mice were used as antileukemic models of therapy [13, 25, 35]. The experimental animals were intraperitoneally implanted with L1210 leukemia cells (1 x 10^5 cells per mouse). The chemotherapy was started 24 h later. The drugs were administered intraperitoneally (0.5 ml per mouse). The treatment schedule and doses were chosen according to previous experiments with araC. Animals were weighed daily and observed for the development of ascites and leukemia related death. Mean survival time (MST) and percentage of increase of life span (% ILS) were calculated and compared with MST of untreated control groups. Statistical significance of the difference was calculated and the effect of the therapeutic regimen was evaluated using a standard *t*-test for unpaired observations.

Statistical analysis. IC$_{50}$ values of drugs were calculated using the CalcuSyn software for Windows.

Statistical significance of the obtained results was determined by unpaired *t*-test.

RESULTS AND DISCUSSION

AraC is a drug that is routinely used in treatment of hematological malignancies as discussed earlier [15]. However, it is relatively easily deaminated to arabinosyluracil that possesses no therapeutic activity and its formation is not beneficial for a patient [9–11]. This represents the main obstacle in its broader use and better therapeutic outcomes achieved. Consequently, the attention is being paid to synthesis and evaluation of anticancer properties of many new arabinosylcytosine derivatives [25], analogs [26, 27] and conjugates [15, 16, 28] as well as to innovative therapeutic regimes that overcome the problem of deamination and that will benefit patients.

In our work we attempted to prepare and evaluate antileukemic activity of two conjugates of arabinosylcytosine and sulfanilamide. The very small-scale synthesis of these two substances was reported earlier [24] but their anticancer potential was not properly evaluated. The conjugation of sulfonamide moiety to nucleoside is interesting because, potentially, it should result in protection of the araC amino group against deamination. Additionally, it is likely to decrease hydrophilicity of araC and increase the life-time of the conjugate in the organism thus increasing the time of araC actions on leukemia cells. Despite of the fact that any anticancer activity of sulfanilamide itself was not reported, sulfonamides as a chemical group are known to inhibit cell proliferation at mitosis [29], arrests cell cycle in the G1 phase, inhibit carbonic anhydrase associated with cancer and alters gene expression [30]. Some of sulfonamides, for example celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzene-sulfonamide) [31, 32], are already recognized for their effects in cancer therapy. Moreover, the combination of nucleoside antimetabolite and sulfonamide in one molecule was justified and our expectations were that sulfonamide, while protecting araC moiety against deamination and modifying its pharmacokinetic, may possibly contribute by specific mechanisms to anticancer activity of araC. This is because various mechanisms of anticancer activity of sulfonamides are reported in scientific literature, including mediating cell-cycle arrest, activation of caspases and downregulation of COX-2 expression [32] or interfering with other enzymes, for example with caspase anhydrase [33, 34] or with microtubule assembly dynamics [29, 34] etc.

AraC cytotoxicity and cytotoxicity of both araC conjugates (A and B) was determined by the Trypan blue exclusion method *in vitro* using L1210 leukemia cells. Table 1 presents results of cytotoxicity study expressed as IC_{50} (μmol/l). The cytotoxicity decreases in the order of araC >> conjugate A > conjugate B. The analogue A is 303-times less active and the analogue B is 757-times less active than araC. This may be expected as the activity of araC part of conjugate can be activated to its active metabolite araC triphosphate only after its releasing from the conjugate.

Microscopic evaluation of cells exposed to tested compounds revealed same changes in morphology of L1210 cells, mainly an increase in their size. This supports the hypothesis that the mechanism of cytostatic/cytotoxic effect of all three tested compounds is the same. This is despite of the fact that this effect was achieved at different concentrations of all this compounds (Table 1).

