Antitumor Activity of P-4055 (Elaidic Acid-Cytarabine) Compared to Cytarabine in Metastatic and s.c. Human Tumor Xenograft Models

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ABSTRACT

The antineoplastic efficacy of P-4055, a 5′-elaidic acid (C18:1, unsaturated fatty acid) ester of cytarabine, a nucleoside antimetabolite frequently used in the treatment of hematological malignancies, was examined in several in vivo models for human cancer.

In initial dose-finding studies in nude mice, the efficacy of P-4055 was highest when using schedules with repeated daily doses. In a Raji Burkitt’s lymphoma leptomeningeal carcinomatosis model in nude rats, the control cytarabine- and saline-treated animals (five in each group) had a mean survival time of 13.2 days, whereas treatment with P-4055 resulted in three of five long-time survivors (>70 days). In a systemic Raji leukemia model in nude mice, 8 of 10 of the P-4055-treated animals survived (>80 days), compared with none of the cytarabine-treated animals (mean survival time, 34.2 days).

In s.c. xenograft models, the effects of maximum tolerated doses of P-4055 and cytarabine, given in four weekly cycles of daily bolus i.v. injections for 5 subsequent days, against seven tumors (three melanomas, one lung adenocarcinoma, one breast cancer, and two osteogenic sarcomas) were investigated. P-4055 induced partial or complete tumor regression of the lung carcinoma, as well as of all three malignant melanomas. In two of the melanomas the activity was highly superior to that of cytarabine, and both P-4055 and cytarabine were, in general, more effective than several clinically established drugs previously tested in the same tumor models. In in vitro studies, inhibitors of nucleoside carrier-dependent transport, nitrobenzylmercaptopurine riboside and dipyridamol, reduced strongly the cellular sensitivity to cytarabine, but not to P-4055, indicating that P-4055 uses an alternative/additional mechanism of internalization into the cell compared with cytarabine. The results explain, at least in part, the observed differences between the two compounds in in vivo efficacy, and together the data strongly support the evaluation of P-4055 in clinical studies.

INTRODUCTION

Cytarabine,1 a nucleoside analogue of deoxycytidine, is frequently used in the treatment of hematological cancers. Its antitumor activity is well proven in leukemias (acute myelogenous leukemia and acute lymphoblastic leukemia) and lymphomas, but not in solid tumors (1). The efficacy of cytarabine is heavily dependent on dose and schedule because of its short biological half-life. This is due to a rapid deamination into uracil arabinoside by the ubiquitous enzyme cytidine deaminase, which is found at highest concentrations in the intestine, liver, and kidneys (2). Recently developed deoxynucleoside analogues are finding an expanding application in cancer chemotherapy, leading to a renewed interest in the use of such antimetabolites also against solid tumors (3).

We have tested a novel approach to improve the efficacy of antimetabolites by constructing ester derivatives of cytarabine and fatty acids designed with the aim of increasing cellular uptake of cytarabine and/or delaying its metabolic deamination and clearance. In vitro testing in murine and human tumor cell lines revealed differential cytotoxic properties of various conjugates of cytarabine and fatty acids, with chain-lengths varying from 16–22 C-atoms and with 0–3 double bonds. The fatty acids were linked to the sugar moiety of cytarabine at the 5′-OH position, and of 11 derivatives tested, P-4055 was found to be the most promising one (4, 5). P-4055 is the 5′-elaidic acid (C18:1Δ9,trans)-unsaturated fatty acid) ester of cytarabine. We studied its antitumor potential in vivo in human tumor models in nude mice and rats and in vitro in several tumor cell lines in the presence or absence of nucleoside carrier inhibitors.

MATERIALS AND METHODS

Animals. Female BALB/c nude (nu/nu) mice, 5–8 weeks of age at the start of the experiment, and Rowett nude (Han/rnu/nu) rats, 4 weeks of age, were used. The animals were maintained under specific pathogen-free conditions, and food and water were supplied ad libitum. Housing and all procedures involving animals were performed according to protocols approved by the animal care and use committee, in compliance with the National Committee for Animal Experiments guidelines on animal welfare.

