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ORIGINAL ARTICLE

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Antitumor activity of troxacitabine (Troxatyl) against anthracycline-resistant human xenografts

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Abstract *Purpose*: We have recently identified a deoxycytidine nucleoside analogue, troxacitabine (β-L-dioxolane cytidine, Troxatyl; Shire BioChem), which has potent antitumor activity against both leukemia and solid tumors. In contrast to the cytidine nucleoside analogues currently in clinical use (cytarabine and gemcitabine), troxacitabine is a poor substrate of nucleoside transporters and enters cells primarily by passive diffusion. This unusual property led us to evaluate the efficacy of troxacitabine in multidrug resistant (MDR) and multidrug resistance-associated protein (MRP) tumors. *Methods*: The in vitro antiproliferative activity of troxacitabine was investigated in the human nasopharyngeal epidermoid carcinoma cell line, KB, and its vincristine-resistant derivative (KBV), as well as in human leukemia cell lines of myeloid and lymphoblastoid origin, HL60 and CCRF-CEM, respectively, and their MDR (HL60/R10 and CCRF-CEM/VLB) and MRP (HL60/ADR) derivatives, using the thymidine incorporation assay. For in vivo studies, we compared the antitumor efficacy of troxacitabine with that of doxorubicin and vinblastine in xenograft models of these solid and hematological human anthracycline-resistant tumor xenografts. *Results*: Troxacitabine demonstrated potent antiproliferative activity against both P-glycoprotein-positive (KBV, HL60/R10, CCRF-CEM/VLB) and P-glycoprotein-negative (HL60/ADR) multidrug-resistant cell lines with IC_{50} values ranging from 7 to 171 n *M*. Tumor regression was observed in the KBV xenograft following a 5-day treatment with 20, 50 and 100 mg/kg of troxacitabine, with percent total growth inhibition (TGI) of 81, 96 and 97, respectively, and some cures at the two highest dose levels. In the HL60, HL60/R10, HL60/ADR and CCRF-CEM/VLB xenografts, the effect of troxacitabine was evaluated on survival time. In the HL60 promyelocytic human xenograft models, troxacitabine treatment (25, 50 and 100 mg/kg per day for 5 days) was initiated 10 days after tumor cell inoculation, once animals had developed disseminated tumors. In all three promyelocytic leukemia xenografts, troxacitabine was quite potent, producing T/C values of 162% to 315% as well as complete cures at the higher dose levels. In the CCRF-CEM/VLB T-lymphoblastoid leukemia xenograft, troxacitabine treatment (10, 30 or 250 mg/kg total doses using different schedules) was initiated 20 days after tumor cell inoculation. Troxacitabine was not as potent in this model but did result in significant antileukemic activity (T/C of 131%) when administered at 10 mg/kg on days 20, 27 and 34. *Conclusions*: These results indicate that troxacitabine has a potent in vivo antitumor activity associated with tumor regressions and complete cures in animals with tumors refractory to current chemotherapeutic agents.

Keywords Nucleoside analogues · Troxacitabine · Multidrug resistance · Xenografts · Cancer

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Introduction

Tumor cell resistance to cytotoxic agents is considered one of the major limiting factors to successful

chemotherapy. Multidrug resistance (MDR) is believed to be one of the relevant mechanisms of primary or acquired resistance in human leukemias and solid tumors [6, 11, 17]. The MDR phenotype has been associated with the overexpression of various members of the ATP-binding cassette (ABC) transporters. The first human ABC transporter identified was P-glycoprotein (Pgp) which is the product of the *mdr*1 gene. The other subfamilies include the multidrug resistance-associated proteins (MRP1–7) (reviewed in ref 18). Although Pgp and MRP1 show little homology, having only 15% identity at the amino acid level, both are ATP-binding proteins and confer resistance to a similar range of chemotherapeutic agents. In various human tumors, including acute leukemias, breast, renal, and esophageal carcinomas, neuroblastomas, and childhood sarcomas, *mdr*1 expression has been shown to correlate with clinical drug resistance [2, 16, 20, 25]. Pgp has been demonstrated to act as an efflux pump for anthracyclines, as well as for various unrelated cytotoxic drugs. This efflux can be circumvented in vitro by a variety of noncytotoxic agents [3, 27], but their clinical use has met with limited success [12, 15]. Therefore, a need still exists for the development of new drugs unaffected by the MDR phenotype.

