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Effect of Daunorubicin, Carminomycin, Idarubicin and 4-Demethoxydaunorubicinol Against Human Normal Myeloid Stem Cells and Human Malignant Cells *In Vitro**†

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Abstract—*The cytotoxic effect of daunorubicin, carminomycin, idarubicin and the major metabolite of idarubicin in man, 4-demethoxydaunorubicinol, was investigated in a human normal progenitor myeloid stem cell assay and in a human tumor stem cell assay. Against normal myeloid progenitor cells, idarubicin and carminomycin were equally potent; both agents were significantly* ($P \leq 0.01$) *more potent than daunorubicin. Idarubicin was approx.* 2.5 *times more potent than 4-demethoxydaunorubicinol. Against malignant tumor cells,* 50% *cell kill after exposure to idarubicin was observed in four out* 24 *samples; this inhibition occurred at a drug concentration of* 0.1 μg/ml. *Two of the samples sensitive to idarubicin were also sensitive to 4-demethoxydaunorubicinol at a concentration of* 0.1 μg/ml. *Overall, idarubicin was active against two out of six ovarian carcinomas and against one out of three breast carcinomas. Our data confirm that 4-demethoxydaunorubicinol may play a role in the biological activity of idarubicin.*

INTRODUCTION

SEVERAL new anthracycline antibiotics have been introduced into clinical trials during the past few years. Idarubicin (4-demethoxydaunorubicin) is a synthetic anthracycline derivative whose chemical structure differs from that of daunorubicin by the removal of the methoxy group in position C-4 of the aglycone [1]. In preclinical investigations, idarubicin demonstrated several potential advantages over daunorubicin, including higher potency, antitumor activity after oral administration and reduced cardiotoxicity [2]. On these grounds, idarubicin has been introduced into phase I and phase II clinical trials. These clinical studies confirmed the higher potency of idarubicin and a significant degree (approx. 30%) of absorption after oral administration of the drug, as predicted by the preclinical studies [2]. With regard to cardiotoxicity, randomized trials comparing idarubicin with daunorubicin or doxorubicin will be necessary to give more information about the cardiotoxic potential of idarubicin in man. Idarubicin has exhibited antitumor activity mainly in acute leukemias and breast cancer [2–5]. Interestingly, antitumor activity has been observed after oral administration too [2, 5].

Several investigators have reported on the clinical pharmacology of idarubicin after intravenous and oral adminstrations [2–4, 6–8]. The pharmacokinetic behavior of idarubicin is characterized by an extensive conversion of the parent drug to its alcohol derivative (4-demethoxydaunorubicinol) [2–4, 6–8]. It is highly likely that 4-demethoxydaunorubicinol has a major role in the biological activity of idarubicin [2]. However, this assumption is based on *in vitro* data obtained with HELA cells and *in vivo* data with the murine P388 leukemia [2, 9]. The present study was designed to investigate the relative effect of idarubicin and 4-demethoxydaunorubicinol on human bone marrow myeloid stem cells (CFU-GM) [10] and against fresh human tumor samples cultured in the clonogenic assay developed by Salmon and Hamburger [11]. In addition, we compared the effect of idarubicin and

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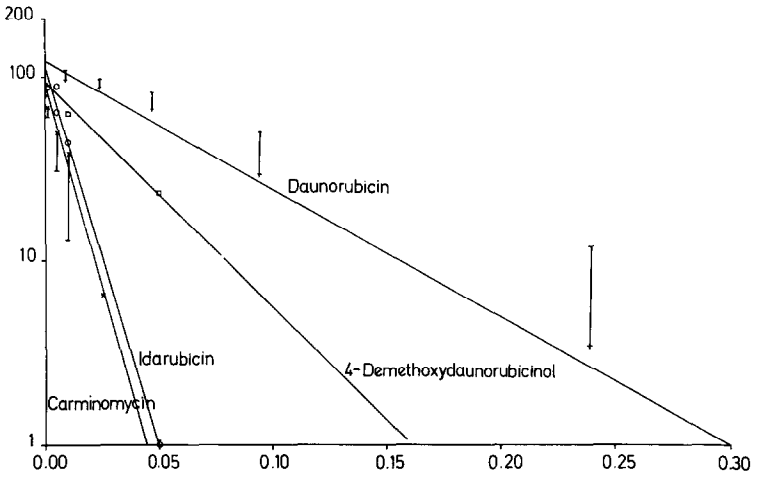

Fig. 1. Effect of daunorubicin (+——+), carminomycin (×——×), idarubicin (○——○) and 4-demethoxydaunorubicinol (□——□) on normal human bone marrow myeloid progenitor cells. The points indicate the mean of five to seven experiments; bars indicate the standard deviations of the mean. Some bars have been omitted for clarity.