Mice were anesthetized with 5 mg/10 g propanidid (Sombrevin; Gedeon Richter Ltd., Budapest, Hungary), given i.p. during tumor tissue implantation, and rats were anesthetized with 0.3 ml/100 g of a mixture with equal parts of fentanyl/fluaniason (Hypnorm; Janssen, Janssen, Beerse, Belgium) and midazolam (Dormicum; Roche, Basel, Switzerland) during surgical procedures.

Tumor Models. Initially, a leptomeningeal carcinomatosis model in nude rats, including the malignant lymphoma cell lines Raji (6) and Molt-4 (7, 8) were tested. Cells (1 × 106) were injected into the spinal fluid in c.m. of nude rats, 4–5 weeks of age.

A systemic leukemia model in nude mice (9, 10) was established by i.v. injection of 1 × 106 Raji cells.

The solid tumor xenograft panel consisted of three malignant melanomas (LOX, THX, and HHMSX; Refs. 11–16), one breast carcinoma (MDA-MB 435; Ref. 17), one non-small cell lung adenocarcinoma (EKVX; Refs. 15 and 16), and two osteogenic sarcomas (KPDX and OHS; Refs. 18–20).

Evaluation of s.c. Tumor Growth. Tumor pieces, about 2 × 2 × 2 mm3, were implanted into each flank of female nude mice. The tumors were allowed to grow to a medium diameter of about 6 mm before the start of treatment (day 0). Each group consisted of at least six tumors in five animals, randomized in a size-matched manner.

At the start of treatment, the minimum tumor diameter was 4 mm, equivalent to a volume of 32 mm3. If a mouse died within 2 weeks after the final injection, this was considered toxic death, and the animal was excluded from the study. Treatment causing more than 10% body weight loss, compared with controls, was considered not evaluable for antitumor efficacy. The drug effects were calculated from growth curves constructed on the basis of median RTVs, where the calculated volume at any day X, based on two perpendicular measurements of the tumor twice weekly, was divided by the tumor volume at the start of treatment (day 0; Refs. 15, 21, 22). Tumor volume was calculated according to the formula: tumor volume = 0.5 × length × width2; and median RTVs were used for construction of growth curves and to calculate treatment efficacy (Table 1; Ref. 15). The time to reach one or two TD times (TD50 and TD90) was calculated for each individual tumor, and the mean values in each...
RESULTS

Doses and Schedules in Rats. Initial treatment of leptomeningeal lymphomas in rats indicated a clear relationship between the response and the number of times the daily bolus dose was repeated, both for P-4055 and cytarabine given at equitoxic or equimolar doses. No cumulative toxicity was observed when a once daily dose, in a 40-μl volume, was given for 4 subsequent days after cell injection.

Doses and Schedules in Mice. The optimal i.p. doses of P-4055 and cytarabine were found to be 20–30 mg/kg and 100–200 mg/kg, respectively, depending on the frequency of dosing. In inhibiting tumor growth, schedules comprising repeated daily injections were clearly favorable over schedules with weekly or every 2nd-day injec-

Table 1. Antitumor effects of P-4055 (25 mg/kg) and cytarabine (100 mg/kg) in human tumor xenografts i.p., days 0–4, 7–11, 14–18, and 21–25

