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Abstract: The P-glycoprotein efflux system located on the apical membrane of brain capillary endothelial cells functions as part of the blood-brain barrier. In this study, primary cultures of bovine brain microvessel endothelial cells (BMECs) were investigated for the presence of a P-glycoprotein system and its contribution in regulating ivermectin distribution across the blood-brain barrier. Results of rhodamine 123 uptake studies with cyclosporin A and verapamil as substrates indicated that a functional efflux system was present on BMECs. Immunoblot analysis with the C219 monoclonal antibody to the product of the multidrug resistant member 1(MDR1) gene also confirmed the expression of MDR1 in the BMECs. Unbound ivermectin was shown to significantly increase the uptake of rhodamine 123 in BMECs, however, the drug only modestly enhanced the transcellular passage of rhodamine. The results of these studies affirmed that unbound ivermectin is an inhibitor of the MDR1 efflux system in BMECs.

Text of paper:

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Evaluation of the Role of P-glycoprotein in Ivermectin Uptake by Primary Cultures of Bovine Brain Microvessel Endothelial Cells

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running head: P-glycoprotein in BMECs and Effects of Ivermectin on Rhodamine 123 Uptake

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INTRODUCTION

The failure of combination chemotherapy in the treatment of many cancers prompted research in the area of multidrug resistance which ultimately lead to the discovery of P-glycoprotein, a 170-180 kDa glycoprotein associated with the plasma membrane of many drug resistant tumor cells. P-glycoprotein is believed to function as an energy-dependent efflux pump and is often overexpressed in cancer cells (1). P-glycoprotein is also expressed in noncancerous tissues including the endothelial cells of the blood-brain barrier (2-5). Specifically, the efflux system has been shown to be present and functional in the membranes of cultured murine (2,4), porcine (2), and bovine brain capillary endothelial cells (5). The blood-brain barrier has many unique characteristics which restrict the exchange of molecules between the systemic circulation and the extracellular fluid compartment of the brain. These characteristics include the presence of tight intercellular junctions, the absence of fenestra, a reduced level of pinocytosis, and a complex glycocalyx. The expression of the P-glycoprotein efflux system contributes an additional defense mechanism to the barrier (6).

Previously our laboratory investigated the uptake of ivermectin by bovine brain microvessel endothelial cells (BMECs) (7) and canine BMECs from ivermectin sensitive and ivermectin tolerant rough-coated collies (8). Ivermectin is a complex macrocyclic lactone which possesses a broad spectrum of activity against several species of nematodes, arachnids, and insects that act as parasites. Ivermectin, known for its relatively high potency and low toxicity, is commonly used for heartworm control in dogs as well as the treatment of various tropical diseases such as onchocerciasis or "river blindness" in humans (9). Our previous studies with

BMECs concluded that ivermectin had only a limited affinity for the endothelial component of the normal blood-brain barrier (7).

Recent work has shown that "knockout" mice which were made deficient for the multidrug resistant member 1 (MDR1) gene are 100 times more sensitive to ivermectin than normal mice (10). Ivermectin has significant retention and distribution in kidney, fat, and liver tissues but achieves its lowest tissue concentrations in the brain (11). Based on the "knockout" mice studies, it has been hypothesized that the P-glycoprotein system at the blood-brain barrier effluxes ivermectin, restricting ivermectin distribution into the brain (10). The objective of this study was to demonstrate the expression and functional activity of MDR1 in primary cultures of bovine brain microvessel endothelial cells in our laboratory, and to demonstrate whether reduced ivermectin uptake by BMECs was due to the presence of MDR1. Rhodamine 123, a known substrate for MDR1, was used as a fluorescent marker to assay for the functional activity of the efflux system (2,12-14).

EXPERIMENTAL PROCEDURE

Materials. Ivermectin (22,23-dihydroavermectin $B1_a$) was donated by Merck & Co., Rahway, NJ. Cyclosporin A, rhodamine 123, and verapamil were purchased from Sigma Chemical Company, St. Louis, MO. All other compounds used were of the highest commercially available quality.