Recently, we have synthesized a nucleoside analogue, troxacitabine (Troxatyl, Shire BioChem), which shows potent and broad antitumor activity in animal models [7, 10, 14, 24, 28]. Unlike other currently used antitumor nucleoside analogues (such as cytarabine and gemcitabine), troxacitabine is in a stereochemically unnatural β-L configuration and has distinct metabolic and pharmacokinetic properties. The rate-limiting step in its intracellular phosphorylation is its conversion to troxacitabine monophosphate catalyzed by deoxycytidine kinase (dCK; EC 2.7.1.74) (with the diphosphate being the major metabolite) and it is resistant to inactivation through deamination [9]. In uptake studies of radiolabelled troxacitabine by cell lines with defined nucleoside transport activities, troxacitabine has been shown to be a poor permeant for the molecularly characterized equilibrative (hENT1, hENT2) and sodium-dependent (hCNT1, hCNT2, hCNT3) nucleoside transporters [8]. Since troxacitabine most likely enters cells by passive diffusion, we were interested to evaluate its efficacy against MDR tumors. The antitumor activity of troxacitabine was therefore evaluated in hematological and solid tumor xenografts expressing the MDR or MRP phenotype.

Material and methods

Drugs

Troxacitabine was synthesized at Shire BioChem [19], vinblastine (Velbé) was purchased from Lilly Labs (St-Cloud, France) and doxorubicin was obtained from Sigma-Aldrich (Mississauga, Ontario, Canada).

Cell culture and cytotoxicity studies

The human hematopoietic cell lines, HL60 and CCRF-CEM, and their MDR (HL60/R10 and CCRF-CEM/VLB) and MRP (HL60/ADR) derivatives were from P. Genne (ONCODESIGN Biotechnology, Dijon, France). The human nasopharyngeal epidermoid carcinoma cell line, KB, and its vincristine-resistant derivative, KBV, were kindly provided by Dr. Moulay Alaoui-Jamali (Lady Davis Institute for Medical Research, Montréal, Québec, Canada). The hematopoietic cell lines were routinely maintained in RPMI-1640 while the KB and KBV carcinoma paired cell lines were cultured in αMEM containing nonessential amino acids. The medium was supplemented with 10% heat-inactivated fetal bovine serum (hiFBS) and cells were incubated under humidified air containing 5% CO_2 at 37°C. Antibiotics were not used and cells were routinely checked for mycoplasma contamination by PCR analysis (Mycoplasma PCR detection kit; Stratagene, La Jolla, Calif.).

For cytotoxicity studies, cells in logarithmic growth phase were plated in 96-well plates at a density of 10^4 cells per well (cell numbers were determined using a hemacytometer). Cells were exposed to troxacitabine or doxorubicin for 3 days at different concentrations. The final drug concentrations in the medium were in tenfold increments ranging from 10^{-10} to 10^{-4} *M*. As a measure of proliferation, [methyl-^{3}H]thymidine (0.5 μCi per well, specific activity 2 Ci/mmol; Amersham Canada) was added during the final 18 h of incubation. At the end of the incubation period, cells were aspirated directly (HL60 and CCRF-CEM cells) or following trypsinization (KB cells) onto glass-fiber filters. Membranes were dried and placed in plastic sample bags containing 6 ml scintillation cocktail. [^{3}H]Thymidine incorporation was measured with a β-scintillation counter (1450 Microbeta, Wallac, Finland). The results are expressed as percent of untreated cells, which was set at 100%. Each point represents a mean value of four to six measurements. The IC_{50} was estimated from individual inhibition curves and represents the concentration of drug that inhibits cell proliferation by 50%.