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Drug</th>
<th>Number of tumors</th>
<th>Body weight loss (%)</th>
<th>Toxicity</th>
<th>TD_{200}</th>
<th>TD_{400}</th>
<th>T/C%</th>
<th>SGD_{200}</th>
<th>SGD_{400}</th>
<th>Efficacy</th>
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<td>LOX (malignant melanoma)</td>
<td>Control</td>
<td>11</td>
<td>0/7</td>
<td>2.0</td>
<td>4.0</td>
<td>100</td>
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<tr>
<td></td>
<td>P-4055</td>
<td>11</td>
<td>2.9</td>
<td>0/7</td>
<td>36.5</td>
<td>38.5</td>
<td>2.8</td>
<td>17.3</td>
<td>8.6</td>
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<td>0/7</td>
<td>2.5</td>
<td>4.5</td>
<td>76.5</td>
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<td>0.1</td>
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<td>70.0</td>
<td>6.6</td>
<td>&gt;22.3</td>
<td>&gt;22.3</td>
<td>&gt;22.3</td>
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</tr>
<tr>
<td></td>
<td>Cytarabine</td>
<td>10</td>
<td>0/6</td>
<td>&gt;70.0</td>
<td>&gt;70.0</td>
<td>&gt;8.6</td>
<td>&gt;9.0</td>
<td>&gt;9.0</td>
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<td>Control</td>
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<td>0/6</td>
<td>7.0</td>
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<td>12.1</td>
<td>0/6</td>
<td>16.5</td>
<td>27.0</td>
<td>3.8</td>
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<tr>
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<td>7</td>
<td>13.4</td>
<td>1/6</td>
<td>16.5</td>
<td>27.0</td>
<td>38.3</td>
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<tr>
<td>EKXX (lung adenocarcinoma)</td>
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<td>0/5</td>
<td>15.0</td>
<td>27.0</td>
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<td></td>
<td>P-4055</td>
<td>6</td>
<td>5.3</td>
<td>&gt;100.0</td>
<td>&gt;100.0</td>
<td>1.9</td>
<td>5.7</td>
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</tr>
<tr>
<td></td>
<td>Cytarabine</td>
<td>6</td>
<td>2.3</td>
<td>0/5</td>
<td>&gt;100.0</td>
<td>&gt;100.0</td>
<td>1.0</td>
<td>&gt;2.7</td>
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<td>++++</td>
</tr>
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<td>MDA-MB 435 (breast cancer)</td>
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<td>11</td>
<td>0/9</td>
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<tr>
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<td>P-4055</td>
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<td>7.1</td>
<td>2/9</td>
<td>12.0</td>
<td>30.0</td>
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<td>0.1</td>
<td>0.3</td>
<td>(+)</td>
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<tr>
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<tr>
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<td>9</td>
<td>0/7</td>
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<td>100</td>
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<tr>
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<td>P-4055</td>
<td>8</td>
<td>0</td>
<td>0/6</td>
<td>7.5</td>
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<td>55.4</td>
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<td>–</td>
</tr>
<tr>
<td></td>
<td>Cytarabine</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>–</td>
</tr>
<tr>
<td>OHS (osteogenic sarcoma)</td>
<td>Control</td>
<td>9</td>
<td>1.3</td>
<td>0/8</td>
<td>5.5</td>
<td>14.5</td>
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<tr>
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<td>P-4055</td>
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<td>0</td>
<td>2/8</td>
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<td>81.8</td>
<td>0.4</td>
<td>0.4</td>
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</table>

* a TDs of test and control groups were defined as the period (days) required to reach a median RTV value of 200 or 400.

b A SGD was calculated as follows: SGD = (T/D treated-T/D control)/TD control.

c Efficacy as defined by: (T/C% < 50% or SGD > 1.0) / T/C% < 40% and SGD > 1.5; ++++ T/C% < 25% and SGD > 3.0. Based on the experience with the model used, a T/C% value of < 50% and a SGD value of >1.0 were defined as minimum criteria for antitumor activity.

EFFECTS OF P-4055 ON s.c. AND METASTATIC HUMAN TUMORS

Fig. 1. Composed figure of mean RTV growth curves of the s.c. LOX melanoma, with results obtained in several experiments with i.p. P-4055 treatment using different doses and schedules. Groups of seven to nine animals with 9–11 tumors: saline control (●); 20 mg/kg, days 0–4 (◇); 30 mg/kg, days 0–4 (□); 30 mg/kg, days 0–9 (●); and 25 mg/kg, days 0–4, 7–11, 14–18, and 21–25 (△).