Cell cultures. BMECs were isolated from gray matter of cerebral cortices by enzymatic digestion and subsequent centrifugation, and seeded into primary culture as detailed by Audus and Borchardt (15,16) and based on the original method by Bowman et al. (17). This method has been extensively characterized (18,19). Isolated BMECs were seeded at a density of 50,000 cells/cm^2 into 12- and 24-well plates, and 100 mm tissue culture dishes (Corning Costar Corporation, Cambridge, MA) that were pretreated with rat-tail collagen and bovine fibronectin (Sigma Chemical Co.). The culture medium consisted of 45% minimum essential medium and 45% F-12 Ham nutrient mixture (Gibco, Life Technologies, Grand Island, NY), 10 mM HEPES, pH 7.4, 13 mM sodium bicarbonate, 10% plasma-derived equine serum, 100 μg/ml heparin, 100 μg/ml streptomycin, 100 μg/ml penicillin G, 50 μg/ml polymyxin B, and 2.5 μg/ml amphotericin B (Sigma Chemical Co.). The cells were cultured at 37°C with 95% humidity and 5% CO_2. The BMECs were fed on the third day after seeding and then every two days until a confluent monolayer was formed (10-14 days).

Human umbilical vein endothelial cells (HUVECs) were purchased from American Type Culture Collection and grown in T-flasks (25 cm^2) according to supplier instructions. Briefly, cells were thawed upon receipt, centrifuged, and resuspended at a concentration of 1.5 x 10^5 cells/ml in culture medium (pH 7.4) containing 90% F-12 Ham nutrient mixture (Gibco), 10% fetal bovine serum (JRH Biosciences, Lenexa, KS), 1.176 mg/ml sodium bicarbonate, 100 μg/ml heparin, and 50 μg/ml endothelial cell growth supplement (Sigma Chemical Co.), pH 7.4. Cells were maintained at 37°C with 95% humidity and 5% CO_2 and were subcultured every 3-4 days at a split ratio of 1:2 to 1:3.

Caco-2 cells were grown to differentiated monolayers in Transwell™ (Corning Costar) growth supports as detailed by Hidalgo et al. (20).

Rhodamine 123 uptake. Primary cultures of BMECs were seeded into 12-well plates or 24-well plates and grown to confluent monolayers. The culture medium was aspirated from each well and 0.9 ml of various concentrations of cyclosporin A, ivermectin, and verapamil were added. Cyclosporin A was first dissolved into 95% ethanol at a concentration of 1 mg/ml and then diluted with MEM, pH 7.4 (MEM, 50 mM HEPES, 100 μg/ml penicillin G, 100 μg/ml streptomycin, 50 μg/ml polymyxin B, and 2.5 μg/ml amphotericin B) to the desired concentrations. Ivermectin was first dissolved in absolute methanol at a concentration of 1 mg/ml and then diluted with MEM to the desired concentrations. Control BMEC wells for cyclosporin A and ivermectin were exposed to equivalent amounts of diluted ethanol and methanol, respectively, as the drug-treated wells. Verapamil was dissolved in MEM, pH 7.4. The plates were incubated for specific times at 37°C. After the incubation, 0.1 ml aliquots of rhodamine 123 were added to each well at a final concentration of 5 μM. The plates were again incubated at 37°C for specific times. After the second incubation period, the medium from each well was decanted off and the monolayers in each well were quickly rinsed three times with 0.01 M phosphate buffered saline (PBS), pH 7.4, 4°C. The cell monolayers were solubilized by the addition of 1.0 ml of 0.2N NaOH / 0.5% Triton X-100. The contents of each well were collected into cuvettes and assayed by fluorescence using a SLM-Aminco 4800 fluorometer (ex = 500 nm, em = 550 nm). After reading the fluorescence, 100 μl was removed from each cuvette and analyzed for protein content with the BCA protein assay kit (Pierce, Rockford, IL).

HUVECs were grown to confluence in T-flasks (25 cm^2). The culture medium was removed from the flasks and the cells were rinsed two times with PBSA, 37°C, pH 7.4 (129 mM NaCl, 2.5 mM KCl, 7.4 mM Na_2HPO_4, 1.3 mM KH_2PO_4, 0.63 mM $CaCl_2$, 0.74 mM $MgSO_4$, 0.1 mM ascorbic acid, and 5.3 mM glucose). The cells were then exposed to 5 ml of PBSA containing 5 μM rhodamine 123 for controls, or 5 μM rhodamine 123 plus 100 μM cyclosporin A or verapamil. The HUVECs were incubated under these conditions at 37°C for 30 minutes at which time the rhodamine 123 solutions were removed and the cells were rinsed twice with 0.01 M PBS, 4°C. The monolayers were solubilized by the addition of 0.2N NaOH / 0.5% Triton X-100 and assayed by fluorescence. The results were normalized for protein content using a BCA protein assay kit (Pierce).

