Original Paper

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2007;20:853-858

Accepted: June 11, 2007

TNF Activates P-Glycoprotein in Cerebral Microvascular Endothelial Cells

Chuanhui Yu, Abba J. Kastin, Hong Tu, Sarah Waters and Weihong Pan

Pennington Biomedical Research Center, Baton Rouge

Key Words

TNF • Endothelial cells • Blood-brain barrier • Microarray • MDR1 • Transport • Drug efflux pump

Abstract

Background/Aims: Multidrug resistance proteins (MDRs, including P-glycoproteins) are efflux pumps that serve important biological functions but hinder successful drug delivery to the CNS. Many chemotherapeutic agents, anti-epileptics, anti-HIV drugs, and opiates are substrates for MDRs. Therefore, understanding the regulation of MDRs in the endothelial cells composing the blood-brain barrier has therapeutic implications. Methods: We used microarray, real time RT-PCR, Western blotting, and uptake of vinblastine by RBE4 cerebral endothelial cells to test the effects of tumor necrosis factor alpha (TNF) on the expression and functions of P-glycoprotein (MDR1). Results: The proinflammatory cytokine TNF specifically induced the expression and enhanced the function of MDR1 in RBE4 cells. The persistent upregulation of MDR1 mRNA was shown by cDNA microarray at 6, 12, and 24 h after TNF treatment. This was confirmed by real-time RT-PCR between 2 and 24 h. MDR1 protein expression was increased 6 to 24 h after TNF treatment and resulted in a significant reduction in the cellular uptake of ^{3}H-vinblastine. Conclusion: The drug efflux transporter in cerebral endothelial cells can be upregulated by TNF. This suggests that adjunctive anti-TNF treatment has novel therapeutic potential in conditions such as brain cancer, epilepsy, neuroAIDS, and chronic pain.

Introduction

The multidrug resistance protein 1 (MDR1, or P-glycoprotein, a product of the MDR1 gene) is one of the most important efflux transporters belonging to the large family of ATP-binding cassette (ABC) transporters. The P-glycoprotein transporter expressed in the intestine, liver, and kidney is essential for drug absorption and disposition, and that at the blood-brain barrier (BBB) limits the permeation of a variety of drugs and toxins from blood to the CNS. Even the protease inhibitors used to treat human immunodeficiency virus type 1 (HIV-1) are substrates for P-glycoproteins . Structurally, the P-glycoproteins are integral membrane glycoproteins, consisting of two similar

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015-8987/07/0206-0853$23.50/0

Accessible online at:
www.karger.com/cpb

Weihong Pan, M.D., Ph.D.
Pennington Biomedical Research Center
6400 Perkins Road, Baton Rouge, LA 70808 (USA)
Tel. +1-225-763-2707, Fax +1-225-763-0261
E-Mail weihong.pan@pbrc.edu, http://labs.pbrc.edu/bloodbrainbarrier

halves of a six membrane spanning domain followed by a cytoplasmic domain where nucleotide binding takes place [2, 3]. There is a wide spectrum of substrates and many isoforms that show differential subcellular distribution.

The induction of MDR1 is largely responsible for the phenomenon of multiple drug resistance. MDR1 is expressed at the CNS barriers as well as in parenchymal tissue [4-10]. In brain tumors as well as many other disorders involving the CNS, drug delivery by peripheral routes is restricted by the presence of the blood-brain, blood-cerebrospinal fluid, and blood-tumor barriers. Once across these initial barriers, drug accumulation in the brain can be further restricted by active efflux transporters, like MDR1. Apart from the wide range of permeabilities related to the nature of the tumor, these efflux transporters actively reduce uptake of the drug [9-11]. Similar considerations apply to the treatment of epilepsy [12] and HIV-1 infection [13]. MDR1 in leukocytes reduces the cell uptake of protease inhibitors [14], and permeation of protease inhibitors across the BBB in neuroAIDS would encounter the same challenge.