Drugs. The preparations of P-4055 were produced at Norsk Hydro Research Centre. Cytarabine (Cytosar; Upjohn, Wilmington, DE) was used for comparative studies. The drugs were kept at 4°C until used, and all drug dilutions were prepared freshly before treatment by adding saline.

Doses and Schedules Used in c.m. Models in Rats. Before the start of the study, dose-finding experiments were carried out with both compounds to determine the MTD. In healthy nude rats, bolus injections (40 μl) possible to administer in this compartment, and

Cell Proliferation Assay. Exponentially growing cells were seeded into 96-well microtiter plates (Falcon; Becton Dickinson) at a final density of 2.5 × 10^5 cells/ml in RPMI-1640-based growth medium. The nucleoside transport carrier inhibitors NBMPR and diprydiamol, both purchased from Sigma Chemical Co., were used at subtoxic levels, and cytarabine and P-4055 were added at 1:5 serial dilutions in the appropriate concentration range. In each well, the final volume was 200 μl. The cells were then allowed to proliferate for 72 h (for approximately three cell generations) at 37°C in a humidified CO2-controlled atmosphere. At the end of the incubation period, the cells were counted with a Coulter Counter (Coulter Electronics, Harpenden Herts, United Kingdom). The IC_{50} value was defined as the concentration of compound that inhibited cell proliferation by 50% compared with the untreated control (23). The cell lines used were: the murine leukemia L1210; the human T-cell leukemias CEM, Molt-4/clone 8 (24), and Molt-4; and the THX melanoma.

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Tumor xenografts and in the Raji systemic leukemia model. For both drugs in inhibiting the growth of solid tumor xenografts are summarized, using the described activity criteria. Partial or complete tumor regression of the lung carcinoma and all three malignant melanomas was obtained after treatment with P-4055. In two of the melanomas, the effect was much stronger than that of cytarabine.

Thus, pronounced regression of the P-4055-treated LOX tumors was observed with 2 of the 11 tumors seemingly eradicated. All tumors started, however, to regrow 7–10 days after the end of treatment (Fig. 3A). Cytarabine did not affect tumor growth, and all of the animals had to be sacrificed on day 16 after the start of treatment, as was the case for the untreated control animals. The optimal T/C% [(RTV treated/RTV control) × 100%] values were 2.8 for the P-4055 and 76.5 for the cytarabine group (Table 1). The calculated SGD over one doubling time (SGD$_{200}$) was 17.3 and 0.3 for P-4055 and cytarabine, respectively.

For the THX malignant melanoma, the latency from tumor implantation until the start of treatment was 19 days. As shown in Fig. 3B, both drugs were highly active, and 5 (P-4055) and 3 (cytarabine) of 10 tumors disappeared and showed no regrowth within 116 days after the start of treatment.

Like LOX, the melanotic HHMSX xenograft line was highly sensitive to P-4055 and moderately sensitive to cytarabine (P < 0.001; Fig. 3C), with T/C% values of 11 and 38.3 and SGD$_{200}$ of 5.6 and 1.4 (Table 1). One death among six animals was observed in each group. This might be caused by a combination of drug toxicity and cachexia, which is known to appear in mice carrying this tumor, reflected in a weight loss of 9.2% in the control group. The rather slowly growing and generally resistant EKVX non-small cell lung cancer was also highly responsive to both P-4055 and cytarabine, with regression of the tumors from the start of treatment, although none of the tumors disappeared completely (Fig. 3D). TD$_{200}$ was not reached within more than 100 days, resulting in SGD of >5.7 for both drugs (Table 1). The optimal T/C% values were 1.9 and 1.0 for P-4055 and cytarabine, respectively.