P-glycoprotein immunoblot. Total cellular extract was prepared using confluent monolayers of Caco-2 cells and BMECs. The cells were lysed via 45 min incubation in ice-cold phosphate buffer saline (PBS) containing 3% sodium dodecyl sulfate (SDS) and protease inhibitors (0.1 mM leupeptin, 73 μM pepstatin A, and 100 μg/ml PMSF). The cell lysate was then centrifuged at 12,000 x g for 15 min and the supernatant containing solubilized membrane proteins was stored at -20°C and used for further analysis. Total cell protein was measured by the Micro BCA protein assay (Pierce, Rockford, IL) using bovine serum albumin as a standard. Cell proteins were electrophoresed on a 10% Tris-Glycine gel (NOVEX, San Diego, CA) and transferred to a PVDF membrane. Immunoreactive protein was detected with the C219 monoclonal antibody (Signet, Deadham, MA) using the enhanced chemiluminescence method (ECL) as per the manufacturer's protocol (Amersham, Downers Grove, IL). Caco-2 cell lysate was used as a positive control (21).

Transendothelial permeability studies in BMECs. Polycarbonate membranes (pore size 3.0 μm) were placed into 100 mm tissue culture dishes and coated with rat-tail collagen and bovine fibronectin. Brain microvessel endothelial cells (BMECs) were grown to confluent monolayers, as determined by inspection of the areas around the membranes using an inverted microscope. The basolateral side of the cells was defined as the side facing the collagen matrix (16,19). Once confluence was attained, the membranes were rinsed with MEM, pH 7.4 and placed in a horizontal Side-bi-Side™ diffusion cell apparatus (Crown Glass, Inc., Somerville, NJ) for transendothelial permeability studies. The area of the diffusion membrane was 0.636 cm^2. The donor and receiver chambers were filled with 3.0 ml of MEM, pH 7.4 and the temperature was maintained with an external circulating water bath. The contents of each chamber were stirred with Teflon coated magnetic stir bars at a speed of 600 r/min driven by an external drive console (Crown Glass, Inc.). Rhodamine 123 was dissolved in PBSA and diluted to a final concentration of 5 μM in the donor chamber. The transendothelial permeability of rhodamine 123 alone was followed for 30 minutes. Several 100 μl samples were taken from the receiver chamber during this time. After each sample, the volume was replaced with fresh MEM. After monitoring rhodamine 123 transport for 30 minutes, ivermectin (250 ng/ml or 1 μg/ml) dissolved in 5 μM rhodamine 123 was added to the diffusion cell donor chambers. Transport was followed for an additional 90 minutes with 100 μl samples taken every 15 minutes from the receiver chamber. Permeability was followed in the presence and absence of serum. All samples were placed into cuvettes and diluted to 1 ml with PBSA. Fluorescence was assayed with a SLM-Aminco 4800 fluorometer (ex = 500 nm, em = 500 nm). The flux was determined by

plotting pmoles vs. minutes. The apparent permeability coefficient was calculated using the equation:

$$P = Flux / (A*C_{Do})$$

where flux is the slope of the line, A is the area of the membrane, and C_{Do} is the initial donor concentration (19).

Statistical analysis. The comparison between mean values within each experimental series was performed by one-way analysis of variance using Dunnett's test to compare several treatments against a control.

RESULTS

Rhodamine 123 uptake experiments. Rhodamine 123 is a fluorescent dye commonly used for the determination of P-glycoprotein function *in vivo* and *in vitro* (2,12-14). Cyclosporin A is a known substrate for the P-glycoprotein efflux system *in vivo* and *in vitro* (3,5,14). In Figure 1 the effect of cyclosporin A on rhodamine 123 uptake in BMECs is shown. All concentrations of cyclosporin A used in this study produced a significant increase in the uptake of rhodamine 123 by BMECs. Figure 2 shows the concentration-dependent effect of verapamil on rhodamine 123 uptake in BMECs where the higher concentrations of verapamil significantly increased the uptake of the fluorescent marker. Verapamil is also a known substrate for the P-glycoprotein system; however, cyclosporin A was shown to have a greater effect on the efflux system in brain capillaries than verapamil (3).

To further establish that the functional activity measured with rhodamine 123 was due to efflux by MDR1, we examined uptake of the fluorescent marker in HUVECs, which do not express P-glycoprotein (2,4) as a negative control. The results of incubating HUVECs in 5 μM rhodamine 123 and100μM cyclosporin A or 100μM verapamil are illustrated in Figure 3. Neither cyclosporin A nor verapamil were shown to have a significant effect on HUVEC uptake of rhodamine 123 at the concentrations studied.

P-glycoprotein immunoblot. The expression of P-glycoprotein in BMEC lysate was determined with anti-MDR1 monoclonal antibody C219 using the ECL method. Figure 4 shows a representative band in both BMEC and Caco-2 cell lysate at ~170 kDa. Caco-2 cells were shown to express P-glycoprotein (21) and were used in this experiment as a positive control. Similar blots using the nonspecific mouse IgG revealed no immunoreactivity (not shown).