Morphine and endorphins are also substrates for P-glycoprotein [15]. By contrast, endomorphin transport across the BBB does not involve P-glycoprotein either in in-vivo [16] or in-vitro experiments [17]. Although endomorphin is more selective for the mu opiate receptor than endorphin, these two peptides have little structural similarity. Inhibition of P-glycoprotein activity by GF120918 prolongs morphine-induced antinociception in rats [18], and another P-glycoprotein inhibitor, cyclosporine, as well as knockout of P-glycoprotein, increases the sensitivity of mice to a variety of opiates [19].

Overall, it seems that many drugs and prodrugs are recognized either as substrates or inhibitors of MDR1. Several of these substances, including chemotherapy agents, anti-epileptics, anti-HIV-1 drugs such as protease inhibitors, and endogenous and exogenous opiates, in turn modulate MDR1 activity and serve as substrates [19-22]. Thus, better understanding of how MDR1 is regulated will have a substantial impact on efficient drug delivery into the CNS.

Here we approach the problem of how neuroinflammation regulates the expression and function of MDR1. This is illustrated by use of tumor necrosis factor α (TNF), a proinflammatory cytokine that is produced with different time courses by all CNS parenchymal cells after trauma, hypoxia, and inflammation, resulting in elevated concentrations in the microcirculation [23]. TNF was originally identified as a macrophage-derived serum factor after endotoxin challenge causing hemorrhagic necrosis of mouse sarcoma Meth A and other transplanted tumors [24]. At the level of the BBB, TNF not only modulates the functions of the constituent endothelial cells and their tight junctions but also exhibits its own receptor-mediated transport [25-27]. We have shown that TNF specifically modulates the expression and functions of the receptors for leukemia inhibitory factor in the cerebral microvessel endothelial cell line RBE4 [28, 29]. How TNF regulates the functions of MDR1, a membrane protein encompassing 12-transmembrane domains [2, 3], has not been fully characterized.

Nonetheless, regulation of MDR1 by proinflammatory cytokines has been observed in parenchymal cells of the liver and colon [30-33]. Since TNF shows variable effects of either inducing or reducing MDR1 activity in these cells, its actions might depend on different cell types and treatment conditions. In BBB cells, one group of investigators initially found that TNF decreases MDR1 transport activity [34], but recently reported that this activity increased at longer times while this manuscript was under initial review [35]. The potential biphasic changes again indicate the dynamics of regulation. As TNF production also shows dynamic changes associated with tumor progression, hypoxia, and inflammation, its induction or suppression of MDR1 activity may directly affect the efficacy of therapeutic interventions at different disease stages.

To better quantify the regulation of MDR1 by TNF and the underlying mechanisms, we used an established rat cerebral microvessel endothelial cell line RBE4 as a model system. RBE4 cells express MDR1 and another ABC transporter, MRP1 (multidrug resistance-associated protein), as do primary cultured brain endothelial cells [36]. RBE4 cells also express TNF receptors, and show a lack of acute toxicity when low doses of TNF are applied [37]. Thus, RBE4 cells constitute a suitable model to study MDR1 expression and function. We found a consistent increase of MDR1, but not MRP1, at different times after TNF treatment in a microarray analysis. The increase of MDR1 was confirmed by real-time RT-PCR and Western blot analysis. Functional correlation with the higher level of expression was shown by upregulated MDR1 transporter activity in endocytosis assays. Thus, therapy of CNS disorders should take into consideration the increased efflux pump activity induced by proinflammatory cytokines.

Materials and Methods

Cells and reagents

RBE4 rat brain microvessel endothelial cells were kindly provided by Dr. Pierre-Oliver Couraud (Institute of Cochin, Paris, France). The cells were maintained in αMEM and F10 containing antibiotics and supplemented with 10% FBS and bFGF as previously reported [38]. Recombinant mouse TNF was obtained from Biosource (Camarillo, CA), and a final concentration of 5 - 25 ng/ml was used to treat the cells. ^{3}H-vinblastine sulfate (10-15 Ci/mmol) was purchased from Amersham Biosciences (Piscataway, NJ).