In the MDA-MB 435 breast carcinoma and the osteogenic sarcoma

One daily injection, repeated for 5 days, with a recovery period of 2 days before the next cycle, was found to be optimal both for cytarabine and P-4055. Therefore, a schedule with injections on days 0–4, 7–11, 14–18, and 21–25 was chosen in efficacy studies in solid tumor xenografts and in the Raji systemic leukemia model. For both compounds, the treatment was generally well tolerated, with a weight loss indicating a dose level close to the MTDs, but with very few toxic deaths (Table 1).

Activity in Leptomeningeal Carcinomatosis Models. In the Raji model, the saline-treated control animals developed central nervous symptoms after 13–14 days (mean, 13.2). The drugs were given as four bolus injections at maximum concentrations on 4 subsequent days after cell inoculation. As can be seen from the survival curves (Fig. 2A), the P-4055 treatment resulted in a strong increase in survival as compared with the other groups. In a one-way ANOVA based on rank sums, the differences were statistically significant (P < 0.05, Kruskal-Wallis test), compared with saline-treated animals, as well as the groups treated with maximum (20 mg/ml) or equimolar doses (10 mg/ml) of cytarabine. In addition, three (60%) of the P-4055-treated rats and none of the cytarabine-treated rats were long-time survivors.

In the Molt-4 model, both P-4055 and cytarabine were effective. The mean latency before the animals developed symptoms of leptomeningeal tumor growth was 22.3, 42 (one survivor), and 39 (one survivor) days in the control, P-4055-, and cytarabine-treated groups, respectively (data not shown).

Activity in the Raji Systemic Leukemia Model. After i.v. injection of 1 × 10$^6$ Raji cells, the mice developed paralysis as a result of systemic growth of tumor cells after a mean latency of 40 days (range, 34–48) in the control group (n = 10; one survivor). The i.p. drug doses were 25 mg/kg P-4055 and 100 mg/kg cytarabine, given on days 7–11, 14–18, 21–25, and 28–32 after cell injection (n = 10 in both groups). A highly significant increase (P < 0.001, Kruskal-Wallis test) in survival was observed for the P-4055-treated group, with 8 of 10 animals living more than 80 days after the start of treatment, compared with none of the cytarabine-treated animals (mean survival time, 34.2 days; Fig. 2B)

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In the MDA-MB 435 breast carcinoma and the osteogenic sarcoma

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KPDX and OHS models, P-4055 and cytarabine were both inactive, except from a marginal effect, against MDA-MB 435 (Table 1).

**Effects of Nucleoside Transport Inhibitors in Vitro.** In attempts to elucidate mechanisms underlying the differential activity of P-4055 and cytarabine *in vivo*, we examined how nucleoside transport carrier inhibitors could influence their antiproliferative capacity *in vitro*. The effects of the two compounds on murine L1210 and human CEM, Molt-4, Molt-4/clone 8, and THX cells were evaluated in the presence or absence of NBMPR and dipyridamol at subtoxic concentrations (100 μM and 4 μg/ml, respectively).

The IC₅₀ values of cytarabine and P-4055 ranged from <0.001 μM to 1.0 μM, depending on the tumor cell line. Importantly, in all cases, the presence of the transport inhibitors (NBMPR or dipyridamol) partially reversed the cytostatic activity of cytarabine, but not that of P-4055. Whereas in L1210 cells the inhibitory effect of cytarabine on cell proliferation was reduced 3–4-fold by NBMPR and dipyridamol, the values were as high as 12–50-fold, 7–26-fold, and 40–75-fold in CEM, Molt-4/clone 8, and THX cell cultures, respectively (Table 2). In striking contrast, neither NBMPR nor

