INTRODUCTION

Paclitaxel (Taxol®) is a chemotherapeutic drug that binds to microtubules within the cell and causes cell cycle arrest at the G2/M phases which leads to apoptosis. Although paclitaxel is lipophilic, it does not cross the blood-brain barrier (BBB) as it undergoes active efflux by P-gp. In patients with malignant brain tumors, the BBB is often disrupted and some chemotherapeutic agents can enter the tumor, however this delivery is quite variable and unpredictable. One approach to overcoming this problem is the development of paclitaxel derivatives which due to structural modifications are no longer substrates for P-gp and furthermore, are substrates for endogenous uptake transporter expressed at the BBB. However, P-gp is able to recognize and transport a wide spectrum of seemingly unrelated, structurally diverse compounds, so deciding which modifications to make is not straightforward. Moreover, many of the synthesizing compounds to be substrates for endogenous uptake systems can be challenging. in vitro assays, such as the rhodamine 123 uptake assay in BMECs, can direct chemistry efforts by being an initial screen for P-gp interaction. This initial screen should then be verified by performing side-by-side diffusion studies using BMEC monolayers. These studies will indicate if P-gp is being avoided and if the new derivative has improved permeability due to an endogenous uptake transporter.

MATERIALS AND METHODS

Materials:
Rhadime 123 was purchased from Sigma Chemical Co. (St. Louis, MO). Paclitaxel derivatives were synthesized by the University of Kansas, Department of Medicinal Chemistry. Minimum Essential Medium, and Ham’s F12 were purchased from Invitrogen (Carlsbad, CA).Media supplements including epidermal growth factor (EGF), ascorbic acid, streptomycin and sodium bicarbonate were also purchased from Sigma Chemical Co. (St. Louis, MO) as well as F12 medium which was used to coat plates. Plates were also coated with rat tail collagen. The rhodamine 123 was prepared in house. Platelet poor horse serum was kindly provided by Bio-Prep-The Shiris Group (Capeville, VA). Lysis buffer was prepared from 0.5% Triton X-100 in 0.2N NaOH. Transwell® polycarbonate 24-well systems were purchased from Corning Costar Company (Cambridge, MA). Ammonium, formamide and sodium thiosulfate were purchased from Fisher Scientific (Pittsburgh, PA).

Cell Culture:
BMECs were grown at 37°C in an atmosphere of 5% CO2 and 95% relative humidity. The cells were grown in 50% Minimum Essential Medium and 50% Ham’s F12 supplemented with 100μg/mL streptomycin, 100μg/mL penicillin, 2mM L-glutamine, and 10% heat inactivated fetal bovine serum. 10% FBS was added to all cultures and solutions of paclitaxel derivatives were added to the wells and allowed to incubate for 30 min. at 37°C in a CO2 incubator. The BMECs were removed and solutions of the paclitaxel derivatives were added to the wells and added to incubate for 30 min. at 37°C in a CO2 incubator. (Paclitaxel derivatives were run at 10μM in PBS). The rhodamine 123 assay was added to all wells at a concentration of 8μM and returned to the hot cells. Cells were incubated for an additional 2 hours. After the incubation was complete, the hot cells and the media were collected. Cells were washed with warm PBS (PBS containing Ca2+, Mg2+, glucose, and lactoside acid) and allowed to incubate in the warm PBS for 10 min. The rhodamine 123 was added to all cells and the cells were analyzed for fluorescence. Concentration dependence was determined for a range of concentrations to identify any possible concentration dependence of permeability values. Concentration may have an effect on previous studies in which the permeability of TX.67 at lower concentrations was substantially increased relative to paclitaxel.

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