Table 1. The cytotoxic activities of araC and two conjugates A and B were compared respectively. Relative values represent the lower measure of cytotoxic activity of araC conjugates *vs* araC

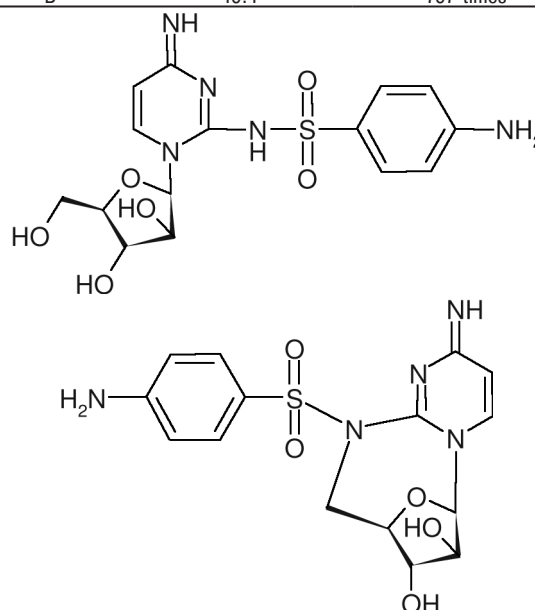|	IC50 (μmol/l)	Relative values
araC	0.06	1-times
A	18.2	303-times
B	45.4	757-times

Fig. 1. Chemical formulas of araC — sulfonamide conjugates A and B

Because studied compounds exhibited different toxicity in experiments *in vitro*, in experiment *in vivo* the dosage of both araC conjugates was increased 10-times (compared to araC) for *in vivo* experiments involving leukemia L1210-bearing mice [13, 35]. The application regime was the same for application of araC and two of its analogues (5 consequent days). Statistically significant therapeutic outcome was observed with all three therapeutic substances. The outcome of the therapy was the best with araC and decreased for araC-analogues: araC >> conjugate A > conjugate B (Table 2).

Table 2. Therapeutic effect of araC and araC – sulfonamide conjugates A and B in leukemia L1210-bearing mice

	N	Dose/day (μmol/kg)	Schedule (days)	Cumulative dose (μmol/kg)	Survival (days ± SD)	ILS (%)	P
Control	6	–	–	–	7.3 ± 1.0	–	–
araC	6	40	1, 2, 3, 4, 5	200	14.2 ± 0.4	95.4	< 0.0001
A	6	400	1, 2, 3, 4, 5	2000	10.8 ± 1.9	48.3	0.0028
B	6	400	1, 2, 3, 4, 5	2000	9.1 ± 0.5	24.3	0.0023

Recorded significantly decreased cytotoxicity of araC analogues in *in vitro* experiment (see Table 1) corresponds well with decreased therapeutic activity

of the analogues *in vivo* (see Table 2). Additionally, the *in vitro* and *in vivo* results recorded for two araC analogues correspond well. Lower cytotoxicity of the conjugate A compared to the cytotoxicity of the conjugate B *in vitro* (~ 2.5-times) is similar to the decreased therapeutic outcome *in vivo* (~ 2.0-times). The difference in cytotoxicity (compared to araC and between two analogues) is most probably caused by necessary liberation of araC from both conjugates. The incorporation of sulfonamide moiety into the conjugate B by two bonds (see the structural formula) also explains decreased antileukemic activity of the conjugate B compared to the conjugate A.
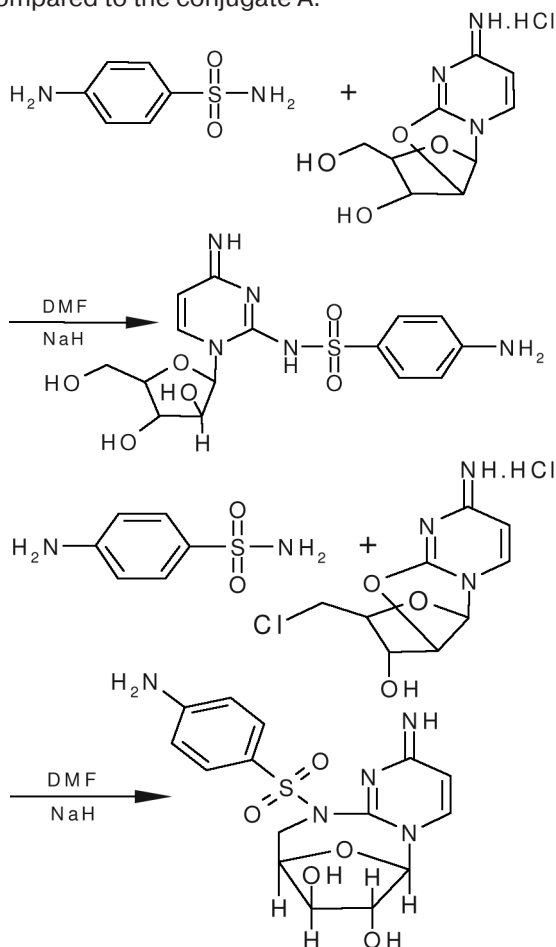

Fig. 2. Scheme of chemical synthesis of the araC – sulfonamide conjugates A and B. *a*. Chemical synthesis of the conjugate A. *b*. Chemical synthesis of the conjugate B.