Pgp detection

Expression of Pgp was measured by flow cytometry. Resistant cell lines were continuously exposed to low concentrations of anthracyclines prior to the start of the experiment in order to ensure proper expression of Pgp. Drug exposure was stopped 48 h before analysis to allow resistant cells to recuperate. Cells (2×10^6) from each cell line were washed once in cold 1×PBS. The cell pellet was then resuspended in 100 μl PBS containing 2% hiFBS and a PE-labeled anti-Pgp antibody or a PE-labeled mouse IgG isotype control (Becton Dickinson, San José, Calif.) at a concentration of 1 μg/10^6 cells. The cells were incubated with the antibody for 1 h at room temperature in the dark. The cells were then washed twice in 1 ml PBS/2% hiFBS, the cell pellet resuspended in 500 μl PBS/2% hiFBS, and 10 μg of propidium iodide was added. The cells were then analyzed on a Coulter EPICS XL-MCL flow cytometer (Beckman Coulter, Fullerton, Calif.).

In vivo studies

BALB/c female nude mice (Charles River, St-Constant, Québec, Canada) at 6–8 weeks of age were injected subcutaneously (s.c.) with 2×10^6 KBV cells (day 0). Prior to inoculation, the KBV cells were passaged in the presence of 1 μ*M* vinblastine. Administration of the drug or vehicle began on day 14 when the median tumor mass ranged from 190 to 300 mg. Tumor-bearing animals were randomized (ten per group) prior to treatment. Troxacitabine was administered intraperitoneally (i.p.) at 10, 25 and 50 mg/kg twice a day (6 h apart) for five consecutive days. Doxorubicin was administered intravenously (i.v.) at 10 mg/kg on days 17 and 24. Tumor measurements were taken twice weekly using calipers and were converted to tumor mass (in milligrams) using the formula:

width^2 (mm)×length (mm)×0.52. Body weights were also recorded twice weekly. The experiment was terminated on day 37 when the average tumor mass in the control group reached a median of 2.3 g. Tumor growth inhibition (TGI) was calculated by subtracting from 100% the mean treated tumor mass/mean control tumor mass×100%.

For the myeloid leukemia survival studies, female SCID mice (Charles River, St-Constant, Québec, Canada) at 3–5 weeks of age were injected i.p. with 1.5×10^7 HL60, 2.5×10^7 HL60/R10, or 2.5×10^7 HL60/ADR tumor cells (day 0). Tumor-bearing animals were randomized (ten per group; saline groups had five animals) and treatment with troxacitabine or doxorubicin was initiated 10 days after tumor cell inoculation once the mice had developed palpable tumors at the site of inoculation. Troxacitabine was administered i.p. at 25, 50, and 100 mg/kg once a day for five consecutive days. Doxorubicin was administered i.p. on days 10, 14, and 18 at 2 mg/kg, which is the maximum tolerated dose for SCID mice.

In the CCRF-CEM/VLB leukemia study, female CB17/IcrIco-SCID mice at 3–5 weeks of age were first irradiated following exposure to 180 rad from a γ source (Co60, INRA, Dijon, France). Mice were injected i.p. 24 h later with 3×10^7 CCRF-CEM/VLB cells. Prior to injection, cells had been passaged in the presence of 0.5 μg/ml vinblastine. The animals were injected i.p. with troxacitabine for five consecutive days (days 20 to 24) twice-daily with 25 mg/kg per injection, or on 3 days with 10 mg/kg per injection at intervals of 7 days (days 20, 27 and 34), or only on day 20 with 10 mg/kg. Vinblastine was administered i.p. at 1 mg/kg on day 20 (this dose is the maximum tolerated dose for irradiated SCID mice).

The results are expressed as percent of mean survival time of treated animals over mean survival time of the control group (treated vs control, T/C%) and as increase in life span (mean survival time of treated animals minus that of control animals over the mean survival time of the control group; ILS%). By NCI criteria, T/C exceeding 125% and ILS exceeding 25% indicate that the drug has significant antitumor activity [23]. The efficacy of the treatment was analyzed using the unpaired Student's *t*-test (at $P<0.05$ to $P<0.001$).

All animals received humane care in compliance with the Canadian Council Guidelines for the Care and Use of Experimental Animals. For the leukemia survival studies, animals were thus killed if the abdominal mass was significantly enlarged or when the body temperature dropped below 34°C. Shire BioChem's Animal Care Committee approved these in vivo experimental protocols and the Canadian Council for Animal Care accredits our animal facility.