Microarray

Groups of RBE4 cells were treated simultaneously with TNF and collected 0, 6, 12, or 24 h after TNF treatment, the 0 time being the untreated control. Total RNA was extracted from pelleted cells with an Absolutely RNA RT-PCR Miniprep kit (Stratagene, La Jolla, CA). Double-stranded cDNA was synthesized by reverse transcription, purified, and transcribed in-vitro into cRNA labeled with digoxigenin. The quantity and quality of purified cRNA were determined in an Agilent 2100 Bioanalyzer (Agilent Biotechnologies, Santa Clara, CA). The labeled sample cRNA was incubated with immobilized oligonucleotides from the whole rat genome in the hybridization plate, followed by sequential incubation with anti-digoxigenin antibody conjugated to alkaline phosphatase and chemiluminescent substrate. The luminescence was detected at an emission wavelength of 458 nm in a 1700 chemiluminescent microarray analyzer from Applied Biosystems (Branchburg, NJ). The original scans were analyzed by ABarray software (R package) and normalized for comparison between microarray chips. Probe IDs which showed more than 10-fold changes ($p < 0.05$) compared with control were uploaded to the Panther (Protein Analysis Through Evolutionary Relationships) Classification System to search for regulated proteins.

Real-time RT-PCR

Primer pairs and fluorescent probes for real-time RT-PCR were designed with Primer Express software (Applied Biosystems) and obtained from Integrated DNA Technologies (Coralville, IA). The primers for rat MDR1 were: forward 5'-AGC ACA CGT CTT TGG GAT CAC -3', reverse 5'-CCG AAC CGG AAA CAA GCA -3'. The fluorescent probe was 5'-FAM-TTC GCC TTC ACC CAG GCC ATG A-TAMRA-3'. Reactions for real-time PCR were performed on an ABI 7900 instrument with Taqman one-step RT-PCR master mix reagents (Applied Biosystems). The quantity of target mRNA in each sample was normalized to the control mRNA of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as described elsewhere [39].

Western blotting

Cells were pelleted and lysed in RIPA buffer (100 mM NaCl, 10 mM Tris, pH 7.2, 0.1 % SDS, 1 % Triton X-100, 1 % deoxycholate, and 5 mM EDTA) in the presence of a protease inhibitor cocktail (Pierce, Rockford, IL). Protein concentrations were measured by bicinchoninic acid (BCA) assay (Pierce). Forty μg of whole cell lysates was separated on 7% SDS - polyacrylamide gels, and proteins were transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA). After blocking with 5% non-fat dry milk, the membranes were probed with rabbit polyclonal antibodies specific to MDR (Santa Cruz Biotechnology, Santa Cruz, CA) and horseradish peroxide-conjugated secondary antibodies (Amersham Biosciences) in phosphate-buffered saline (PBS) containing 0.1% Tween 20 and 0.5% milk. β-actin was also probed as a loading control. The signals were developed with enhanced chemiluminescence (ECL)-plus Western blotting detection reagents (Amersham Biosciences). All findings were confirmed in replicate studies.