Additional evaluation of the obtained results led us to the following comparisons: The ration between therapeutic activity and cytotoxicity (a cumulative dose *vs* IC_{50}) for araC is 3333 (Table 3). This is the ratio that characterizes the rate of araC inactivation (deamination) in organism. When the same calculation is performed for the conjugate A and for the conjugate B, the ration of therapeutic activity *vs* cytotoxicity for the conjugates investigated is significantly lover (110 and 44) compared to the same parameter obtained for araC (Table 3). Based on this and also on the hypothesis that sulfanilamide as such does not possess any anti-cancer activity, it can be concluded that the inactivation/deamination of the conjugate A is 30-times

lower (3333 : 110) and of the conjugate B is 75-times lower (3333 : 44) compared to araC.

Table 3. The ratio of cytotoxicity (IC_{50}) *versus* antileukemic activity (cumulative doses) for the araC and araC-sulfonamide conjugates A and B was assessed in *in vitro* and *in vivo* experimental models (data are presented in Table 1 and Table 2). The relative values characterize the rate of araC inactivation/deamination in organism

Drugs	IC50 (µmol/l)	Cumulative doses (µmol/kg)	Relative values
araC	0.06	200	3333-times
A	18.2	2000	110-times
B	45.4	2000	44-times

It is known that araC as such possesses a very low toxicity as documented by the results presented in Fig. 3. The weight monitoring of the experimental animals during chemotherapy confirmed that the used doses of the araC analogues tested (400 µmol/kg per day) were not toxic in the experimental mice (see Fig. 3). Our previous experiments revealed that 400 µmol of araC/kg per day applied in the regime of 5 consecutive days is sublethal (results not shown). The weight monitoring performed during the *in vivo* experiment (during the application of araC and araC conjugates) aimed at registering possible toxicity of the tested substances. This would be recorded as a decrease in the weight of experimental animals. However, toxicity was not observed with any of two tested araC analogues. Additionally, the conjugates A and B did not affect any important organ or function in the used animals at used dosage. This is an additional support for the hypothesis that tested araC conjugates retained the same mechanism of antileukemic activity as did araC.
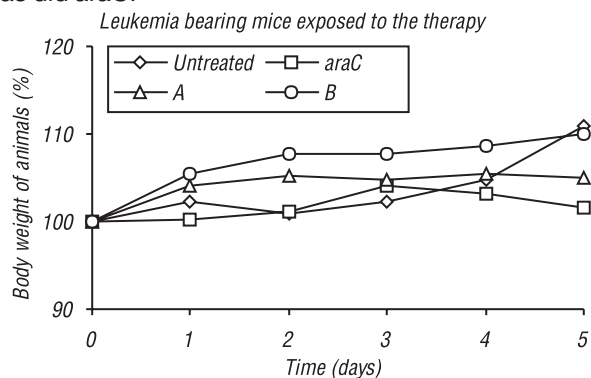

Fig. 3. Monitoring of weight of the experimental animals during the araC and araC-sulfonamide conjugates A and B application as an indicator of toxicity demonstrated by tested compounds

In conclusion, it is demonstrated that araC conjugates A and B possess the cytotoxic and therapeutic activity similar to araC but at significantly higher concentrations. This is possibly caused by their high intracellular stability. On the other hand, the inactivation (deamination) of the araC moiety of the conjugates A and B is also substantially decreased compared to araC deamination *in vivo*. Originally, araC-containing analogues were prepared with the aim that they should protect araC amino groups towards deamination and serve as depot forms of araC. This investigation confirmed that araC-conjugation is a suitable way for protecting araC stability.

REFERENCES

1. **Karp JE, Passaniti A, Gojo I, Kaufmann S, Bible K, Garimella TS, Greer J, Briel J, Smith BD, Gore SD, Tidwell ML, Ross DD, Wright JJ, Colevas AD, Bauer KS.** Phase I and pharmacokinetic study of flavopiridol followed by 1-beta-D-arabinofuranosylcytosine and mitoxantrone in relapsed and refractory adult acute leukemias. Clin Cancer Res 2005; **11**: 8403–12.

