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

E. Anderson1, V.C. Salisbury1, M. Conway1, M.A. Smith2, M. Ruddock3, A. Martin4, J. Lamont3, H.M. Alloush1, P. Mehta4, J.G. Smith5

1Centre for Research in Biomedicine, University of the West of England, Bristol, UK. 2Royal Marsden NHS Foundation Trust, Surrey, UK. 3Randox Laboratories Ltd, Belfast, UK. 4Bristol Haematology and Oncology Centre, Bristol, UK. 5Frimley Park NHS Foundation Trust, Surrey, UK.

Email: elizabeth.ah.andsen@uwe.ac.uk

Introduction

The nucleoside analogue cytose lignoside arac-A (ara-C) remains the mainstay treatment of acute non-lymphocytic 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 5-oxouracil (5-OH). 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 assessed ara-C levels in leukemic cell lines and patient samples independently of the cause of patient resistance (Anderson et al., Biochim 2009; 114(2)): p649). 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 CON-EM (ALL), HL-60 (APL), HEL (erythrolkaemia), THP-1 (M6 AML), KG-1 to KG-6 (AML), K562 (CML), M1411 (thymocytic & myelocytic leukaemia) cell lines and compared with the commercially available 3-day cytotoxicity Cell-Titer-Glo assay (Promega).

Patients samples

Patients samples - peripheral blood (57%) or bone marrow (43%) from patients with presentation with ANLL (n=7). Patient ages ranged from 27 to 71 (median 53 years). FAB sub-type distribution M4 (30%), M4Eo (25%), secondary AML (14%), M0 (12%), biphagous AML (14%) and Ph+ AML (14%). Samples were provided blind and the bioluminescent 8-hour assay was performed in two separate centres to control for between-assay variations. 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 5CK for conversion of ara-C to the active metabolite ara-CTP, inducible using SigD (O-1 thigalactosyrophosphatase (TIGT). The biosensor also contains a luc-expressing plasmid and produces increased light output in response to ara-C. The biosensor is lysated for ease of use and storage.

Patient blasts (4 x 10⁶) were incubated for 30 minutes with ara-C at a clinically relevant dose, the equivalent of 2g/ml. Blasts were washed and lysed prior to exposure to the biosensor. Pre-incubation with ara-C (10 μM) was for 4 hours. Fludarabine was prepared in DMSO so that cells were exposed to a final concentration of 0.1% DMSO. Blasts from sensitive patients produced high light output, whereas those from resistant patients produced low light output (Figure 1).

Statistics

One-way ANOVA with Bonferroni’s post-hoc test was used to assess significance for cell line and patient samples.

Figure 1: Schematic of the biosensor system showing cellular responses to Ara-C

> Ara-CTP itself cannot enter the bacterial cell and must first be converted to Ara-C. This is accomplished by adding alkaline phosphatase (AP) to the sample.

Comparison between 4 AP indicates the proportion of Ara-C converted from Ara-CTP, and thus the patient’s ability to import and metabolise the drug to the active metabolite.

Conclusions

> Assays produces a result within 6 hours of sampling allowing same day indication of patient sensitivity to Ara-C. Testing with 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 tests 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=97). 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 THP-1 and K562 cell lines, and patient samples.

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