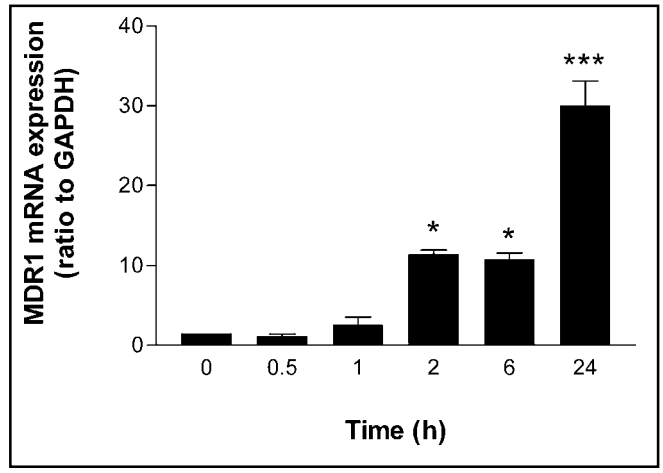
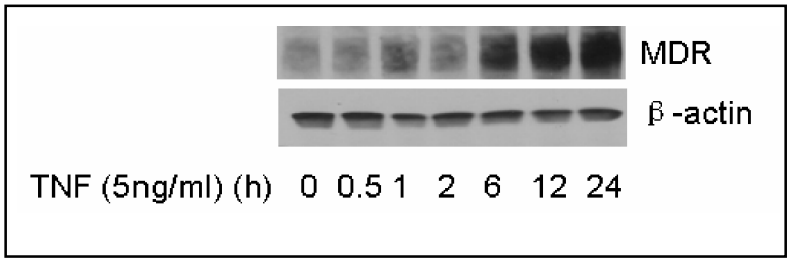


Fig. 1. Real-time RT-PCR measurement of MDR1 mRNA, normalized by the level of GAPDH mRNA. There was a significant increase at 2, 6, and 24 h after TNF treatment (5 ng/ml). *: $p < 0.05$; ***: $p < 0.005$ compared with the control without TNF.

Drug accumulation assay to determine MDR1 function

RBE4 cells were seeded onto a 12-well plate and allowed to grow to 90% confluence. Two groups of cells were studied simultaneously (n = 3 wells/group) for each time point: PBS treatment or TNF treatment (5 ng/ml) for 6 h. The cells were pre-equilibrated in 0.25 ml transport buffer (α-MEM and F-10 containing 0.5 % bovine serum albumin and 20 mM HEPES) at 37°C for 30 min immediately before the assay. Cellular uptake of ^{3}H-vinblastine (19 nM) was determined by incubation of the cells in 0.25 ml of transport buffer containing ^{3}H-vinblastine at 37°C for 10, 30, 60, and 90 min. At the end of the assay, the remaining extracellular ^{3}H-vinblastine was collected, and the cells were quickly washed with ice-cold PBS three times. The cells were collected in 0.25 ml of PBS containing 1% SDS, and further solubilized by addition of 0.5 ml of Solvable (Packard Biosciences, Meriden, CT) with overnight incubation at 50°C in a shaking water bath. After addition of UltimaGold scintillation cocktail (PerkinElmer, Waltham, MA) and incubation at room temperature overnight, the β-radioactivity in the samples was measured in an LS5000 scintillation counter (Beckman Coulter, Fullerton, CA). The percentage of internalized radioactivity was calculated. Group differences were determined by one-way analysis of variance.

Fig. 2. Immunoblot of MDR1 showed that protein expression was increased in cells 6, 12, and 24 h after TNF treatment.



Results

TNF upregulates MDR1, 2, 3 as shown by microarray

Panther analysis showed that there were 72 genes having more than a 10-fold upregulation ($p < 0.05$) after 6 h of TNF treatment, 48 genes at 12 h, and 35 genes at 24 h. The gene products included those involved in cellular membrane function, transport, cytokine signaling, cell adhesion, growth, differentiation, and extracellular matrix functions. Among these proteins, only a few showed persistent changes at all time points tested. MDR1, 2, 3 showed consistent upregulation, most pertinent to our studies on blood-brain and blood-tumor barriers. The more than 10-fold increase of the mRNA of MDRs suggests the specificity of the findings.

Real-time RT-PCR verifies increased MDR1 mRNA

To verify the findings of increased MDR1 mRNA shown by microarray, RBE4 cells were treated with an even lower dose of TNF (5 ng/ml) for 0, 0.5, 1, 2, 6, and 24 h, the 0 time being the PBS vehicle-treated control. Real-time RT-PCR showed that TNF induced a time-dependent increase of MDR1, which was significant by 2 h and was most pronounced at 24 h (Fig.1). The results were normalized to the mRNA of the housekeeping gene GAPDH.