4-demethoxydaunorubicinol to that of two structurally close anthracycline antibiotics, daunorubicin and carminomycin (4-demethyldaunorubicin) against human normal bone marrow myeloid stem cells.

MATERIALS AND METHODS

Drug supply

Idarubicin and 4-demethoxydaunorubicinol were supplied by Farmitalia Carlo Erba (Milan, Italy). Carminomycin was obtained from Bristol Myers Company (Syracuse, New York). Daunorubicin was purchased from Rhone Poulenc Belgique (Brussels, Belgium). All drugs were dissolved in sterile water.

Drug sensitivity of bone marrow progenitor cells

Four to 5 ml bone marrow samples were obtained from the sternum or posterior iliac crest in healthy volunteers after having obtained informed consent. Mononuclear cells were separated from the whole marrow samples as previously described [12].

The effect of the various drugs on CFU-GM growth was tested by incubating 0.5×10^6 mononuclear marrow cells for 30 min at 37°C in the presence of various drug concentrations. The latter were selected from the plasma drug concentrations observed in man after intravenous administration [2–4, 6–8, 13–15]. Preliminary experiments further defined the range of concentrations inducing 0–100% cell kill for each drug. The incubations were terminated by the addition of a 6-fold excess of Dulbecco tissue culture medium at 4°C. Cells were then centrifuged at 800 ***g*** for 10 min. The cellular pellets were resuspended in culture medium and cultured in soft agar as described previously [12].

At least 30 colonies per control plate were required for an experiment to be considered evaluable. Colony counts of the three plates for a particular drug concentration were averaged to obtain one data point; the standard error of the mean for individual data points did not exceed 15% of the mean. To determine cell sensitivity to a particular drug, the percentage surviving colonies relative to the number of control colonies was plotted vs. drug concentration.

Drug sensitivity of malignant cells

Malignant cells were isolated from effusions by centrifugation and from solid tumors by mechanical dissociation as previously described [16]. Nucleated viable cells at a concentration of 3.0×10^6 cells/ml were exposed to 1, 0.1 and 0.01 μg/ml of idarubicin and 4-demethoxydaunorubicinol. These concentrations were based on the peak plasma concentrations observed in man after treatment with idarubicin [2–4, 6–8]. When the cell yield was sufficient (six cases), cells were also exposed to doxorubicin at concentrations of 1 and 0.1 μg/ml [17]. Cells were incubated with or without drug for 1 h at 37°C in Hank's balanced salt solution; the cells were then washed twice before culture, which was performed according to a previously described technique [16]. All experiments were conducted in triplicate.

Cell kill was measured as the percentage of the mean number of colonies after drug exposure relative to the mean number of control colonies. A minimum mean of 20 colonies was required in

Table 1. In vitro *effect of daunorubicin (DNR), carminomycin (CMM), idarubicin (IDA) and 4-demethoxydaunorubicinol (ID-OL) on normal human bone marrow myeloid progenitor cells*

Drug	Mean ± S.E. (ng/ml)		
	IC_{10}*	IC_{50}	IC_{90}
CMM	1.1 ± 0.4	4.9 ± 0.8	18.0 ± 1.9
IDA	2.7 ± 1.6	7.1 ± 2.16	19.1 ± 4.5
ID-OL	6.8 ± 3.05	17.0 ± 4.1	47.4 ± 7.5
DNR	24.0 ± 2.7	54.0 ± 0.9	156.0 ± 3.7

*IC_{10}, IC_{50}, IC_{90}: concentrations inducing a 10, 50 and 90% cell kill, respectively.

triplicate control plates. *In vitro* efficacy was defined as a 50% decrease in tumor colony forming units.

Statistical analyses

All statistical comparisons were done with Student's *t*-test.

RESULTS

Effect on normal myeloid progenitor cells

A linear relationship was observed between the logarithm of colony survival and concentration (Fig. 1). The coefficients of correlation were −0.996, −0.990, −0.998 and −0.994 for idarubicin, 4-demethoxydaunorubicinol, daunorubicin and carminomycin, respectively. The slope (k) of the best fit curve was significantly steeper for idarubicin ($k = -205.6 \pm 79.9/\mu g/ml$) (mean ± S.D.) and for carminomycin ($k = -133.6 \pm 16.8/\mu g/ml$) than for daunorubicin ($k = -24.0 \pm 6.4/\mu g/ml$) ($P \leq 0.001$ for both comparisons). There was no significant difference between the slopes for idarubicin and carminomycin. The slope of the best fit curve was also steeper for idarubicin than for 4-demethoxydaunorubicinol ($P \leq 0.02$).

