APHIDICOLIN MARKEDLY INCREASES THE IN VITRO SENSITIVITY TO ARA-C OF BLAST CELLS FROM PATIENTS WITH AML

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Keywords: ara-C, aphidicolin, AML, drug resistance, DNA repair.

1. ABSTRACT

Drug resistant cells often have an increased capacity to repair their DNA after damage by cytotoxic agents. Aphidicolin can inhibit this DNA repair. We describe a study of the effect of aphidicolin to modulate the sensitivity to cytotoxic drugs of blast cells from 13 patients with AML, 11 with de novo disease on presentation and 2 secondary to MDS. Three patients had relapsed following previous therapy and samples were received from 1 patient both on presentation and relapse. Blast cells were exposed to anthracyclines, antimetabolites or etoposide ± aphidicolin (15μM) for 48 hours. The MTT assay was used to measure cell survival and the LC$_{50}$ (concentration of drug required for 50% cell kill) was calculated. Overall, there was a significant increase in sensitivity to ara-C on co-incubation with aphidicolin in 12/14 samples (p = 0.007). The median increase in sensitivity was 3.88-fold (range 1.26- to 80-fold). Interestingly, when patients were grouped according to in vitro sensitivity to ara-C, cells from resistant patients demonstrated the greatest increase in sensitivity (median 14-fold compared to 2-fold for the sensitive group, p = 0.02). Despite the documented evidence for altered DNA repair as a mechanism of resistance to the topoisomerase II inhibitors, we found no significant increase in sensitivity to daunorubicin, doxorubicin or etoposide on co-incubation with aphidicolin. Nevertheless, we believe the unparalleled modulation of ara-C warrants further investigation.
2. INTRODUCTION

Whilst prognosis has improved considerably in childhood ALL, this is not the case in adult AML and drug resistance remains a major reason for the failure of chemotherapy. Whilst initial remission induction rates are around 70–80%, most patients will relapse early and only <25% can expect to have a sustained long term survival.

These patients are usually treated with combination chemotherapy which almost invariably includes cytosine arabinoside (ara-C). Using chemosensitivity testing, we, and others, have shown that it is the \textit{in vitro} sensitivity of the anthracycline in the combination that most often predicts response to treatment.\textsuperscript{1} Initial resistance to ara-C, however, has been shown to correlate with early relapse in this disease.\textsuperscript{2}

Resistance to ara-C can be conferred by many different mechanisms including alteration of DNA repair.\textsuperscript{3} for review} Aphidicolin is an inhibitor of the DNA polymerases involved in this repair mechanism and the glycinate ester of aphidicolin has entered clinical trials where limited toxicity was seen.\textsuperscript{4} However, the anti-tumour effect was minimal and combination studies with cisplatin were suggested. These clinical trials were never completed and therefore the true worth of this compound has never been established.

We have previously shown aphidicolin markedly increased the \textit{in vitro} sensitivity of platinum drugs using cells from patients with ovarian cancer\textsuperscript{5} and we have extended this study of inhibition of DNA repair to the blast cells from patients with AML.

3. MATERIALS AND METHODS

3.1. Patients

Fourteen samples, 7 bone marrows and 7 peripheral blood, were collected from 13 patients with AML. Eleven patients had \textit{de novo} disease, 8 on first presentation and 3 on relapse following previous cytotoxic therapy. Two patients were secondary to MDS. Two samples were taken from one patient, on presentation and relapse.

3.2. Cell Preparation and Drug Exposure

Blast cells were separated using density gradient centrifugation and a cell suspension in RPMI 1640 plus 10% FCS and antibiotics was prepared. There were >80% blast cells in these final preparations as measured by May Grünwald Giemsa staining. Blast cells were exposed, in triplicate, in a microtitre plate to 4 concentrations of drugs ± aphidicolin at a fixed dose of 151\textmu M for 48 hours at 37°C, 5% CO\textsubscript{2}. Drugs included daunorubicin (DNR), doxorubicin (DOX), ara-C and etoposide. Experiments were controlled by cells incubated in medium ± aphidicolin.

3.3. MTT Assay

Drug exposure was followed by removal of the medium plus drug and a further 4 hour incubation in 2mg/ml MTT.\textsuperscript{6} Acid/alcohol (0.04N HCl in isopropanol) was used to dissolve any formazan crystals and the plate was read at 570nm (reference 690nm). The LC\textsubscript{50} (concentration required to kill 50% of the cells) was calculated for each experiment. The sensitivity ratio of the LC\textsubscript{50} of drug over that for drug + aphidicolin gave a measure of any modulation effect.