Results and discussion

The in vitro toxicity of troxacitabine against pairs of parental and corresponding MDR cells was compared with that of doxorubicin by assessing their relative abilities to inhibit proliferation during 72-h continuous exposures (Table 1). The MDR cell lines are of hematopoietic and epithelial origin and have different MDR phenotypes. The human promyelocytic leukemia cell line HL60/R10, the human T-lymphoblastoid leukemia cell line CCRF-CEM/VLB and the human nasopharyngeal epidermoid vincristine-resistant carcinoma cell line, KBV, have alterations in the *mdr*1 gene [1, 13, 26], while HL60/ADR has alteration in the *MRP* gene [21, 22]. These cell lines were chosen because we had previously shown that troxacitabine is effective in the parental (non-MDR) tumor xenografts [7]. As shown in Fig. 1, flow cytometric analyses using a specific Pgp monoclonal antibody indicated that KB/V, HL60/R10 and CEM/VLB express high levels of Pgp when compared to their respective parental cell lines (note the shift of the cell population to the right). In contrast, HL60/ADR, although resistant to doxorubicin (Table 1), was Pgp-negative (the fluorescence intensity was similar to that of the HL60 parental cell line; Fig. 1).

Table 1 Comparative in vitro antiproliferative activity of troxacitabine and doxorubicin on HL60, CCRF-CEM and KB parental and anthracycline-resistant subclones. IC_{50} values were measured after 72 h continuous exposure to the drugs. Antiproliferative activity of the drugs was determined in terms of [^{3}H]thymidine uptake during the final 18 h of a 3-day incubation. The values are the means ± SD of three experiments in which each data point was the average of six measurements

Cell line	IC_{50} (n*M*)	
	Troxacitabine	Doxorubicin
KB	171.2 ± 86	4.6 ± 1.5
KBV	63.4 ± 8.5	257 ± 121
HL60	53.7 ± 10.9	1.26 ± 1.7
HL60/R10	7.4 ± 0.6	6530 ± 947
HL60/ADR	61.2 ± 7.0	396 ± 109
CCRF-CEM	35.8 ± 14.8	4.7 ± 1.2
CCRF-CEM/VLB	80.7 ± 3.1	40.3 ± 8.4

Our in vitro studies demonstrated that doxorubicin was more potent than troxacitabine in inhibiting proliferation of parental KB, HL60 and CCRF-CEM cell lines, with IC_{50} values of 4.6, 1.3 and 4.7 n*M* vs 171, 54 and 36 n*M*, respectively (Table 1). As expected, doxorubicin was less active in inhibiting the proliferation of the anthracycline-resistant cell lines HL60/R10, HL60/ADR, CCRF-CEM/VLB and KBV (Table 1). Resistance ratios varied from cell to cell, differing by 56-fold in the KB pair, 5183- and 314-fold in the HL60 pair, and 8.6-fold in the CCRF-CEM pair. In contrast, the anthracycline-resistant cell lines remained sensitive to troxacitabine, with resistance ratios of 0.37 (KB pair), 0.13- and 1.1-fold (HL60 pair) and 2.3-fold (CCRF-CEM pair).

We then evaluated the antitumor efficacy of troxacitabine against these anthracycline-resistant tumor cell lines xenografted in immunodeficient mice. BALB/c female nude mice at 6–8 weeks of age were injected s.c. with KBV cells and treatment with troxacitabine (20, 50, 100 mg/kg per day for five consecutive days) or doxorubicin (10 mg/kg per day on days 14 and 21) was initiated when tumors had reached a palpable size (200–300 mg). Treatment of mice with troxacitabine at 50 and 100 mg/kg per day resulted in a highly significant tumor regression occurring from day 25 through day 37 with TGIs of 96.4% and 97.4% on day 37 (tumor size observed with the different treatments is shown in Fig. 2). At each of these two dose levels, we observed a complete regression in three animals. The 20 mg/kg per day dose was also effective, with a TGI of 81.4%. However, no cures were evident at that dose. A TGI of 50% was observed in the doxorubicin-treated group, but

