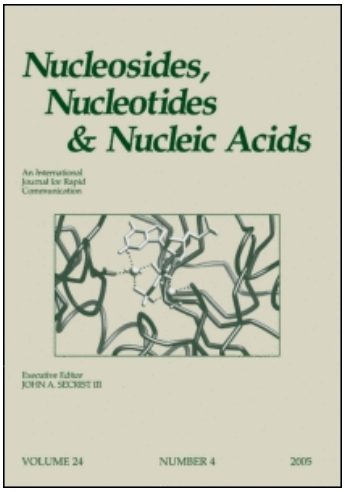

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Online Fluorescent Method to Assess BCRP/ABCG2 Activity in Suspension Cells

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ABSTRACT

An online method was developed to monitor BCRP mediated efflux of fluorescent substrates in suspension cells. To this end, a 2-compartment system consisting of a transwell cup and a cuvette was used. In this system we were able to observe differences in efflux kinetics between BCRP overexpressing RPMI 8226/MR cells and parental myeloid RPMI 8226(s) cells using only 50,000 cells per experiment. 8226/MR cells displayed a larger cellular efflux rate of the BCRP substrate Hoechst 33342, as compared to the wildtype cells. This difference in efflux rate was completely decreased in the presence of the BCRP inhibitor Ko143.

Key Words: ABC-transporters; BCRP/ABCG2; Efflux rate; Hoechst 33342; Ko143.

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INTRODUCTION

The assessment of cellular efflux kinetics is an important subject in the study of plasma membrane associated ATP binding cassette transporters (ABC-transporters). Some of these proteins, such as the Breast Cancer Resistance Protein (BCRP/ABCG2), contribute in cellular resistance against chemotherapy, by effluxing drugs.[1,2]

In this study we describe a new and simple method to monitor cellular efflux of fluorescent drugs/compounds in suspension cells, online and real-time. We measured the cellular efflux of the BCRP substrate Hoechst 33342 in the human myeloid cell line RPMI 8226(s) and its mitoxantrone resistant and BCRP overexpressing subline 8226/MR.

MATERIALS AND METHODS

Cells

The human myeloid cell line RPMI 8226(s) and its mitoxantrone resistant and BCRP overexpressing subline 8226/MR were cultured in RPMI medium supplemented with 10% FCS (both GIBCO, Grand Isl., NY) without HEPES, with 2 mM glutamine and 100 μg/ml penicillin/streptomycin. Every 2 passages 80 nM mitoxantrone was added to the 8226/MR line.

Cellular Drug Efflux

Efflux of fluorescent BCRP substrates (Hoechst 33342) was measured using an online computerized method, which was based on an earlier described method for adherent cells,[3] with several modifications. Suspension cells were cultured and collected in a density of 50.000 cells / 100 μl. Cells were loaded with 10 μM substrate (Hoechst 33342) in culture medium (37°C) for at least 90 minutes, until steady-state was reached. After loading, the cells were put on ice until further use. For an efflux experiment 1 ml of the cell suspension was washed rapidly twice with ice-cold medium A (RPMI medium without phenol red). This did not largely influence the cellular Hoechst accumulation (not shown). Then 100 μl of the suspension was placed in a transwell cup (6.5 mm diameter, 0.4 μm pore size, Costar, USA), and the cup was placed on top of a quartz cuvette. Fluorescence of the effluxed substrate, which diffused into the medium of the lower compartment, was monitored with a spectrafluorometer (FluoroMax, SPEX Industries, Edison, NJ). Fluorescence of Hoechst 33342 was measured every second at excitations and emission wavelengths of 350 nm, and 480 nm respectively. At the end of the experiment 25 μM digitonine was added to permeabilize the cells, and the permeable membrane of the transwell cup was cut to determine the total amount of intracellular Hoechst 33342.

RESULTS AND DISCUSSION

To measure real-time cellular efflux of fluorescent drugs in suspension cells we developed a 2-compartment system, in which drug-loaded cells stay out of the lightway (Fig. 1). The method is based on passive diffusion of effluxed drugs/compounds to the lower detection compartment.