MDR1 protein is upregulated by TNF treatment

Time-dependent induction of MDR1 protein in RBE4 cell lysate was shown by semi-quantitative Western blot analysis with β-actin as an internal control. The increase was pronounced at 6 h, and continued to increase at 12 and 24 h (Fig.2). This is consistent with the induction of MDR1 mRNA which exhibited an earlier onset.

Increased MDR1 function results in reduced cellular uptake of vinblastine

In RBE4 cells, the baseline uptake of ^{3}H-vinblastine was high. The accumulation of radioactivity inside the cells was most rapid in the first 30 min, and gradually reached a plateau. In cells 6 h after TNF treatment, ^{3}H-vinblastine uptake was significantly lower than in the control group at all time points tested ($p < 0.005$) (Fig.3). Since the extracellular concentration (amount of vinblastine available for uptake) was significantly higher than that inside the cells, the nonlinear relationship between the %endocytosis and time of incubation indicates the presence of an efflux pump for vinblastine. This efflux system showed a greater capacity in the presence of TNF, reflected by the lower percent accumulation of vinblastine at all time points tested and a similar plateau.

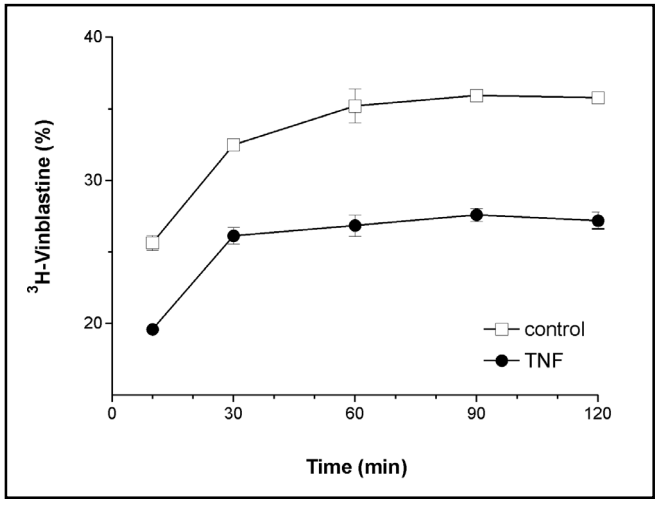


Fig. 3. The function of MDR1 was determined by the cellular uptake of vinblastine. TNF treatment (6 h) caused a significant reduction of vinblastine uptake at 10, 30, 60, 90, and 120 min ($p < 0.005$).

Discussion

Our results show that TNF treatment induced a pronounced increase of MDR1 mRNA and protein expression. This led to upregulation of transporter activity, with less vinblastine taken up by the cells. Vinblastine is a known substrate for MDR1, and its reduced uptake by the cells reflects activation of MDR1 [40]. The findings

suggest that increased production of TNF, such as by CNS tumor or inflammation, can activate efflux transport at the BBB and alter the efficacy of CNS therapeutics.

RBE4 cerebral microvessel endothelial cells express receptors for TNF and show dose- and time-related changes in γGT activity and ATP production in response to TNF. Overall, TNF treatment results in a reduction of cellular metabolism shown by decreased levels of ATP [37]. MDR1 activity is dependent on binding and utilization of ATP [41]. Thus, in the presence of reduced cellular ATP production, the upregulation of MDR1 by TNF assumes added significance. Since brain tumors are often associated with necrosis and inflammation of the surrounding brain parenchyma, TNF produced in-situ may serve as an autocrine or paracrine factor to activate MDRs.

We showed by microarray that MDR1,2,3 were upregulated in RBE4 cells by TNF treatment, and focused on MDR1 in the current study. Regina et al. reported that another ABC transporter, MRP1 (multidrug resistance-associated protein), as well as MDR1, are present in primary cultured brain endothelial cells [36]. However, our microarray analysis did not detect more than a 10-fold of increase of MRP1 or other ABC transporters. This makes the finding of MDR1 upregulation more specific.