For each compound, the concentrations inducing 10% (IC_{10}), 50% (IC_{50}) and 90% (IC_{90}) cell kill were calculated (Table 1). There is no statistical difference between the inhibitory concentrations for idarubicin and carminomycin. In contrast, carminomycin is significantly more potent than daunorubicin ($P \leq 0.001$); similarly, idarubicin is approximately eight times more potent than daunorubicin ($P \leq 0.001$). Finally, 4-demethoxydaunorubicinol was slightly less potent than idarubicin against the myeloid progenitor cells; the difference is statistically significant for the IC_{50} and IC_{90} ($P \leq 0.001$).

Effect on malignant tumor cells

Twenty-four samples grew sufficiently (more than 20 colonies in the control plates) for drug testing. Among these samples, the most common tumor types were ovarian cancers (six cases), adenocarcinomas of unknown origin (four cases) and breast carcinomas (three cases). There were eight solid samples and 16 pleural effusions or ascites. Eight samples were obtained from patients previously treated with chemotherapy, including an anthracycline antibiotic in all these cases.

Cell kill by 50% or more after exposure to idarubicin was observed in four samples. The characteristics of the responding samples are indicated in Table 2; three of these responses were seen in patients previously untreated with chemotherapy; the fourth response was observed in a sample from a patient with breast cancer previously treated with cyclophosphamide, methotrexate, fluorouracil, doxorubicin and vincristine. No significant inhibition occurred at 0.01 μg/ml; the four responses were seen at a concentration of 0.1 μg/ml.

Two of the samples sensitive to idarubicin were also sensitive to 4-demethoxydaunorubicinol (Table 2). No other sample was sensitive to 4-demethoxydaunorubicinol. The two responses were observed at a concentration of 0.1 μg/ml; no response was seen at 0.01 μg/ml.

Overall, idarubicin was active against two out of six ovarian carcinomas and against one out of three breast carcinomas; 4-demethoxydaunorubicinol was active against two of the six ovarian samples and against none of the three breast samples. Idarubicin was active against one of the four adenocarcinomas of unknown origin. No activity was observed against the other tumor types. Doxorubicin did not induce any *in vitro* response in this study. The cell yield was sufficient to test doxorubicin against three ovarian carcinomas, three breast carcinomas and three adenocarcinomas of unknown origin and none responded (Table 2).

DISCUSSION

The inhibitory effect of daunorubicin, carminomycin, idarubicin and 4-demethoxydaunorubic-

Table 2. Effect of idarubicin (IDA), 4-demethoxydaunorubicinol (ID-OL) and doxorubicin (DOX) against malignant tumor cells

Tumor	*In vitro* inhibition (%)							
	IDA			ID-OL			DOX	
	1*	0.1	0.01	1	0.1	0.01	1	0.1
Ovary	70	63	17	53	63	4	33	47
Ovary		68			80			
Breast†	70	62	−19‡	39	8	1	7	0
Adenocarcinoma of unknown origin		53			31			

*Concentrations are expressed in μg/ml.

†This sample was obtained from a patient previously treated with cyclophosphamide, methotrexate, fluorouracil, doxorubicin and vincristine. All other samples were derived from previously untreated patients.

‡A negative value indicates an apparent stimulation.

inol, the major metabolite of idarubicin in man [2–4, 6–8] against normal human myeloid progenitor cells was studied with a CFU-GM assay. Theoretically, this experimental model appears attractive to predict for the relative clinical potency of these derivatives since their dose-limiting toxicity in man is leukopenia [2, 13, 18, 19]. Hematopoietic cells at an earliest stage of development than the CFU-GM may be grown *in vitro* [20]. Whether such cells are more appropriate to predict for the myelosuppressive potential of cytotoxic agents is unknown, as the precursors that are primarily affected by those agents have not been identified.

A single exponential pattern of CFU-growth inhibition was observed for the four compounds. Correlation coefficients of 0.99 or greater are consistent with this type of relationship between drug concentration and CFU-GM survival. Similar findings were reported for the anthracene derivatives ametantrone, mitoxantrone and bisantrene [12]. Relatively marked individual variations in *in vitro* sensitivity to daunorubicin, carminomycin, idarubicin and 4-demethoxydaunorubicinol were found in our study. These observations are consistent with the variable hematologic tolerance reported clinically with these compounds. However, comparisons of *in vitro* and *in vivo* data should be interpreted with caution since our *in vitro* studies were conducted with samples from normal volunteers whereas clinical results were obtained in heterogeneous populations of cancer patients.