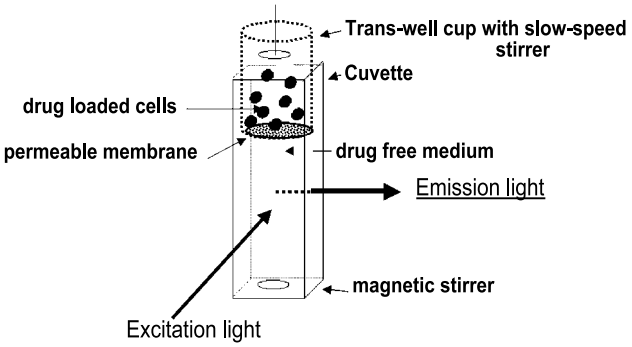

Figure 1. Experimental set-up for online and real-time detection of cellular efflux of fluorescent drugs. A 2-compartment system consisting of transwell cup (upper compartment) in which drug-loaded cells are incubated, and a cuvette in which effluxed drugs are detected was used.

Figure 2 shows the timeframe of passive diffusion of 100 μM Hoechst 33342 (without cells) from the upper to the lower compartment. For the use of drug-loaded cells this timeframe is extended, since the drugs first have to be effluxed/tranported out of the cells.

Next, we determined whether this online fluorescent method is suitable to monitor cellular efflux of drugs in suspension cells. To this end, we used a relatively small amount (~50,000) of cells loaded with Hoechst 33342 (Fig. 3). The initial efflux rate from the BCRP overexpressing cell line 8226/MR was 2-fold higher (~0.64 pmol/min/10^6 cells) compared to the efflux rate from wildtype 8226(s) cells (~0.30 pmol/min/10^6 cells) (Fig. 3). The BCRP inhibitor Ko143[4] (100 nM, kindly provided by prof. G.J. Koomen, Univ. Amsterdam) decreased the efflux in 8226/MR cells to a similar efflux rate as in the wildtype cells, but did not affect efflux kinetics in the wild type cells (Fig. 3).

Initial efflux was measured as function of time. Signals presented have been normalized for the amount of cells. 8226/MR overexpress BCRP, whereas 8226(s) do not show BCRP expression on western blot (not shown). After 2500 sec 100 nM of the BCRP inhibitor Ko143 was added. The stripped line indicates the efflux rate before Ko143 addition.

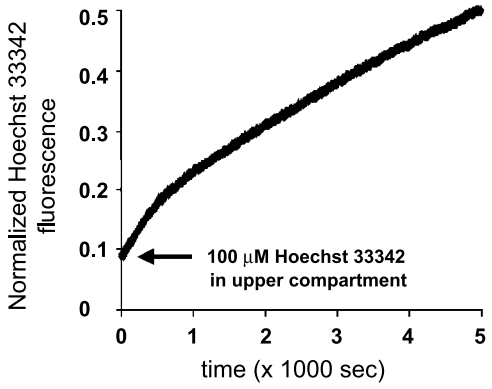

Figure 2. Passive diffusion of 100 μM Hoechst 33342 from upper to lower compartment.

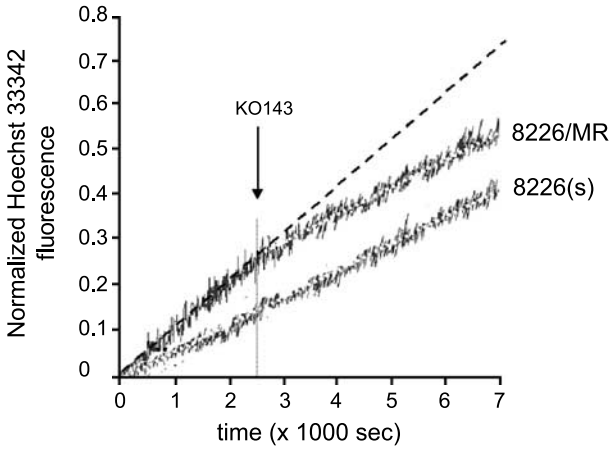

Figure 3. Cellular efflux of Hoechst 33342 from 8226(s) cells and 8226/MR cells.

The Ko143 independent component of the total cellular Hoechst efflux consisted predominantly of passive diffusion out of the cells, but possibly also of active transport due to marginal expresion of other transporters present in both cell lines.

In conclusion, we here showed a new, simple and rapid online method to asses in suspension cells the transport of fluorescent compounds mediated by ABC-transporters.

ACKNOWLEDGMENT

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