The TNF-mediated upregulation of MDR1 expression and function in endothelial cells may be involved in the variability of tumor responses to chemotherapy. MDR1 is mainly located at the apical surface of endothelial cells[42]. TNF can be produced by cancer cells, microglia and other cell types in the CNS parenchyma, and it enters the cerebral microcirculation through a relatively leaky barrier as a result of tissue necrosis, inflammation, and hypoxia. Thus, TNF can activate MDR1 at the luminal side of the endothelial cells, where most BBB efflux transporters are located, to reduce drug penetration. Similarly, TNF concentration in the microcirculation may be elevated in systemic inflammation leading to brain abscess, neuroAIDS, epilepsy, and chronic pain syndromes. Since the permeability of the BBB to TNF shows a circadian rhythm, particularly in the spinal cord region [43], the activity of MDR1 also may be subject to circadian regulation.

The RBE4 in-vitro BBB system also serves as a good model for dissection of the downstream events after TNF binding to its receptors that eventually lead to transcriptional regulation of MDR1, as we showed by microarray and real-time PCR. One of the major effector molecules for TNF signaling is nuclear factor (NF)-κB. An NF-κB binding site has been identified within the rat MDR1b promoter [44]. This has facilitated our ongoing investigation of MDR1 promoter regulation by TNF in RBE4 cells.

The finding that MDR1 in endothelia was subject to regulation by TNF also points to the possibility that other proinflammatory cytokines may play regulatory roles in the uptake of vinblastine and other substrates for MDR1. This opens the new prospect of anti-TNF or anti-inflammatory treatment to enhance the efficacy of delivery of CNS drugs that are affected by the MDR1 efflux transporter, such as some chemotherapeutic agents, anti-epileptics, anti-AIDS, analgesics, and anti-Alzheimer's disease medications.

Acknowledgements

Supported by NIH (NS45751, NS46528, and DK54880).

References

1 Kim RB: Drug transporters in HIV Therapy. Top HIV Med 2003;11:136-139.

2 Croop JM: P-glycoprotein structure and evolutionary homologies. Cytotechnology 1993;12:1-32.

3 Germann UA: Molecular analysis of the multidrug transporter. Cytotechnology 1993;12:33-62.

4 Cutler L, Howes C, Deeks NJ, Buck TL, Jeffrey P: Development of a P-glycoprotein knockout model in rodents to define species differences in its functional effect at the blood-brain barrier. J Pharm Sci 2006;95:1944-1953.

5 Kubota H, Ishihara H, Langmann T, Schmitz G, Stieger B, Wieser HG, Yonekawa Y, Frei K: Distribution and functional activity of P-glycoprotein and multidrug resistance-associated proteins in human brain microvascular endothelial cells in hippocampal sclerosis. Epilepsy Res 2006;68:213-228.

6 De Boer AB, De Lange EL, Van dS, I, Breimer DD: Transporters and the blood-brain barrier (BBB). Int J Clin Pharmacol Ther 1998;36:14-15.

7 Tanaka Y, Abe Y, Tsugu A, Takamiya Y, Akatsuka A, Tsuruo T, Yamazaki H, Ueyama Y, Sato O: Ultrastructural localization of P-glycoprotein on capillary endothelial cells in human gliomas. Virchows Arch 1994;425:133-138.