Our laboratory has previously asked the question whether the comparison of *in vitro* toxicity on marrow CFU-GM of various chemotherapeutic analogs might help predict their hematologic toxicity *in vivo* [12]. Clearly, the predictivity of this *in vitro* test is insufficient: for example, our data would suggest that carminomycin is between eight and 21 times more potent than daunorubicin while clinically, carminomycin is approximately four times more potent than daunorubicin [18, 19]. A similar discrepancy was observed for the comparison of idarubicin vs. daunorubicin. We reported similar findings with anthracene derivatives [12]. These discrepancies may be related to the large variability in the *in vitro* data. Alternatively, incorrect predictions may be related to factors such as biotransformation, tissue distribution and other pharmacokinetic parameters that may vary from one agent to another *in vivo* and that are not taken into account *in vitro*.

In contrast, and similarly to the conclusions of our study on the anthracene derivatives [12], the test might provide important information for the interpretation and design of other *in vitro* models, such as the human tumor stem cell assay. In this test, malignant cells are exposed usually for 1 h to a fraction (one-tenth) of the peak plasma concentration of the cytotoxic agent, which implies that clinical studies including pharmacokinetic determination of the new agent must be available before *in vitro* studies can be performed. After intravenous administration, the peak plasma concentration of idarubicin and 4-demethoxydaunorubicinol range between 0.02 and 0.2 μg/ml [2–4, 6–8]. One-tenth of these peak plasma concentrations is in fact in excellent agreement with the IC_{90} determined in our study. The IC_{90} determined in the CFU-GM assay could therefore be used to select the appropriate concentrations for the stem cell assay, independently of any pharmacokinetic determinations. The validation of this concept for other groups of anticancer agents is the object of another publication [21].

The small number of samples for each tumor type does not allow one to determine precisely the *in vitro* response rates to idarubicin and comparisons with the clinical activity of the drug should be made cautiously. However, the occurrence of one response among three breast cancer samples is consistent with the clinical activity of idarubicin in this disease

[2]. To our knowledge, only one phase II study of idarubicin in ovarian cancer has been published [22]. No antitumor response was seen among 17 evaluable patients but all of them had received prior chemotherapy including usually cyclophosphamide, cisplatin and doxorubicin. In our *in vitro* data, a response was observed in two out of six samples; one responding sample was obtained from a patient with no prior chemotherapy; the other responding sample was obtained from a patient previously exposed to a multiagent regimen including doxorubicin; three of the four non-responding samples were obtained from patients previously exposed to multiagent chemotherapy.

A major goal of our study was to assess the cytotoxic potential of 4-demethoxydaunorubicinol against human cells. Our data indicate that 4-demethoxydaunorubicinol does have a cytotoxic effect against both human tumor cells and normal bone marrow progenitor cells *in vitro*. Have these findings any relevance *in vivo*? Against tumor cells, idarubicin and 4-demethoxydaunorubicinol were active at the same *in vitro* concentration (0.1 μg/ml). This concentration is achieved only for idarubicin after intravenous treatment [2, 3, 7]. However, the *in vitro* concentration times time product (0.1 μg/ml × h) was well below the *in vivo* area under the plasma concentration vs. time curve (AUC) for both idarubicin and 4-demethoxydaunorubicinol after intravenous and oral administration. Thus, 4-demethoxydaunorubicinol may play a role in the activity of idarubicin against tumor cells *in vivo*.

Against bone marrow progenitor cells, 4-demethoxydaunorubicinol is 2.5–3 times less potent than idarubicin. Interestingly, Dessypris *et al.* have reported that doxorubicin is also more cytotoxic than its alcohol derivative, doxorubicinol [23]. The concentrations producing a 50% *in vitro* growth inhibition, 7.1 and 17.0 ng/ml for idarubicin and 4-demethoxydaunorubicinol respectively, are achieved *in vivo*; the *in vitro* concentration times time products (3.6 and 8.5 ng/ml × h) are also below the plasma AUCs achieved *in vivo*. Finally, after an intravenous dose of 15 mg/m^2 of idarubicin, plasma AUCs are approx. 0.25 and 1.0 μg/ml × h for idarubicin and 4-demethoxydaunorubicinol, respectively [7, 24–26]. Assuming an arbitrary biological effect of 1 for idarubicin, our data indicate that 4-demethoxydaunorubicinol would have a biologic effect of 0.33. The overall biologic effect after an intravenous dose of 15 mg/m^2 is therefore 0.58. After an oral dose of 45 mg/m^2 of idarubicin, plasma AUCs are approx. 0.15 and 0.85 μg/ml × h for idarubicin and 4-demethoxydaunorubicinol, respectively [7, 24–26]. The overall biologic effect is therefore 0.43. Similar biologic effects are consistent with a similar degree of myelosuppression after an intravenous dose of 15 mg/m^2 and an oral dose of 45 mg/m^2. Thus, our data suggest that 4-demethoxydaunorubicinol plays a role in the effect of idarubicin against normal bone marrow progenitor cells too.

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