Ivermectin effects on rhodamine 123 uptake in BMECs. The uptake of rhodamine 123 in BMECs was studied alone and after a one hour preincubation with ivermectin. After the preincubation period, the cells were incubated one hour with rhodamine 123. In Figure 5, the typical concentration-dependent effect of ivermectin on rhodamine 123 uptake by BMECs is illustrated in the absence of serum. The higher concentrations (25 ng/ml or greater) of ivermectin were found to significantly increase the uptake of rhodamine 123 in BMECs. Figure 6A shows the effect of cyclosporin A, a known P-glycoprotein inhibitor, compared with selected concentrations of ivermectin on rhodamine 123 uptake in BMECs with medium without serum. The uptake of rhodamine 123 in BMECs with cyclosporin A and ivermectin was also studied with medium containing 10% equine serum and Figure 6B shows that cyclosporin A still inhibited

rhodamine 123; however, ivermectin's effects were completely inhibited in the presence of serum.

Transendothelial permeability studies in BMECs. The effects of BMEC monolayer pretreatment and subsequent exposure to ivermectin on rhodamine 123 transport through the BMEC monolayers was determined. Transendothelial permeability studies were performed in medium and medium containing 10% equine serum. A sample of the results obtained are illustrated in Figure 7. Ivermectin at 250 ng/ml reproducibly increased the transmonolayer permeability of rhodamine 123 in medium that did not contain serum although this increase was not statistically significant. Concentrations nearer reported circulating levels of ivermectin (≈10-100 ng/ml) had no effect on the permeation of rhodamine 123 across the BMEC monolayers. By contrast, there was no increase on rhodamine 123 permeability observed with 1µg/ml ivermectin treatment when the medium contained serum.

DISCUSSION

The first part of this study confirmed the functionality and specific expression of P-glycoprotein in primary cultures of BMECs in our laboratory. Both cyclosporin A and verapamil were shown to increase the uptake of rhodamine 123, a fluorescent marker of the P-glycoprotein efflux system (2,12-14). Cyclosporin A and verapamil are known inhibitors of the P-glycoprotein efflux system that are commonly used to distinguish the existence of this efflux system on cells (2,3,5,13,14). Our results compare favorably, in a qualitative manner, with the studies of Wang et al. (14) who showed about a 3-fold enhancement of rhodamine 123 uptake

in rat brain in the presence of cyclosporin A. Enhancement of rhodamine 123 uptake in the presence of verapamil was also consistent with the ability of calcium influx blockers to modulate drug efflux by MDR1 positive cells (2,13,22). The lack of effect of cyclosporin A or verapamil on rhodamine 123 uptake in HUVECs, cells negative for MDR1 (2,4), further supported the use of rhodamine 123 as a specific marker to evaluate the role of MDR1 efflux in BMECs in this study. Finally, the existence of a BMEC P-glycoprotein was further evidenced by the results of immunoblots which affirmed expression of an immunoreactive protein with the C219 monoclonal antibody, previously shown to be specific for MDR1 expression (10).

Despite being relatively lipophilic, ivermectin typically achieves its lowest concentrations in the brain (11). Supporting that observation was our previous work which demonstrated that the uptake of ivermectin by bovine BMECs was a limited process and subject to further restrictions in the presence of serum (7). Until recently, however, an exception existed in that certain rough-coated collies, exhibit higher brain concentrations of ivermectin compared with "normal" rough-coated collies (23). One can also distinguish between ivermectin sensitive and ivermectin tolerant collies on the basis of a greater verapamil-sensitive uptake of the drug by primary cultures of BMECs isolated from the ivermectin-sensitive collies (8). Schinkel et al.'s (10) work subsequently showed that in mice deficient in MDR1a, high levels of ivermectin accumulate in the brain, implicating a significant role for blood-brain barrier P-glycoprotein in regulating ivermectin distribution into the brain.