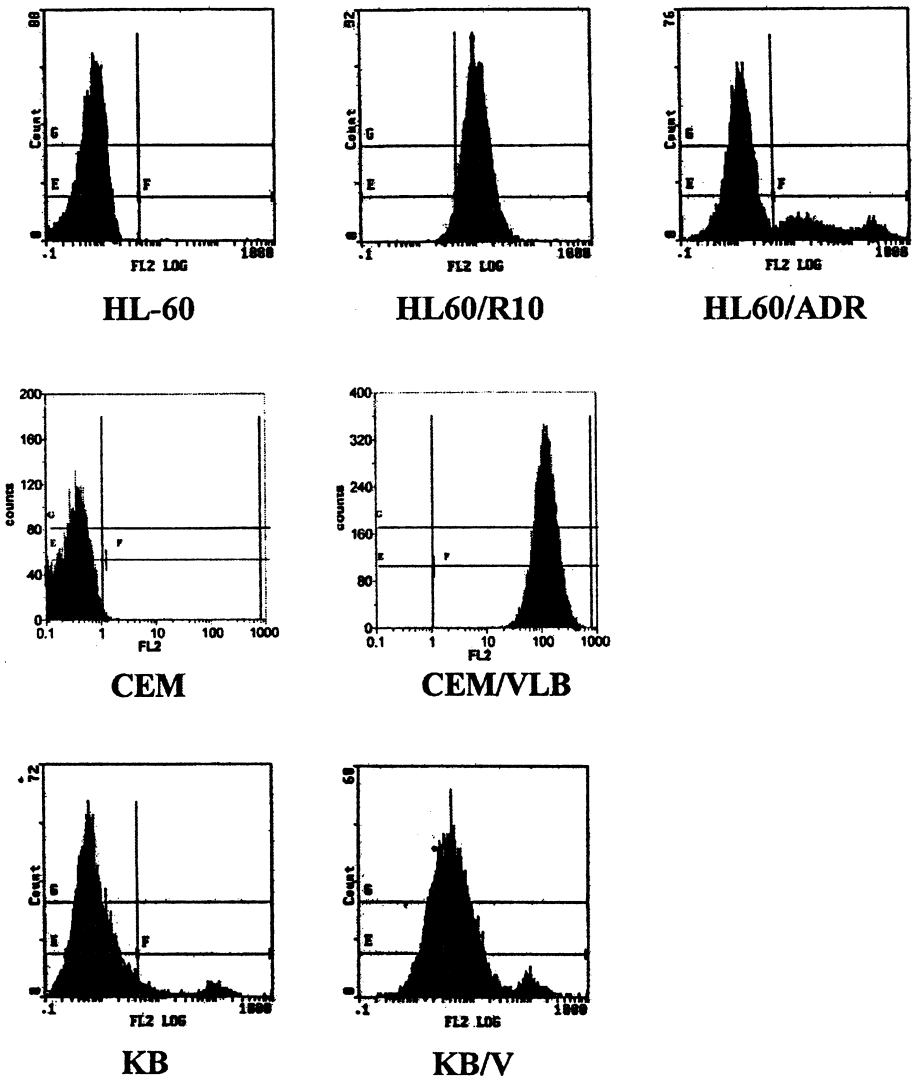

Fig. 1 Flow cytometry analysis of Pgp expression of wild-type and anthracycline-resistant cell lines. Cells were incubated with a PE-labeled specific Pgp antibody as described in Material and methods and analyzed by flow cytometry

the average tumor size had increased from 291 to 1192 mg; in the saline-treated control group the tumor size went from 280 to 2361 mg during this period, representing a 400-fold and 840-fold increase, respectively. Troxacitabine was therefore substantially more effective than doxorubicin in the KBV xenograft at doses that were well tolerated and did not produce any undesirable side effects (significant weight loss was not observed in any of the treated groups).

HL60, HL60/R10, and HL60/ADR cells were inoculated into SCID mice and treatment with troxacitabine (25, 50 or 100 mg/kg i.p. per day for 5 days) and doxorubicin (2 mg/kg i.p. on days 10, 14 and 18) was initiated 10 days after tumor cell inoculation, when animals had developed disseminated tumors (survival curves are shown in Fig. 3, and the results are summarized in Table 2). The mice in the saline control group died within 20 days (HL60 and HL60/R10) or 40 days (HL60/ADR) after tumor inoculation. At morbidity, SCID mice with leukemia were noted to have massive hepatosplenomegaly, enlarged kidneys and large masses in the pelvic fat. There was general enlargement of peritoneal and mediastinal lymph nodes.

