Acute Lymphatic Leukemia

A comparison of the *in vitro* cytotoxicity of daunorubicin and liposomal daunorubicin in pediatric acute leukemia

Anthracyclines are effective in the treatment of leukemia, but their use is limited because of cardiotoxicity. Liposomal daunorubicin (L-DNR) is potentially less cardiotoxic than daunorubicin (DNR). We compared *in vitro* cytotoxicity in pediatric acute leukemia samples and found no significant differences between cytotoxicity of DNR and L-DNR.

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Anthracyclines and cytarabine play a central role in the treatment of acute myeloid leukemia (AML). The use of anthracyclines is limited by their side-effects. The most serious late side-effect is cardiac dysfunction, which occurs in around 10% of children surviving AML.[1] Daunoxome® is the liposomal formulation of daunorubicin (L-DNR). Animal studies showed that L-DNR preferentially accumulated in tumor tissue, sparing tissues such as the heart.[2,3] In addition, L-DNR did not cause significant alterations in cardiac function, in contrast to DNR.[3] Trials in adult AML found an acceptable toxicity profile and significant antileukemic activity.[4] Clinical studies in pediatric patients are limited and data on cardiotoxicity are conflicting, possibly because many patients have been heavily pretreated.[5-8] An international phase III trial was designed to evaluate the clinical efficacy and toxicity of L-DNR when added to FLAG (fludarabine, cytarabine, granulocyte colony-stimulating factor) in children with relapsed/refractory AML (I-BFM-SG Relapsed AML 2001/01). This ongoing trial is randomizing children to treatment with FLAG with or without L-DNR (60 mg/m^2/day x 3). However, it was not known whether the cytotoxicity of L-DNR and DNR differ and we, therefore, compared the *in vitro* cytotoxicity of these two agents.

This study was performed using bone marrow or peripheral blood samples from 66 children (0-≤18 years) and included samples from 16 children with newly diagnosed AML, 9 with relapsed AML and 19 with newly diagnosed acute lymphoblastic leukemia (ALL) treated with DCOG, AML-BFM and MRC protocols. In addition, 14 normal bone marrow and 8 peripheral blood samples from healthy children were tested. Samples were taken with informed consent. The study was approved by the Medical Ethical Committee and the Dutch Central Committee for Medical Research in Humans. Drug resistance testing was performed using a 4-day total cell kill MTT assay.[9] DNR and L-DNR were tested at equivalent DNR concentrations (0.002-2 μg/mL). The LC_{50} value, the drug concentration that kills 50% of the leukemic cells, was used as a measure of resistance. To assess differences in the distribution of continuous data, the non-parametric Mann-Whitney U test was used for independent samples and the Wilcoxon signed-rank test for paired samples. Cross-resistance was analyzed with the Spearman's correlation coefficient (ρ). *p*-values of ≤0.05 were considered statistically significant (two-tailed test). The patients' characteristics are presented in Table 1. Both DNR and L-DNR were cytotoxic to leukemic cells in a dose-dependent fashion. There were large interpatient differences in the sensitivity to DNR (>480 times) and L-DNR (>200) and strong cross-resistance between DNR and L-DNR (Spearman's ρ=0.89, $p<0.0001$). Within AML and ALL samples, there was no statistically significant difference between the sensitivity to DNR or L-DNR (AML median LC_{50} 0.035 vs. 0.028 μg/mL, $p=0.55$, ALL median LC_{50} 0.018 vs. 0.021 μg/mL, $p=0.60$) (Figure 1). In some paired samples a difference was observed between DNR and L-DNR, but most differences were within one dilution step (which is the variability normally seen in reproducibility experiments), and these differences were not statistically significant. Leukemic samples (n=44) were nine times more sensitive to DNR ($p<0.0001$) and six times more sensitive to L-DNR ($p<0.0001$) than were normal bone marrow samples and similarly more sensitive to DNR (15 times, $p<0.0001$) and L-DNR (12 times, $p<0.0001$) than normal peripheral blood samples, reflecting the therapeutic index of these drugs.

Table 1. Patients' characteristics. Clinical characteristics of the patient samples included in this study.

