Predictive value of an in vitro bioluminescent assay for rapid assessment of response to cytarabine and fludarabine in patients with acute leukaemia

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Introduction

The nucleoside analogue cytosine arabinoside (Ara-C) remains the mainstay treatment of acute non-lymphoblastic leukaemia (ANLL) even although up to 30 percent of patients fail to respond. Furthermore a large proportion of patients fail to achieve long-term remission and develop resistance to subsequent therapy.

Resistance to treatment is multi-factorial, including increased export of the parent compound from cells, insufficient conversion of Ara-C to the active metabolite Ara-CTP, and increased deamination of Ara-C to the inactive Acorusol (Acor). There is a requirement for a test to identify the extent of resistance, independent of cause, which in combination with cytogenetic screening could allow tailoring of the dose and/or selection of combination therapy.

Currently there is no rapid, inexpensive test to assess patient sensitivity to Ara-C prior to treatment. We have previously reported a bioluminescent 8-hour assay which assesses Ara-C resistance levels in leukaemic cell lines and patient samples independently of the cause of patient resistance (Anderson et al., 2009; 2010). In theory any agent capable of potentiating generation of Ara-CTP from Ara-C can also be tested with this assay system. Here we present results using the 8-hour assay for combination therapy screening, as tested on seven ANLL cell lines and an initial cohort of seven patients with ANLL, dosed with Ara-C alone or in conjunction with the purine analogue fludarabine.

Methods

Cell lines (assay validation)

This assay was validated using CONF-EEM (ALL), HL60 (APL), HEL (erythroblastoma), THP-1 (M6 AML), KG-1 (M0 AML), K562 (CML), M4A11 (phenotypic & molecular acute myeloid leukaemia) cell lines and compared with the commercially available 3-day cytotoxicity CellTiter-Glo assay (Promega).

Patients samples

Patients samples - peripheral blood (57%) or bone marrow (43%) from patients at presentation with ANLL (n=1): Patient ages ranged from 27 to 71 (median 53 years), FAB sub-type distribution M4 (30%), M2 (20%), secondary AML (14%), M0 (14%), biphenotypic AML (14%) and Ph1- ALL (14%). Samples were provided blind and the bioluminescent 8-hour assay was performed in two separate centres (Bristol and Frimley). Test results were verified using the commercially available 3-day Cell Titer-Glo assay (Promega) and compared with clinical outcome where known.

8-hour assay principle

The biosensor used in the assay is a non-pathogenic strain of E. coli, genetically modified to express human aCK for conversion of Ara-C to the active metabolite Ara-CTP, inducible using isopropyl-β-D-thiogalactopyranoside (IPTG). The biosensor also contains a luc-expressing plasmid and produces increased light output in response to Ara-C. The biosensor is lysified for ease of use and storage. Patient blasts (4 x 10⁷) were incubated for 30 minutes with Ara-C at a clinically relevant dose, the equivalent of 2g/m². Blasts were washed and lysed prior to exposure to the biosensor. Pre-incubation with Ara-C (10⁻³) was for 4 hours. Fluorescence was determined in DMSO so that cells were exposed to a final concentration of 0.1% DMSO.

Fluorescence from sensitive patients produced high light output, whereas those from resistant patients produced low light output (Figure 1).

Statistics

One-way ANOVA with Bonferonni's posthoc test was used to assess significance for cell line and patient samples.

Results

Cell lines

Validation studies were performed using leukaemic cell lines exposed to fludarabine and Ara-C and calculated using the 8-hour assay (Figure 2A) and the 3-day cytotoxicity assay (Figure 2B). A high degree of reproducibility was achieved from replicate assays (n=10). The Thp1 and K562 cell lines showed marked improvement in sensitivity index with combination therapy over Ara-C alone.

The sensitivity index is calculated from AP:AP results for treated and untreated samples. It is a measure of the level of Ara-C to Ara-CTP conversion achieved by the cell type. Immortalised cell lines achieve high values due to their homogenous nature.

Patient samples

Figure 3 shows typical 8-hour assay results from two patients using bone produced following in vitro treatment of samples with Ara-C alone or fludarabine (5µM) pre-treatment for 4 hours followed by Ara-C (FLA-regime). The sensitivity index (%) is shown. Figure 3A shows an increased sensitivity index in response to the combination therapy over Ara-C alone (12% versus 49%). This indicates a significant improvement in vitro sensitivity to Ara-C following pre-treatment with fludarabine (p<0.0001) versus Ara-C alone (p=0.0086). Figure 3B shows a patient for whom combination showed no improvement in sensitivity index (28% versus 27%) over Ara-C alone (p=0.05).

Conclusions

Assay produces a result within 8 hours of sampling allowing same day indication of patient sensitivity to Ara-C. Combination therapy requires 6 or 24 hour pre-incubation.

Assay is simple to perform, without the requirement for cell culture equipment - necessary for the 3-day Cell Titer-Glo assay.

Assay can determine patient resistance independent of the specific cause of resistance, unlike other kits specific to one resistance mechanism, for example assessment of drug efflux (MultiDrugQuant™).

Proof of principle analysis for Ara-C testing has shown 94% correlation with clinical outcome in ANLL patient samples (n=32) and 100% with the 3-day Cell Titer-Glo assay (n=47). Combination testing has shown 100% correlation with clinical outcome to date (n=2).

This assay may be useful in predicting the beneficial effect of using compounds such as fludarabine in association with Ara-C to maximise the generation of Ara-CTP, as validated in Thp1 and K562 cell lines, and patient samples.

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