Our results provided further evidence that unbound ivermectin is at least an inhibitor of MDR1. We observed a significant increase in rhodamine 123 uptake when BMECs were

preincubated for one hour with 50 and 500 ng/ml ivermectin. Therefore, increased rhodamine 123 accumulation by BMECs occurred with treatments within the 10-100 ng/ml drug concentration range coinciding with the normal circulating levels of ivermectin (24,25). The ivermectin-induced increase in rhodamine 123 uptake was comparable to the increase seen with cyclosporin A and expected since these two drugs have similar apparent affinities for the MDR1 efflux system of other cell types (26). By contrast, in the presence of 10% serum in the medium, the highly protein bound ivermectin did not alter the uptake of rhodamine 123 by BMECs whereas the effect of cyclosporin A on rhodamine 123 uptake was still significant. This is an interesting observation since cyclosporin A is both highly protein bound and effectively trapped by brain endothelia (27). This observation was consistent with work in myeloma cell lines where Lehnert et al. (28) demonstrated that cyclosporin A concentrations above those achievable in humans effectively inhibited MDR1 even in the presence of serum. However, Lehnert et al. also observed that the actions other MDR1 inhibitors, amiodarone and trifluorperazine, were extremely sensitive over wide concentration ranges to the presence of serum (28). The uptake and entrapment of ivermectin by BMECs in the presence of serum (7), is at least 100-fold lower than the reported entrapment of cyclosporin A (27) and therefore, differences in inhibition of rhodamine 123 uptake by ivermectin and cyclosporin A in the presence of serum may reflect a combination of factors including differential protein binding and BMEC affinity for the two drugs. As pointed out by other researchers (28), the presence of serum *in vivo* and *in vitro* is an important variable in characterizing the inhibitory activity of some agents towards MDR1.

In an earlier study, we demonstrated that permeation of 30 ng/ml of ^{3}H-ivermectin across bovine BMEC monolayers was not altered by 10 µg/ml unlabeled ivermectin (7). The MDR1 efflux system would have been implicated as the sole regulator of ivermectin passage across bovine BMECs had the substantial excess of unlabeled ivermectin enhanced permeation of the labeled form of the drug. In fact, the effect of apparent P-glycoprotein inhibition on the ehanced permeation of drugs across bovine BMECs has been recently demonstrated in our laboratory for other drugs (29). Although high concentrations of ivermectin enhanced the permeation of the MDR1 substrate, rhodamine 123, across BMEC monolayers reproducibly, the effect was not statistically significant. Serum protein in the medium again did block the modest enhancing effect of ivermectin on rhodamine 123 permeation across the BMEC monolayers. These *in vitro* studies are short term relative to *in vivo* studies where drug distribution into the brain tissue and neurotoxicity is examined over a period of partial days to weeks (10,26). Thus, despite only a modest effect of ivermectin on the rhodamine permeation **rate**, over time one could expect greater **accumulation** of the marker on the brain tissue side of BMECs when MDR1 is inhibited.

Clearly, not all drugs have the same affinity for MDR1, MDR1 systems do vary somewhat among species (10,26), and there are some indications that MDR1 may vary in localization at the blood-brain barrier, particularly in the human, where there is conflicting evidence of MDR1 localization from the typical luminal membrane (30) to an abluminal and astrocyte location (31). Consequently, explanations for our observations of the only modestly enhanced rhodamine 123 diffusion across the BMECs in the presence of ivermectin may also include the possibility that the drug does inhibit the MDR1 on the apical membrane, but additional factors,

including other transport systems or binding sites in the cell control rhodamine123 transfer across bovine BMECs.

In summary, we conclude that there is an MDR1 efflux system associated with primary cultures of bovine BMECs and that unbound ivermectin is an inhibitor for this efflux system. This observation provides further support for the contributions of the MDR1 efflux system in the neurotoxicity of ivermectin and other drugs associated with some species of animals.

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FIGURE LEGENDS

Figure 1. Percentage of rhodamine 123 (initial concentration 5 μM) uptake in bovine brain microvessel endothelial cells alone (shaded bar) and in the presence of cyclosporin A (hatched bar) for 60 minutes at 37°C, pH 7.4. Each bar represents the mean ± SD of n = 6. ** represents statistically significant changes ($P < 0.01$).

Figure 2. Percentage of rhodamine 123 (initial concentration 5 μM) uptake in bovine brain microvessel endothelial cells alone (shaded bar) and in the presence of verapamil (hatched bar) for 40 minutes at 37°C, pH 7.4. Each bar represents the mean ± SD of n = 5. ** represents statistically significant changes ($P < 0.01$).

Figure 3. Rhodamine 123 (initial concentration 5 μM) uptake in human umbilical vein endothelial cells alone (shaded bar) and in the presence of 100 μM cyclosporin A or verapamil (hatched bars) for 30 minutes at 37°C, pH 7.4. Each bar represents the mean ± SD of n = 3.

Figure 4. Immunoblot analysis of P-glycoprotein (170 kDa) expression in BMEC and Caco-2 cell monolayers.