8 Torok M, Huwyler J, Gutmann H, Fricker G, Drewe J: Modulation of transendothelial permeability and expression of ATP-binding cassette transporters in cultured brain capillary endothelial cells by astrocytic factors and cell-culture conditions. Exp Brain Res 2003;153:356-365.
9 Zhang W, Mojsilovic-Petrovic J, Andrade MF, Zhang H, Ball M, Stanimirovic DB: The expression and functional characterization of ABCG2 in brain endothelial cells and vessels. FASEB J 2003;17:2085-2087.
10 Aronica E, Gorter JA, Redeker S, van Vliet EA, Ramkema M, Scheffer GL, Scheper RJ, van d, V, Leenstra S: Localization of breast cancer resistance protein (BCRP) in microvessel endothelium of human control and epileptic brain. Epilepsia 2005;46:849-857.
11 Begley DJ: ABC transporters and the blood-brain barrier. Curr Pharm Des 2004;10:1295-1312.
12 Abbott N, Khan EU, Rollinson C, Reichel A, Janigro D, Dombrowski S, Dobbie M, Begley DJ: Drug resistance in epilepsy: the role of the blood-brain barrier. Novartis Found Symp 2002;243:38-47.
13 Chaillou S, Durant J, Garraffo R, Georgenthum E, Roptin C, Clevenbergh P, Dunais B, Mondain V, Roger PM: Intracellular concentration of protease inhibitors in HIV-1-infected patients: correlation with MDR-1 gene expression and low dose of ritonavir. HIV Clin Trials 2002;3:493-501.
14 Srinivas RV, Middlemas D, Flynn P, Fridland A: Human immunodeficiency virus protease inhibitors serve as substrates for multidrug transporter proteins MDR1 and MRP1 but retain antiviral efficacy in cell lines expressing these transporters. Antimicrob Agents Chemother 1998;42:3157-3162.
15 King M, Su W, Chang A, Zuckerman A, Pasternak GW: Transport of opioids from the brain to the periphery by P-glycoprotein: peripheral actions of central drugs. Nat Neurosci 2001;4:268-274.
16 Kastin AJ, Fasold MB, Zadina JE: Endomorphins, Met-Enkephalin, Tyr-MIF-1, and the P-glycoprotein efflux system. Drug Metab Disp 2002;30:231-234.
17 Somogyvari-Vigh A, Kastin AJ, Liao J, Zadina JE, Pan W: Endomorphins exit the brain by a saturable efflux system at the basolateral surface of cerebral endothelial cells. Exp Brain Res 2004;156:224-230.
18 Letrent SP, Pollack GM, Brouwer KR, Brouwer KL: Effect of GF120918, a potent P-glycoprotein inhibitor, on morphine pharmacokinetics and pharmacodynamics in the rat. Pharm Res 1998;15:599-605.
19 Thompson SJ, Koszdin K, Bernards CM: Opiate-induced analgesia is increased and prolonged in mice lacking P-glycoprotein. Anesthesiology 2000;92:1392-1399.
20 Mercier C, Decleves X, Masseguin C, Fragner P, Tardy M, Roux F, Gabrion J, Scherrmann JM: P-glycoprotein (ABCB1) but not multidrug resistance-associated protein 1 (ABCC1) is induced by doxorubicin in primary cultures of rat astrocytes. J Neurochem 2003;87:820-830.
21 Loscher W: Animal models of drug-resistant epilepsy. Novartis Found Symp 2002;243:149-159.
22 Dupuis ML, Flego M, Molinari A, Cianfriglia M: Saquinavir induces stable and functional expression of the multidrug transporter P-glycoprotein in human CD4 T-lymphoblastoid CEMrev cells. HIV Med 2003;4:338-345.
23 Pan W, Zadina JE, Harlan RE, Weber JT, Banks WA, Kastin AJ: Tumor necrosis factor α: a neuromodulator in the CNS. Neurosci Biobehav Rev 1997;21:603-613.
24 Carswell EA, Old LJ, Dassel RL: An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci U S A 1975;72:3666-3670.