**Table 2. Effects of nucleoside transport inhibition on the in vitro cytotoxic effect of cytarabine and P-4055**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Nucleoside transport inhibitor</th>
<th>IC₅₀(μM)</th>
<th>Cytarabine</th>
<th>P-4055</th>
</tr>
</thead>
<tbody>
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<td>L1210</td>
<td>As such</td>
<td>0.04 ± 0.009</td>
<td>0.04 ± 0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ NBMPR</td>
<td>0.12 ± 0.01</td>
<td>0.06 ± 0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ Dipyridamole</td>
<td>0.16 ± 0.01</td>
<td>0.07 ± 0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>As such</td>
<td>0.04 ± 0.006</td>
<td>0.07 ± 0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ NBMPR</td>
<td>0.49 ± 0.05</td>
<td>0.06 ± 0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ Dipyridamole</td>
<td>1.9 ± 0.15</td>
<td>0.09 ± 0.008</td>
<td></td>
</tr>
<tr>
<td>Molt-4</td>
<td>As such</td>
<td>0.0001 ± 0.00002</td>
<td>0.0009 ± 0.00002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ NBMPR</td>
<td>4.35 ± 1.53</td>
<td>0.036 ± 0.014</td>
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</tr>
<tr>
<td></td>
<td>+ Dipyridamole</td>
<td>2.82 ± 0.18</td>
<td>0.038 ± 0.005</td>
<td></td>
</tr>
<tr>
<td>Molt-4/clone 8</td>
<td>As such</td>
<td>1.00 ± 0.12</td>
<td>0.12 ± 0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ NBMPR</td>
<td>7.65 ± 0.75</td>
<td>0.038 ± 0.006</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ Dipyridamole</td>
<td>25.6 ± 5.4</td>
<td>0.076 ± 0.003</td>
<td></td>
</tr>
<tr>
<td>THX</td>
<td>As such</td>
<td>0.32 ± 0.18</td>
<td>0.23 ± 0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ NBMPR</td>
<td>12.6</td>
<td>0.072 ± 0.039</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ Dipyridamole</td>
<td>23.5 ± 6.3</td>
<td>0.17 ± 0.05</td>
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</tbody>
</table>

*NBMPR was used at a subtoxic concentration of 100 μM.*  
*Dipyridamole was used at a subtoxic concentration of 4 μg/ml.*
dipryidamol had any appreciable influence on the cytostatic activity of P-4055 in any of the cell lines, except in Molt-4 cells, where the activity decreased 40–43-fold, compared with >43,000- to >28,000-fold for cytarabine.

DISCUSSION

In a number of in vivo human tumor models, P-4055, a novel conjugate of cytarabine and elaidic acid, was superior in antitumor activity compared with its parent compound cytarabine. Differential activity was seen in several experimental metastasis and s.c. models in nude mice and rats, including models for leptomeningeal carcinomatosis and systemic leukemia, as well as two of the four s.c. xenografts that responded to these drugs. Because no differences in effects were found in some of the models, the observed selectivity may reflect tumor-associated differences in cellular uptake and/or metabolism of the compounds.

Hardly any data have been reported on cytarabine having an effect against solid tumors in the clinic (1, 25–27), and part of our findings with P-4055 and cytarabine were, therefore, surprising. However, another nucleoside analogue of deoxycytidine 2′,2′-difluorodeoxy- cytidine (dFdC, gemcitabine) has proven effective in the treatment of nonhematological tumors, both preclinically and in clinical trials in patients with lung, ovarian, breast, and pancreatic cancers (3, 28, 29), indicating that deoxycytidine analogues possess a potential for a wider spectrum of activity than leukemias and lymphomas. This might be dependent on optimal scheduling of the treatment.

Because of the rapid inactivation of cytarabine by cytidine deaminase, and its phase-dependent tumor cell killing, the drug is usually administered to patients as a continuous infusion of 100–200 mg/m² for 5–10 days. An alternative high-dose regimen for patients with refractory forms of acute leukemia is to use 2–3 g/m², given as a 2-h infusion twice daily for 6 days; but, it is not clear to us whether similar dosages and schedules have been tested in patients with solid cancer.