2. **Lie SO, Abrahamsson J, Clausen N, Forestier E, Hasle H, Hovi L, Jonmundsson G, Mellander L, Siimes MA, Yssing M, Zeller B, Gustafsson G.** Nordic Society of Pediatric Hematology and Oncology (NOPHO); AML Study Group Nordic Society of Pediatric Hematology and Oncology (NOPHO); AML Study Group. Long-term results in children with AML: NOPHO-AML Study Group — report of three consecutive trials. Leukemia 2005; **19**: 2090–100.

3. **Sung WJ, Kim DH, Sohn SK, Kim JG, Baek JH, Jeon SB, Moon JH, Ahn BM, Lee KB.** Phase II trial of amsacrine plus intermediate-dose Ara-C (IDAC) with or without etoposide as salvage therapy for refractory or relapsed acute leukemia. Jpn J Clin Oncol 2005; **35**: 612–6.

4. **Zwierzina H, Suciu S, Loeffler-Ragg J, Neuwirtova R, Fenaux P, Beksac M, Harousseau J, Nuessler V, Cermak J, Solbu G, Willemze R, de Witte T, Amadori S; EORTC Leukemia Cooperative Group.** Low-dose cytosine arabinoside (LD-AraC) vs LD-AraC plus granulocyte/macrophage colony stimulating factor vs LD-AraC plus Interleukin-3 for myelodysplastic syndrome patients with a high risk of developing acute leukemia: final results of a randomized phase III study (06903) of the EORTC Leukemia Cooperative Group. Leukemia 2005; **19**: 1929–33.

5. **Smith FO, Alonzo TA, Gerbing RB, Woods WG, Arceci RJ; Children's Cancer Group.** Long-term results of children with acute myeloid leukemia: a report of three consecutive Phase III trials by the Children's Cancer Group: CCG 251, CCG 213 and CCG 2891. Leukemia 2005; **19**: 2054–62.

6. **Robak T, Korycka A, Kasznicki M, Wrzesien-Kus A, Smolewski P.** Purine nucleoside analogues for the treatment of hematological malignancies: pharmacology and clinical applications. Curr Cancer Drug Targets 2005; **5**: 421–44.

7. **Nakayama T, Sakamoto S, Sassa S, Suzuki S, Kudo H, Nagasawa H.** Paradoxical effect of cytosine arabinoside on mouse leukemia cell line L1210 cells. Anticancer Res 2005; **25**: 157–60.

8. **Krett NL, Ayres M, Nabhan C, Ma C, Nowak B, Nawrocki S, Rosen ST, Gandhi V.** *In vitro* assessment of nucleoside analogs in multiple myeloma. Cancer Chemother Pharmacol 2004; **54**: 113–21.

9. **Gandhi V, Xu YZ, Estey E.** Accumulation of arabinosyluracil 5'-triphosphate during arabinosylcytosine therapy in circulating blasts of patients with acute myelogenous leukemia. Clin Cancer Res 1998; **4**: 1719–26.

10. **Kirch HC, Schroder J, Hoppe H, Esche H, Seeber S, Schutte J.** Recombinant gene products of two natural variants of the human cytidine deaminase gene confer different deamination rates of cytarabine *in vitro*. Exp Hematol 1998; **26**: 421–5.

11. **Burk M, Heyll A, Arning M, Volmer M, Fartash K, Schneider W.** Pharmacokinetics of high-dose cytarabine and its deamination product — a reappraisal. Leuk Lymphoma 1997; **27**: 321–7.

12. **Stankovicova M, Rauko P, Bachrata M, Blesova M, Sveda P.** *In vitro* antileukemic activity and chemical transformation of the 5'-chloro-5'-deoxy derivative of cyclocytidine. Neoplasma 1995; **42**: 255–8.

13. **Saiko P, Horvath Z, Bauer W, Hoechtl T, Grusch M, Krupitza G, Rauko P, Mader RM, Jaeger W, Schott H, Novotny L, Fritzer-Szekeres M, Szekeres T.** *In vitro* and *in vivo* antitumor activity of novel amphiphilic dimers consisting of 5-fluorodeoxyuridine and arabinofuranosylcytosine. Int J Oncol 2004; **25**: 357–64.