Doxorubicin, administered i.p. at the maximum tolerated dose, had a potent antitumor effect (T/C 140%, ILS 40%) in the HL60 tumor model, but, as expected, was not effective in the HL60/R10 and HL60/ADR tumor models (mean survival times were comparable with that of controls). In the HL60 and HL60/ADR models, troxacitabine was very effective at all doses tested. On day 63, the termination point of the experiment, 100% of all animals remained alive in all three different treatment groups (this gave T/C 315% and ILS 215% in the HL60 model, and T/C 162% and ILS 62% in the HL60/ADR model). At autopsy, these animals did not reveal any tumors (the animals had very little ascites and no other sign of tumor mass in their kidneys, intestines, or liver), except for two animals in the HL60/ADR model at the 25 mg/kg per day dose which showed an enlarged abdominal mass.

Significant antitumor activity was observed in the HL60/R10 tumor model receiving 25 mg/kg per day of

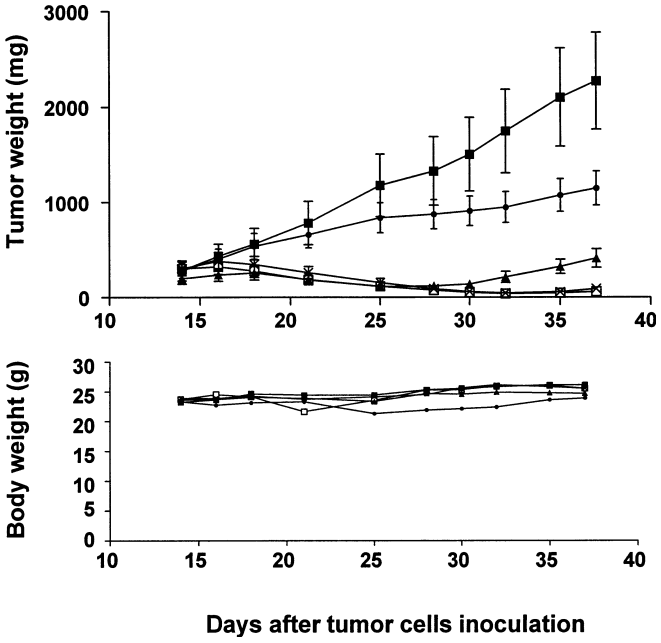

Fig. 2 Activity of troxacitabine and doxorubicin against the KBV human nasopharyngeal tumor xenograft. BALB/c female nude mice at 6–8 weeks of age were injected s.c. with 2×10^6 KBV cells (day 0). Treatments were started on day 14, once mice had developed palpable tumors: *filled squares* saline i.p. days 14 to 18, *filled circles* doxorubicin 10 mg/kg i.v. days 17 and 24, *filled triangles* troxacitabine 10 mg/kg twice daily i.p. days 14 to 18, *crosses* troxacitabine 25 mg/kg twice daily i.p. days 14 to 18, *open triangles* troxacitabine 50 mg/kg twice daily i.p. days 14 to 18