Figure 5. Concentration dependent effect of ivermectin on rhodamine 123 (initial concentration 5 μM) uptake by bovine brain microvessel endothelial cells at 60 minutes at 37°C. Each point is the mean ± SD for n = 4. ** represents statistically significant changes ($P < 0.01$).

Figure 6. A) Rhodamine 123 (initial concentration 5 μM) uptake by bovine brain microvessel endothelial cells alone (shaded bar) and after a one hour preincubation with 1 μg/ml cyclosporin A or selected concentrations of ivermectin (hatched bars) for 60 minutes at 37°C. Each bar represents the mean ± SD of n = 4. ** represents statistically significant changes ($P < 0.01$). **B)** Rhodamine 123 (initial concentration 5 μM) uptake in bovine brain microvessel endothelial cells alone (shaded bar) and after a one hour preincubation with 1 μg/ml cyclosporin A or different concentrations of ivermectin (hatched bars) in medium containing 10% equine serum for 60 minutes at 37°C. Each bar represents the mean ± SD of n = 4. ** represents statistically significant changes ($P < 0.01$).

Figure 7. Apparent permeability of rhodamine 123 alone (Rhod; shaded bars) and in the presence of ivermectin (Iver; hatched bars). Rhod* and Iver* represent apparent permeability of rhodamine 123 with 10% equine serum added to the medium. Each bar represents the mean $\pm$ SD of n = 3.

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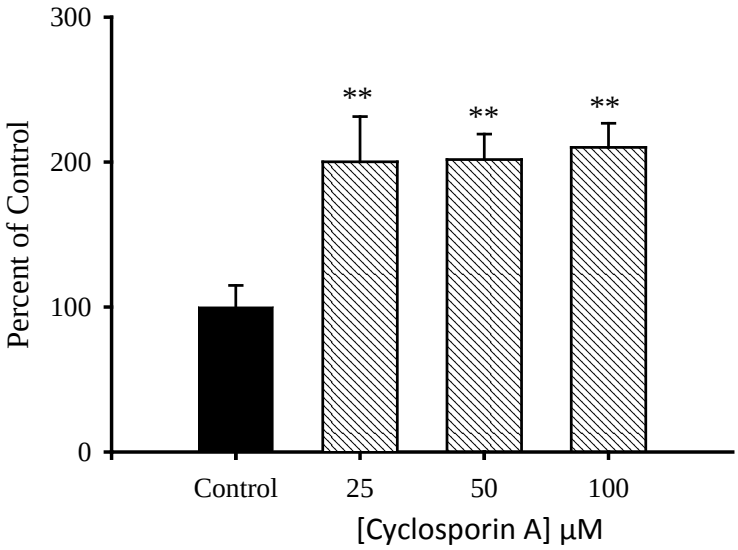
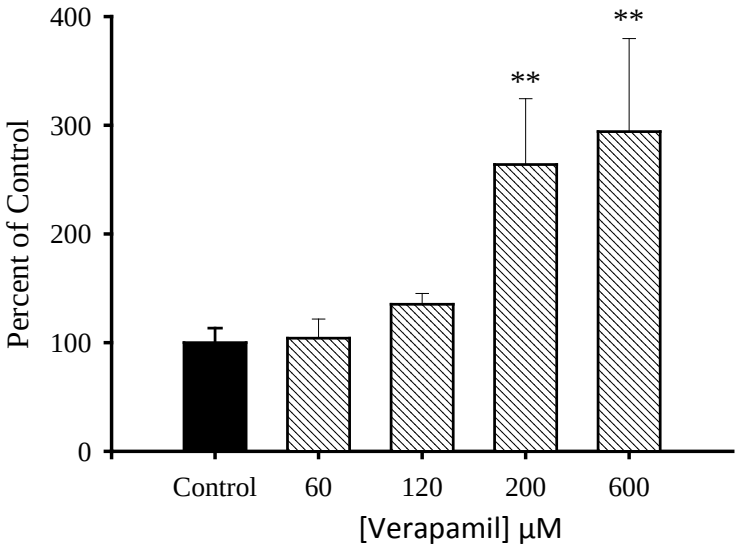
Rose et al., Figure 1