25 Gutierrez EG, Banks WA, Kastin AJ: Murine tumor necrosis factor alpha is transported from blood to brain in the mouse. J Neuroimmunol 1993;47:169-176.
26 Pan W, Banks WA, Kastin AJ: Permeability of the blood-brain and blood-spinal cord barriers to interferons. J Neuroimmunol 1997;76:105-111.
27 Pan W, Kastin AJ: TNFa transport across the blood-brain barrier is abolished in receptor knockout mice. Exp Neurol 2002;174:193-200.
28 Yu C, Kastin AJ, Tu H, Pan W: Opposing effects of proteasomes and lysosomes on LIFR: modulation by TNF. J Mol Neurosci 2007;in press.
29 Yu C, Kastin AJ, Pan W: TNF reduces LIF endocytosis despite increasing NFkappaB-mediated gp130 expression. J Cell Physiol 2007;213:161-166.
30 Stein U, Walther W, Shoemaker R: Reversal of multidrug resistance by transduction of cytokine genes into human colon carcinoma cells. J Natl Cancer Inst 1996;88:1383-1392.
31 Hartmann G, Kim H, Piquette-Miller M: Regulation of the hepatic multidrug resistance gene expression by endotoxin and inflammatory cytokines in mice. Int Immunopharmacol 2001;1:189-199.
32 Lee G, Piquette-Miller M: Cytokines alter the expression and activity of the multidrug resistance transporters in human hepatoma cell lines; analysis using RT-PCR and cDNA microarrays. J Pharm Sci 2003;92:2152-2163.
33 Belliard L, Lacour B, Farinotti R, Leroy C: Effect of tumor necrosis factor-alpha and interferon-gamma on intestinal P-glycoprotein expression, activity, and localization in Caco-2 cells. J Pharm Sci 2004;93:1524-1536.
34 Hartz A, Bauer B, Fricker G, Miller D: Rapid modulation of P-glycoprotein-mediated transport at the blood-brain barrier by tumor necrosis factor-alpha and lipopolysaccharide. Mol Pharmacol 2006;69:462-470.
35 Bauer B, Hartz A, Miller D: Tumor necrosis factor alpha and endothelin-1 increase P-glycoprotein expression and transport activity at the blood-brain barrier. Mol Pharmacol 2007;71:667-675.
36 Regina A, Koman A, Piciotti M, El HB, Center MS, Bergmann R, Couraud PO, Roux F: Mrp1 multidrug resistance-associated protein and P-glycoprotein expression in rat brain microvessel endothelial cells. J Neurochem 1998;71:705-715.
37 Yu C, Kastin AJ, Ding Y, Pan W: Gamma glutamyl transpeptidase is a dynamic indicator of endothelial response to stroke. Exp Neurol 2007;203:116-122.
38 Pan W, Yu Y, Cain CM, Nyberg F, Couraud P-O, Kastin AJ: Permeation of growth hormone across the blood-brain barrier. Endocrinol 2005;146:4898-4904.
39 Pan W, Ding Y, Yu Y, Ohtaki H, Nakamachi T, Kastin AJ: Stroke upregulates TNF alpha transport across the blood-brain barrier. Exp Neurol 2006;198:222-233.
40 Wang F, Wang L, Yang J, Nomura M, Miyamoto K: Reversal of P-glycoprotein-dependent resistance to vinblastine by newly synthesized bisbenzylisoquinoline alkaloids in mouse leukemia P388 cells. Biol Pharm Bull 2005;28:1979-1982.
41 Horio M, Gottesman M, Pastan I: ATP-dependent transport of vinblastine in vesicles from human multidrug-resistant cells. Proc Natl Acad Sci 1980;85:3580-3584.
42 Lee G, Dallas S, Hong M, Bendayan R: Drug transporters in the central nervous system: brain barriers and brain parenchyma considerations. Pharmacol Rev 2001;53:569-596.
43 Pan W, Cornelissen G, Halberg F, Kastin AJ: Selected contribution: circadian rhythm of tumor necrosis factor-alpha uptake into mouse spinal cord. J Appl Physiol 2002;92:1357-1362.
44 Zhou G, Kuo MT: NF-kappaB-mediated induction of mdr1b expression by insulin in rat hepatoma cells. J Biol Chem 1997;272:15174-15183.