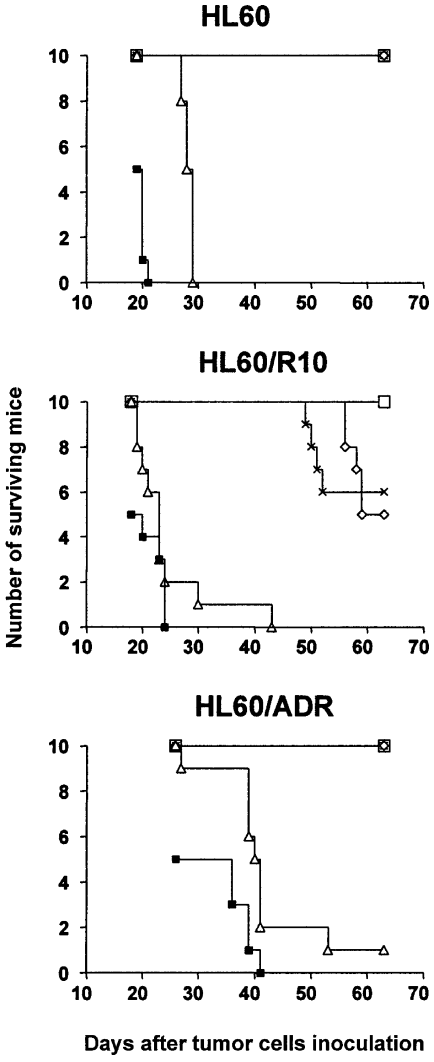

Fig. 3 Comparative effects of troxacitabine and doxorubicin on life span of mice bearing wild-type and anthracycline-resistant leukemias. Female SCID mice were injected i.p. with 1.5×10^7 HL60, 2.5×10^7 HL60/R10, or 2.5×10^7 HL60/ADR tumor cells (day 0). Treatment with troxacitabine or doxorubicin was started 10 days after tumor cell injections: *closed squares* saline i.p. days 10 to 14, *open triangles* doxorubicin 2 mg/kg i.p. days 10, 14 and 18, *diamonds* troxacitabine 25 mg/kg daily i.p. days 10 to 14, *crosses* troxacitabine 50 mg/kg daily i.p. days 10 to 14, *open squares* troxacitabine 100 mg/kg daily i.p. days 10 to 14

troxacitabine, with T/C and ILS values of 251% and 146%, respectively, and 40% of the animals surviving on day 63. In the 50 mg/kg per day group, T/C and ILS values were 242% and 163%, respectively, with 50% of the animals still alive on day 63. Mortality in these animals was caused by the development of leukemia. In the 100 mg/kg per day group, the T/C and ILS values were 263% and 163%, respectively, with all animals remaining alive until the end of the study. Of the surviving animals, two out of six at 25 mg/kg, three out of five at 50 mg/kg, and seven out of ten at 100 mg/kg had no clinical symptoms, while the other surviving mice had an increase in their abdominal mass.

CCRF-CEM/VLB cells were inoculated into irradiated SCID mice and these animals were treated with troxacitabine once they had developed disseminated tumors. Dosing was with a single 10 mg/kg i.p. injection on day 20, three 10 mg/kg i.p. injections on days 20, 27 and 34, or five consecutive 25 mg/kg twice-daily i.p. injections on days 20 to 24. A separate group of animals was treated with vinblastine (1 mg/kg i.p. on day 20). The results of these studies are summarized in Table 3. Sometimes, mice developed visible tumors at the site of inoculation after 10 days and a small amount of ascites. Gross examination at autopsy showed massive swelling of paraortic lymph nodes, mesenterium and pelvic fat. Tumor-invaded tissues included the liver, spleen, pancreas, fallopian tubes, pericardium, peribronchial and abdominal lymph nodes, bone marrow, mediastinum and mesentery. Solid tumors of various sizes were also found inside the peritoneal cavity, usually involving the entire mesentery. Both kidneys were embedded in solid tumor tissue.

All of the control saline-treated animals died within 44 days after CCRF-CEM/VLB cell inoculation. As expected, vinblastine, administered i.p. at the maximum tolerated dose, had no effect (T/C $<125\%$ and ILS $<25\%$) and did not significantly increase survival when compared with saline treatment ($P>0.05$; summarized in Table 3). Three different schedules and doses of troxacitabine were evaluated. The results showed that a

Table 2 Comparative in vivo antitumor activity of troxacitabine and doxorubicin in wild-type and anthracycline-resistant HL60 promyelocytic human leukemia xenografts. Female SCID mice were injected i.p. with 1.5×10^7 HL60, 2.5×10^7 HL60/R10, or 2.5×10^7 HL60/ADR tumor cells (day 0). Treatment with troxacitabine or doxorubicin was started 10 days after tumor cell injection once the mice had developed visible tumors at the site of inoculation (*T/C* treated vs control, *ILS* increase in life span)

