Abstract

We are interested in the anti-inflammatory effects of natural products, Black Cohosh (Actaea racemosa), St. John’s wort (Hypericum perforatum), and Skullcap (Scutellaria baicalensis) in mammalian tissues. Mouse brain capillaries serve as a model to examine the anti-inflammatory properties of these herbs in the brain. Previous research has shown that inflammation in the brain may be modeled by treating harvested brain capillaries with lipopolysaccharide (LPS). One endpoint to examine brain inflammation is to assess how the activity of the efflux transporter, p-glycoprotein (P-gp), has been altered under inflammation. While function of this protein is known in the presence of LPS (inflammation) and in the presence of various herbs (without inflammation), no work has been done to observe the effects of the herbs with LPS treated brain capillaries. Our current work utilizes confocal microscopy to observe P-gp function and Western blotting analysis to observe P-gp expression.

Introduction

Inflammation is linked to a wide variety of diseases and conditions ranging from Alzheimer’s disease to obesity. Given the alternative medicine market, our research has far-reaching implications in the efficacy of anti-inflammatory herbs and in the clinical treatment of inflammation. P-glycoprotein (P-gp) is located within the plasma membrane of many tissues, particularly the brain, liver, kidney and intestine, where it effluxes xenobiotics and interferes with drug absorption (Miller DS et al., 2008). P-gp has also been identified as a multidrug resistance (MDR) factor in tumor cells, transporting various chemotherapeutic agents out of the cell. By modulating the effects of P-gp with these herbs we may be able to modify the pharmacokinetics of drugs at the blood-brain barrier.

Several natural products are already known to change P-gp activity, as shown in Table 1. However, no study to date observes the effects natural products have on brain capillaries under conditions of inflammation.

<table>
<thead>
<tr>
<th>Latin Name</th>
<th>Genus Name</th>
<th>Effects on Protein Transport</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actaea racemosa</td>
<td>Black Cohosh</td>
<td>No significant change</td>
<td>Drug metabolism and disposition 2006; 34(1): 69-74</td>
</tr>
<tr>
<td>Hypericum perforatum</td>
<td>St. John’s wort</td>
<td>Transport decreased</td>
<td>Gauging the clinical significance of p-glycoprotein, Mol Nutr Food Res. 2008 July; 52(7): 772-779</td>
</tr>
<tr>
<td>Scutellaria baicalensis</td>
<td>Skullcap</td>
<td>Increased transport</td>
<td>Evaluation of the Flavonoid Oroxylin A, J Nat Prod. 2009 Sep; 72(9): 1616-9</td>
</tr>
</tbody>
</table>

Table 1. Transport observed from natural products

In Figures A and B, control and LPS treated brain capillaries are detected with the P-gp C219 antibody. P-gp expression levels do not appear to change in bands detected at 50 and 110 kDa. In Figures C and D, control blood-brain barrier capillaries were incubated with the fluorescent substrate rhodamine (red) and visualized with confocal microscopy. Rhodamine accumulates in the capillary lumen and is an indicator of P-gp expression. Preliminary studies have suggest appreciable accumulation of rhodamine within the lumen of brain capillaries. This fluorescence in control brain capillaries will be compared to that of LPS and natural product treated capillaries to quantitatively assess p-glycoprotein function.

Experimental Procedure

Summary and Future Work

The blood-brain barrier, composed of capillary endothelial cells joined at tight junctions, functions as an interface between peripheral and CNS circulation (Hawkins BT et al., 2005). LPS-induced inflammation has been shown to modulate transport at the BBB (Miller DS et al., 2008). Found in the outer membrane of Gram-negative bacteria, LPS stimulates microglial NADPH oxidase (Sumi N et al., 2010) and leads to increased CNS concentrations of cytokines, platelet-activating factor, thromboxane A2, prostaglandins, leukotrienes, nitric oxide, proteases, toxic oxygen radicals, and vasoactive amines (Olson NC et al., 1995). These mediators of inflammation alter expression of P-gp. However, several natural products are promising agents for attenuating CNS inflammation (Suk K, 2005). We hope to observe LPS and natural product effects on P-gp transport in brain capillaries.

The natural products we are currently investigating have varied effects on P-gp transport in the absence of inflammation: skullcap (Scutellaria baicalensis) increases transport, St. John’s wort (Hypericum perforatum) decreases transport, and Black Cohosh (Actaea racemosa) has no significant effect on transport. After additional experiments that suggest effects of inflammation on P-gp function and protein expression, we will investigate if these natural products return P-gp function and protein expression to control levels. Additional tools of immunohistochemistry and RT-PCR will also be used to investigate how natural products modulate P-gp during inflammation.

Acknowledgements

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References