14. **Erion MD, van Poelje PD, Mackenna DA, Colby TJ, Montag AC, Fujitaki JM, Linemeyer DL, Bullough DA.** Liver-targeted drug delivery using HepDirect prodrugs. J Pharmacol Exp Ther 2005; **312**: 554–60.

15. **Lee KH, Jung YJ, Hong CI, Cho MH, Bai DH, Kim SH, Choi KY, Yu JH, Kim CM.** Activation of protein kinase C by 1-beta-D-arabinofuranosylcytosine conjugates of phospholipid. Int J Oncol 2004; **24**: 193–9.

16. **Alexander RL, Morris-Natschke SL, Ishaq KS, Fleming RA, Kucera GL.** Synthesis and cytotoxic activity of two novel 1-dodecylthio-2-decyloxypropyl-3-phosphatidic acid conjugates with gemcitabine and cytosine arabinoside. J Med Chem 2003; **46**: 4205–8.

17. **Cavallaro G, Pitarresi G, Licciardi M, Giammona G.** Polymeric prodrug for release of an antitumoral agent by specific enzymes. Bioconjug Chem 2001; **12**: 143–51.

18. **Bardenheuer W, Lehmberg K, Rattmann I, Brueckner A, Schneider A, Sorg UR, Seeber S, Moritz T, Flasshove M.** Resistance to cytarabine and gemcitabine and in vitro selection of transduced cells after retroviral expression of cytidine deaminase in human hematopoietic progenitor cells. Leukemia 2005; **19**: 2281–8.

19. **Lofgren C, Albertioni F, Paul C.** High activity and incomplete cross resistance of nucleoside analogues cladribine and fludarabine versus Ara-C on leukemic cells from patients with AML. Ther Drug Monit 2005; **27**: 641–6.

20. **Han T, Fernandez M, Chou TC, Agarwal RP.** Quantitation of synergism of arabinosylcytosine and cladribine against the growth of arabinosylcytosine-resistant human lymphoid cells. J Cancer Res Clin Oncol 2005; **131**: 609–16.

21. **Hubeek I, Stam RW, Peters GJ, Broekhuizen R, Meijerink JP, van Wering ER, Gibson BE, Creutzig U, Zwaan CM, Cloos J, Kuik DJ, Pieters R, Kaspers GJ.** The human equilibrative nucleoside transporter 1 mediates in vitro cytarabine sensitivity in childhood acute myeloid leukaemia. Br J Cancer 2005; **93**: 1388–94.

22. **Braess J, Fiegl M, Lorenz I, Waxenberger K, Hiddemann W.** Modeling the pharmacodynamics of highly schedule-dependent agents: exemplified by cytarabine-based regimens in acute myeloid leukemia. Clin Cancer Res 2005; **11**: 7415–25.

23. **Puccetti L, Fasolis G, Cecchi A, Winum JY, Gamberi A, Montero JL, Scozzafava A, Supuran CT.** Carbonic anhydrase inhibitors: synthesis and inhibition of cytosolic/tumor-associated carbonic anhydrase isozymes I, II, and IX with sulfonamides incorporating thioureido-sulfanilyl scaffolds. Bioorg Med Chem Lett 2005; **15**: 2359–64.

24. **Novotny L, Hrebabecky H, Beranek J.** Synthesis of the sulfonamido derivatives of arabinonucleosides. Collect Czech Chem Commun 1985; **50**: 383–92.

25. **Schwendener R, Schott H.** Lipophilic arabinofuranosyl cytosine derivatives in liposomes. Methods Enzymol 2005; **391**: 58–70.

26. **Liu X, Guo Y, Li Y, Jiang Y, Chubb S, Azuma A, Huang P, Matsuda A, Hittelman W, Plunkett W.** Molecular basis for G2 arrest induced by 2'-C-cyano-2'-deoxy-1-beta-D-arabino-pentofuranosylcytosine and consequences of checkpoint abrogation. Cancer Res 2005; **65**: 6874–81.

27. **Richardson KA, Vega TP, Richardson FC Moore CL, Rohloff JC, Tomkinson B, Bendele RA, Kuchta RD.** Polymerization of the triphosphates of AraC, 2',2'-difluorodeoxycytidine (dFdC) and OSI-7836 (T-araC) by human DNA polymerase alpha and DNA primase. Biochem Pharmacol 2004; **68**: 2337–46.