P-4055 was synthesized with the aim of reducing the deamination of cytarabine, possibly lowering the clearance, and increasing the intracellular level of the drug in tumor cells. The results obtained here suggest that, in part, this goal may have been reached. When calculated at a molar level of active nucleoside, the results were obtained at an 8-fold lower dose of cytarabine in the form of P-4055. After testing different doses and schedules to establish the MTD at a schedule that also resulted in the best antitumor effects, both drugs were given as daily bolus injections. With this approach, significant activity with cytarabine, as such, was also seen.

Cytarabine differs from its physiological counterpart only by the presence of a hydroxyl group in β-configuration, at the 2′ position of the arabinosyl sugar moiety. Cytarabine is converted intracellularly to its active derivative by phosphorylation into a three-phosphate pyrimidine nucleoside by different kinases, where the 5′monophosphorylation catalyzed by deoxycytidine kinase is the rate-limiting step. In its active form, cytarabine acts as an antimetabolite and, as a competitive inhibitor of DNA polymerase, it interferes with the synthesis of DNA. The formation and retention of intracellular cytarabine-three-phosphate are the most important determinants of the cytotoxicity of cytarabine, and is further found to correlate with the number of transport sites for cytarabine (30), because the drug penetrates cells by a carrier-mediated process (31).

The superior activity of P-4055 may be explained by differences in cellular transport mechanisms, as demonstrated indirectly by in vitro experiments where the cytotoxicity of the two compounds to different human tumor cell lines was measured, with or without blocking of the active transport sites for cytarabine. The data obtained in five different tumor cell lines clearly indicate that cytarabine is, at least partially, dependent on its uptake by the nucleoside transport carrier, whereas P-4055 is almost completely independent. These observations may have two different implications. First, P-4055, in contrast to cytarabine, enters the cells presumably by alternative/additional mechanisms, one of which may be passive diffusion, a nonsaturable process. Therefore, it might be expected that uptake of P-4055 by the tumor cells will not be limited by saturation of, or by competition of other compounds for the nucleoside transport carrier. Second, whereas resistance of tumor cells against cytarabine can develop at the level of transport into the tumor cells, this would not affect P-4055 uptake giving P-4055 an additional benefit over cytarabine in the clinical setting.

Preliminary pharmacokinetic data in male rats (data not shown) indicate a higher plasma level of free cytarabine administered as P-4055, compared with cytarabine given as such at an equimolar dose. Functional studies to examine hydrolysis of the ester bond between elaidic acid and cytarabine, and to study whether this is taking place in the plasma compartment only, and/or after transport into the cells, will be initiated. Results with various conjugates of different fatty acids, with C-atoms ranging from 16–22, however, indicate that both the chain-length of and the degree of saturation of the fatty acid is important (4, 5). The natural formation of micelles from the lipophilic cytarabine and a possibly delayed clearance because of capillary entrapment might be contributing factors, because formulations with simple trapping of cytarabine into small liposomes yielded increased activity in some studies (32–34).

When cytarabine is administered by continuous i.v. infusion to patients, it distributes rapidly into total body water with concentrations in the CSF reaching 50% of simultaneous plasma levels after 2 h. P-4055 has in this study proven effective against lymphomas growing in the CNS compartment. However, because the models used here were examined with bolus injections given intrathecally to directly reach the CSF, the efficacy of systemic P-4055 treatment against metastases to the CNS is not yet determined.

In conclusion, P-4055 is a new analogue of cytarabine, with a different or at least an additional mechanism of transport into the cell. The compound was active against experimental human lymphomas growing systemically or in the CNS of nude mice and rats, as well as against solid human tumor xenografts growing s.c. in immunodeficient mice. The superior and differential activity compared with cytarabine suggests that P-4055 might be effective also in the treatment of solid tumors in the clinical setting.

REFERENCES


Antitumor Activity of P-4055 (Elaidic Acid-Cytarabine) Compared to Cytarabine in Metastatic and s.c. Human Tumor Xenograft Models

Knut Breistøl, Jan Balzarini, Marit Liland Sandvold, et al.


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