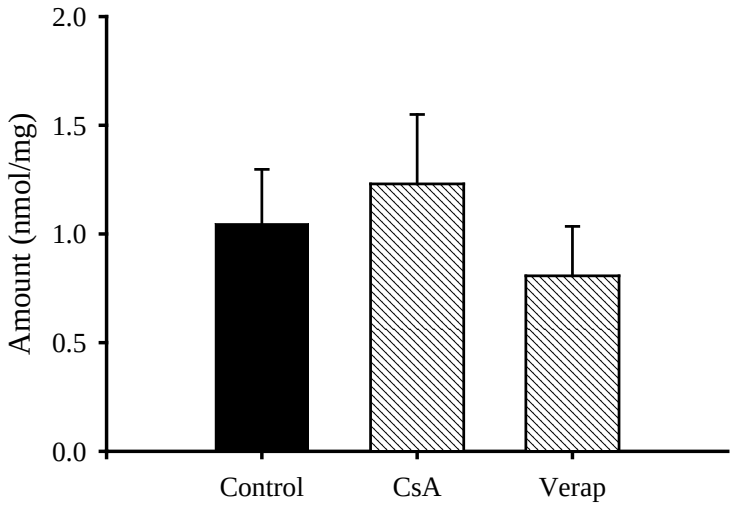
Rose, J.M., Peckham, S.L., Scism, J.L., and Audus, K.L. (1998) Evaluation of the role of P-glycoprotein in ivermectin uptake by primary cultures of bovine brain microvessel endothelial cells. Neurochem. Res. 23, 203-209. PMID: 9475515. Publisher's official version: <http://dx.doi.org/10.1023/A:1022485026198> . Open Access version: http://kuscholarworks.ku.edu/dspace/.

Rose et al., Figure 2



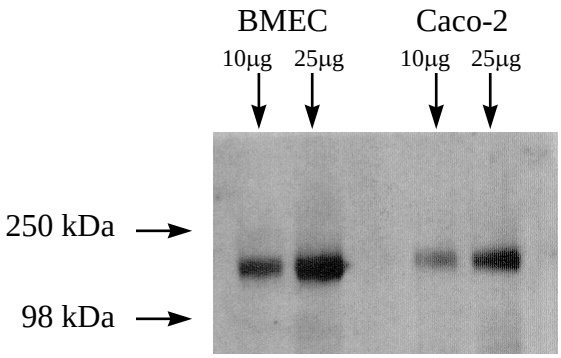
Rose, J.M., Peckham, S.L., Scism, J.L., and Audus, K.L. (1998) Evaluation of the role of P-glycoprotein in ivermectin uptake by primary cultures of bovine brain microvessel endothelial cells. Neurochem. Res. 23, 203-209. PMID: 9475515. Publisher's official version: <http://dx.doi.org/10.1023/A:1022485026198> . Open Access version: http://kuscholarworks.ku.edu/dspace/.

Rose et al., Figure 3



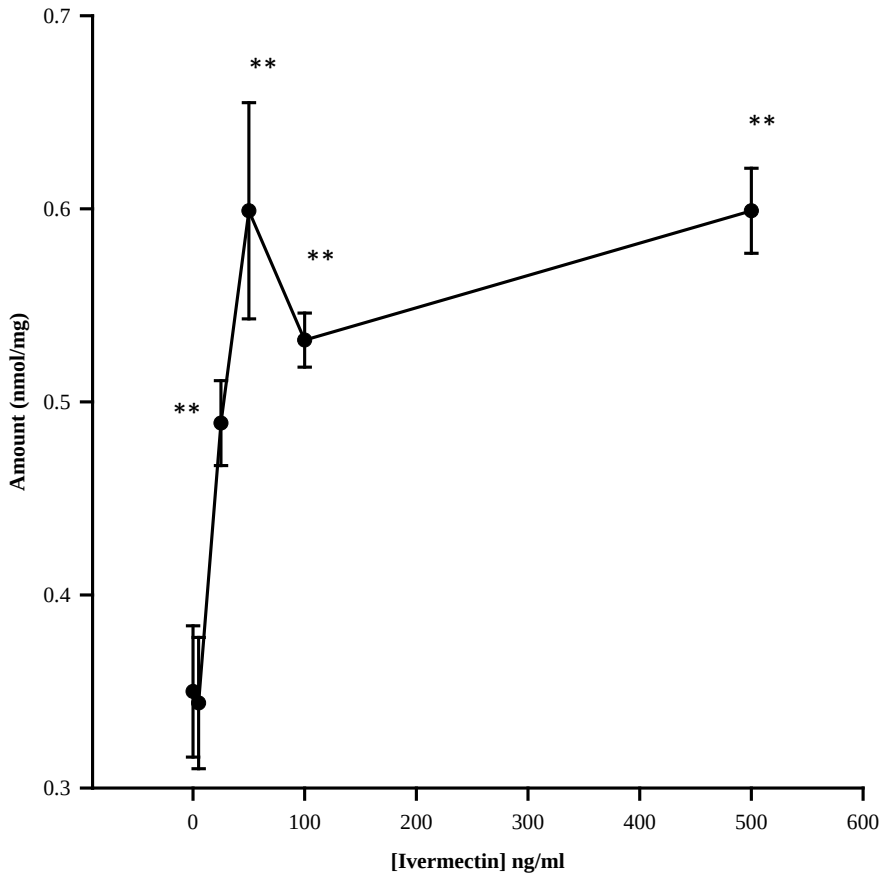
Rose, J.M., Peckham, S.L., Scism, J.L., and Audus, K.L. (1998) Evaluation of the role of P-glycoprotein in ivermectin uptake by primary cultures of bovine brain microvessel endothelial cells. Neurochem. Res. 23, 203-209. PMID: 9475515. Publisher's official version: <http://dx.doi.org/10.1023/A:1022485026198> . Open Access version: http://kuscholarworks.ku.edu/dspace/.