	AML	*ALL*	*N BM*	*N PB*
Number	25	19	14	8
previously untreated	16	19		
relapsed	9			
Sex (% male)	72	58	65	50
Age (years) (median, p25-p75)	12.0 (7.4-14.2)	6.8 (4.8-8.9)	7.6 (6.3-11.2)	8.6 (5.6-9.8)
WBC (x10^9/L) (median, p25-p75)	44.5 (20.2-135.0)	18.8 (5.9-59.8)		
FAB	M0 1 M1 4 M2 4 M4 8 M5 5 Unknown 3			
Immunophenotype		BCP 13 T-cell 4 unknown 2		

WBC: white blood cell count; FAB: French-American-British morphology classification; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; BCP: B-cell precursor; ALL: T T-cell ALL; N BM normal bone marrow, N PB; normal peripheral blood.

In this study we showed that there were no statistically significant differences in sensitivity to L-DNR and DNR in ALL or AML *in vitro*. Apart from cellular drug resistance, a clinically relevant factor in the comparison of DNR and L-DNR is the difference in their pharmacokinetics. Peak plasma concentrations of L-DNR are higher than those of free DNR, resulting in a significant increase of the area under the curve (AUC) for L-DNR.[7,10] Thus, leukemic cells *in vivo* are exposed to higher concentrations of L-DNR when equivalent doses of DNR and L-DNR are used. In addition, L-DNR is frequently employed at higher dosages in clinical trials (for example L-DNR 80 mg/m^2×3 in AML-BFM 2004 vs. DNR 50 mg/m^2×3 in MRC AML15) because of fewer short-term side-effects. Therefore, one could hypothesize that, given the favorable pharmacokinetics, and similar *in vitro* cytotoxicity, L-DNR will be more clinically effective than DNR. Unfortunately no randomized clinical trials comparing L-DNR and DNR in leukemia are available to prove this concept. In addition to the pediatric Relapsed AML 2001/01 study comparing FLAG with FLAG+L-

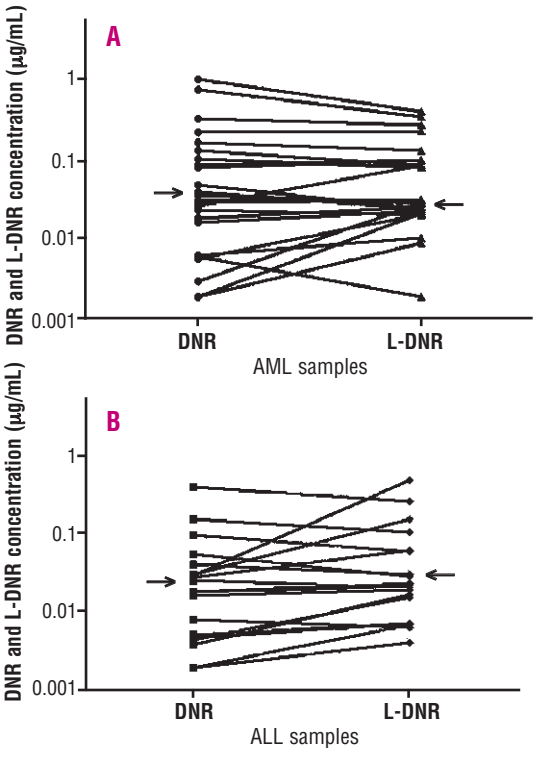

Figure 1. The cytotoxicity of free (DNR) and liposomal (L-DNR) daunorubicin in paired AML and ALL samples. Results are depicted as LC_{50} values (μg/mL), the concentration of DNR or L-DNR needed to kill 50% of the cells. Each symbol represents the LC_{50} value of an individual sample; the line connects the paired samples. The arrow indicates the median LC_{50} value. A. In AML there was no statistically significant difference between the sensitivity to DNR or L-DNR (median LC_{50} 0.035 vs. 0.028 μg/mL, p=0.55). B. In ALL there was no statistically significant difference in sensitivity to DNR or L-DNR (median LC_{50} 0.018 vs. 0.021 μg/mL, p=0.60).

DNR, the current AML-BFM Study Group 2004 protocol for pediatric newly diagnosed AML is randomizing idarubicin (12 mg/m²/daily x 3) vs. L-DNR (80 mg/m²/daily × 3) in induction. These studies may answer some of the remaining questions regarding the clinical efficacy and cardiotoxicity of L-DNR in pediatric AML.

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