28. **Schiavon O, Pasut G, Moro S, Orsolini P, Guiotto A, Veronese FM.** PEG-Ara-C conjugates for controlled release. Eur J Med Chem 2004; **39**: 123–33.

29. **Mohan R, Banerjee M, Ray A, Manna T, Wilson L, Owa T, Bhattacharyya B, Panda D.** Antimitotic sulfonamides inhibit microtubule assembly dynamics and cancer cell proliferation. Biochemistry 2006; **45**: 5440–49.

30. **Supuran CT.** Indisulam: an anticancer sulfonamide in clinical development. Expert Opin Investig Drugs 2003; **12**: 283–87.

31. **Zhang GS, Liu DS, Dai CW, Li RJ.** Antitumor effects of celecoxib on K562 leukemia cells are mediated by cell-cycle arrest, caspase-3 activation, and downregulation of Cox-2 expression and are synergistic with hydroxyurea or imatinib. Am J Hematol 2006; **81**: 242–55.

32. **Chow LW, Loo WT, Wai CC, Lui EL, Zhu L, Toi M.** Study of COX-2, Ki67, and p53 expression to predict effectiveness of 5-flurouracil, epirubicin and cyclophosphamide with celecoxib treatment in breast cancer patients. Biomed Pharmacother 2005; **59**: S298–301.

33. **Pastorekova S, Parkkila S, Pastorek J, Supuran CT.** Carbonic anhydrases: current state of the art, therapeutic applications and future prospects. J Enzyme Inhib Med Chem 2004; **19**: 199–229.

34. **Scozzafava A, Owa T, Mastrolorenzo A, Supuran CT.** Anticancer and antiviral sulfonamides. Curr Med Chem 2003; **10**: 925–53.

35. **Rauko P, Bauer W, Horvath Z, Hochtl T, Saiko P, Karl D, Schott H, Fritzer-Szekeres M, Novotny L, Szekeres T.** Combination effects of Ara-C and 5-fluorodeoxyuridine against leukemia cells *in vitro* and in mice. Anticancer Res 2003; **23**: 3841–46.

ПРОТИВОЛЕЙКЕМИЧЕСКАЯ АКТИВНОСТЬ СУЛЬФОНАМИДНЫХ КОНЪЮГАТОВ АРАБИНОЗИЛЦИТОЗИНА

Цитозинарабинозид (araC) используют при терапии лейкемии и лимфомы, однако ввиду его активной метаболической инактивации и лимитированной активности создаются и испытываются новые аналоги araC. *Цель* работы — синтез двух конъюгатов araC и оценка их цитотоксической/противолейкемической активности. *Методы:* синтез сульфонамидных конъюгатов araC A и B проводили в безводном режиме с использованием 5'-хлорциклоцитидина в качестве исходного материала. Для подтверждения структуры использовали элементный анализ, ЯМР, ИК и УФ спектрометрию. Синтезированные конъюгаты araC испытывали на цитоксичность против клеток лейкемии линии L1210 *in vitro,* а также на наличие терапевтической активности и токсичности *in vivo* на мышах с экспериментальной опухолью L1210. *Результаты:* цитоксическую активность araC и конъюгатов A и B выражали как IC_{50} (μM/л). Конъюгат A оказался в 303 раза менее активным, а конъюгат B — в 757 раз менее активен, чем araC. Противоопухолевую активность и острую токсичность соединений исследовали в экспериментах *in vivo*. Статистически значимые различия в терапевтической эффективности препаратов отмечали при условии, если дозы конъюгатов были в 10 раз выше, чем таковые araC. Отмечено, что в то время как соотношение между цитоксичностью и терапевтической активностью для araC было 3333, то для конъюгатов A и B эта величина была значительно ниже (110 и 44 соответственно), что указывает на то, что инактивация конъюгатов A и B araC происходит в 30 и 70 раз медленнее соответственно, чем инактивация araC. *Выводы:* различия в цитоксической и терапевтической активности araC и конъюгатов вероятнее всего определяются медленным высвобождением araC, а продолжительный процесс высвобождения araC защищает активное вещество от инактивации и увеличивает продолжительность терапевтического действия конъюгатов.

Ключевые слова: арабинозилцитозин, цитарабин, сульфаниламид, конъюгат, противолейкемическая активность, лейкемия L1210.