Rose et al., Figure 4



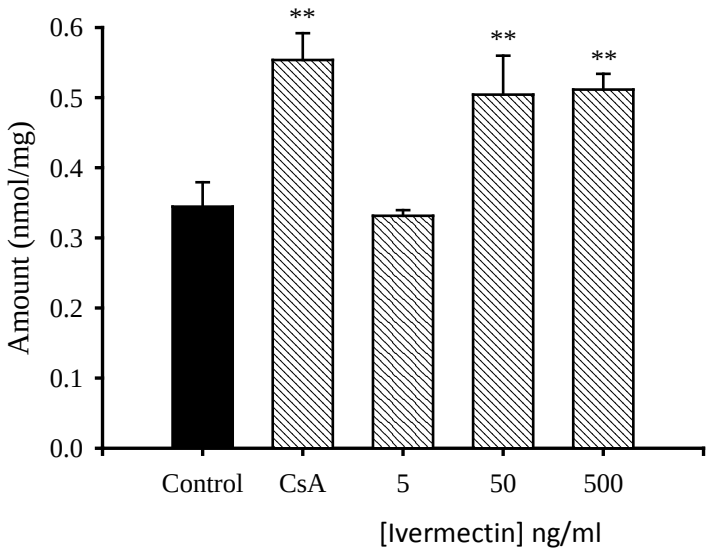
Rose, J.M., Peckham, S.L., Scism, J.L., and Audus, K.L. (1998) Evaluation of the role of P-glycoprotein in ivermectin uptake by primary cultures of bovine brain microvessel endothelial cells. Neurochem. Res. 23, 203-209. PMID: 9475515. Publisher's official version: <http://dx.doi.org/10.1023/A:1022485026198> . Open Access version: http://kuscholarworks.ku.edu/dspace/.

Rose et al., Figure 5



Rose, J.M., Peckham, S.L., Scism, J.L., and Audus, K.L. (1998) Evaluation of the role of P-glycoprotein in ivermectin uptake by primary cultures of bovine brain microvessel endothelial cells. Neurochem. Res. 23, 203-209. PMID: 9475515. Publisher's official version: <http://dx.doi.org/10.1023/A:1022485026198> . Open Access version: http://kuscholarworks.ku.edu/dspace/.

Rose et al., Figure 6A



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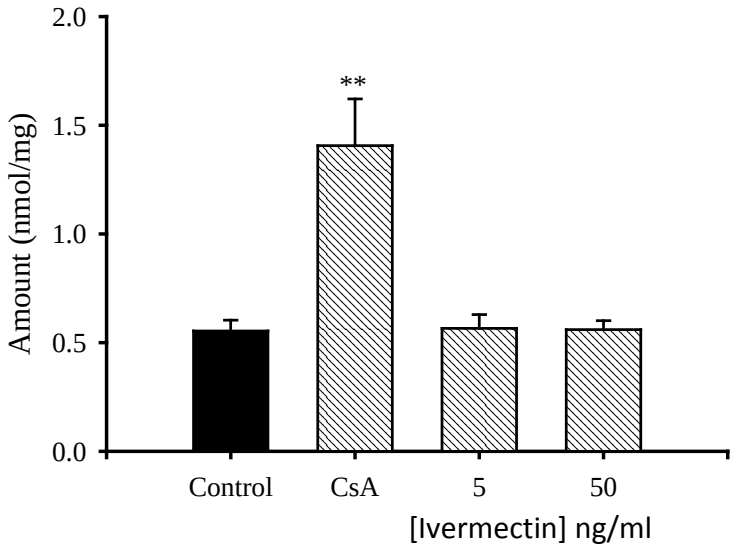
Rose et al., Figure 6B



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Rose et al., Figure 7