Treatment group	Cell line	Mice per group	Dose (mg/kg) and route	Schedule	Survival (days)		ILS (%)	T/C (%)
					Range	Median		
Control	HL60	5	Saline i.p.	Daily ×5	20–21	20		
Doxorubicin	HL60	10	2 i.p.	Every 4 days ×3	27–29	28	40	140
Troxacitabine	HL60	10	25 i.p.	Daily ×5	63	63	215	315
Troxacitabine	HL60	10	50 i.p.	Daily ×5	63	63	215	315
Troxacitabine	HL60	10	100 i.p.	Daily ×5	63	63	215	315
Control	HL60/R10	5	Saline i.p.	Daily ×5	20–24	24		
Doxorubicin	HL60/R10	10	2 i.p.	Every 4 days ×3	19–43	23	–4.2	102
Troxacitabine	HL60/R10	10	25 i.p.	Daily ×5	56–63	59	146	251
Troxacitabine	HL60/R10	10	50 i.p.	Daily ×5	49–63	63	163	242
Troxacitabine	HL60/R10	10	100 i.p.	Daily ×5	63	63	163	263
Control	HL60/ADR	5	Saline i.p.	Daily ×5	36–41	39		
Doxorubicin	HL60/ADR	10	2 i.p.	Every 4 days ×3	27–63	40	2.6	103
Troxacitabine	HL60/ADR	10	25 i.p.	Daily ×5	63	63	61.5	162
Troxacitabine	HL60/ADR	10	50 i.p.	Daily ×5	63	63	61.5	162
Troxacitabine	HL60/ADR	10	100 i.p.	Daily ×5	63	63	61.5	162

Table 3 Comparative in vivo antitumor activity of troxacitabine and vinblastine in CCRF-CEM/VLB T-lymphoblastoid human leukemia. Irradiated female CB17/IcrIco-SCID mice were injected i.p. with 3×10^7 CCRF-CEM/VLB tumor cells (day 0). Treatment with troxacitabine or vinblastine was started 20 days after tumor cell injection once mice had developed visible tumors at the site of inoculation (*T/C* treated vs control, *ILS* increase in life span)

Treatment group	Mice per group	Dose (mg/kg) and route	Schedule	Survival (days)		ILS (%)	T/C (%)
				Range	Median		
Control	10	Saline i.p.	Daily ×5	40–50	41		
Vinblastine	10	1 i.p.	Single bolus	38–54	45	10	110
Troxacitabine	10	10 i.p.	Single bolus	42–62	47	15	114
Troxacitabine	10	10 i.p.	Every 7 days ×3	48–60	54	32	131
Troxacitabine	10	25 i.p.	Twice daily ×5	25–68	28	–32	68

single bolus i.p. administration of 10 mg/kg was not effective while moderate but significant antitumor activity was obtained when troxacitabine was administered at the same dose but given three times (T/C 131% and ILS 32%). Five consecutive twice-daily i.p. injections of 25 mg/kg of troxacitabine proved to be toxic, resulting in the death of seven animals on the last day of treatment. Irradiated SCID mice were more sensitive to troxacitabine, and our previous results have shown that troxacitabine is less active in T-lymphoblastoid leukemia than in promyelocytic leukemia xenografts [7].

Development of the MDR tumor phenotype often accompanies chemotherapy. A major determinant of MDR is the overexpression of the drug efflux pumps, Pgp and MRP. We showed in this study that troxacitabine was very effective in inhibiting cell proliferation of the human nasopharyngeal carcinoma cell line KB and its vincristine-resistant derivative KBV. Troxacitabine was also effective in the HL60 promyelocytic leukemia cell line, and its Pgp-positive (HL60/R10) and Pgp-negative (HL60/ADR) MDR derivatives, and in the CCRF-CEM T-lymphoblastoid leukemia cell line and its MDR derivative (CCRF-CEM/VLB). More importantly, troxacitabine showed significant in vivo antitumor activity including complete regressions and cures in both MDR leukemic and solid tumor xenografts. This finding is consistent with studies showing that troxacitabine is a substrate of neither nucleoside transporters [8] or drug efflux pumps (shown in this study). This efficacy in models of MDR leukemia and solid tumors suggests that troxacitabine may have an advantage over current chemotherapeutic drugs. The results of this study are consistent with the results of clinical studies showing that troxacitabine has significant activity in patients with acute myelogenous leukemia, refractory or relapsed acute myeloid leukemia and chronic myeloid leukemia (blast phase) [4, 5].

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