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Insulin suppresses the expression and function of breast cancer resistance protein in primary cultures of rat brain microvessel endothelial cells

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Abstract:
The aim of this study was to investigate the role of insulin in the regulation of breast cancer resistance protein (BCRP) function and expression using primary cultured rat brain microvessel endothelial cells (rBMECs) as an *in vitro* model of the blood brain barrier (BBB). The prazosin uptake assay and western blot analysis were used to assess the function and expression of BCRP, respectively. It was noted that the uptake of prazosin by rBMECs was time-, concentration- and temperature-dependent. The BCRP inhibitors novobiocin and imatinib mesylate significantly increased the uptake of prazosin by the cells in a concentration-dependent manner. The cells were also incubated with sera from diabetic rats for 72 h, serving as a diabetic *in vitro* model. We found that the uptake of prazosin by rBMECs incubated in the diabetic rat sera was 39.8% of that in normal rat sera, and insulin treatment reversed this decrease. Further results showed that insulin down-regulated the function and expression of BCRP in rBMECs in a concentration-dependent manner. Treatment with an antibody against the insulin receptor abolished the down-regulation of BCRP function and expression that was induced by insulin. These results indicate that insulin suppressed the function and expression of BCRPs in rBMEC primary cultures.

Key words:
breast cancer resistance protein, diabetes, insulin, rat brain microvessel endothelial cells

Abbreviations: ABC – ATP-binding cassette, ANOVA – analysis of variance, BBB – blood brain barrier, BCRP – breast cancer resistance protein, CNS – central nervous system, DM – diabetes mellitus, HBSS – Hanks' balanced salt solution, IL – interleukin, LRP – lung resistance protein, MRP – multidrug resistance-associated protein, PBST – phosphate-buffered saline containing 0.1% Tween-20, P-GP – P-glycoprotein, rBMECs – rat brain microvessel endothelial cells, STZ – streptozotocin, TNF-α – tumor necrosis factor-α

Introduction

Breast cancer resistance protein (BCRP), a member of the ATP-binding cassette (ABC) superfamily of drug transporters, is involved in multi-drug resistance and actively transports various drugs out of the cell. This protein is expressed not only in anticancer drug-resistant cells, but

also in various normal tissues, including brain capillary endothelial cells [8–10, 28]. The tissue distribution and substrates of BCRP show extensive overlap with those of P-glycoproteins (P-GP), suggesting that BCRP may have a similar role as P-GP in the pharmacological handling of drugs. In the brain, BCRP is mainly expressed in the luminal membrane of microvessel endothelial cells [7, 10], which is an important component of the BBB. An absence of functional BCRP in the BBB may result in more drugs penetrating the brain, which may result in increased neurotoxicity or altered pharmacological effects of the drugs on the central nervous system (CNS).

Accumulating evidence has shown that diabetes mellitus (DM) may alter the function and expression of some ABC transporters [13, 15, 17, 19, 25]. These alterations are often transporter species- and tissue-dependent. One report has shown that multidrug resistance-associated protein 2 (MRP2), MRP4, and BCRP were increased by 5.4 fold, 2 fold, and 1.6 fold, respectively, in the kidneys of type 2 diabetic rats [19]. Streptozotocin-induced (STZ-induced) diabetic states have resulted in increased hepatic gene and protein expression of type 2 P-GPs by 105% and 530%, respectively [25]. Fluorescein, a MRP2 substrate, was found to lower brain distribution in STZ-induced diabetic rats, indicating enhanced MRP2 function in the brain [13]. On the contrary, our previous studies showed an impaired function and expression of P-GP in the brains of diabetic rats induced by STZ, which was reversed by insulin treatment [14, 15]. Data from our *in vitro* study demonstrated that insulin up-regulated the function and expression of P-GP in a concentration-dependent manner in primary cultured rBMECs [14]. It is known that insulin deficiency is a key sign of DM, and many reports have suggested that insulin deficiency may be an important factor in the impairment of P-GP function and expression in the BBB during diabetic states. Our previous study showed that STZ-induced diabetes may impair the function and expression of BCRP in the cortex of rats, and insulin treatment reversed this down-regulation of BCRP [17], indicating that insulin may also play an important role in the function and expression of BCRP. However, an accurate role of insulin in BCRP function and expression in the BBB is not fully understood. The aims of this study were to investigate the function and expression of BCRP in rBMECs and to further investigate the role of insulin in BCRP expression and function using rBMECs as an *in vitro* model. The expression and function of BCRP were measured using western blot analysis and by measuring the uptake of prazosin by rBMECs, respectively.

Materials and Methods

Isolation and culturing of rBMECs

The isolation and culturing of rBMECs from 7- to 10-day-old Sprague-Dawley rats was performed according to previous methods in our laboratory [24, 27]. The primary cultures of rBMECs were used for the BBB experiments when cells reached confluency. Positive staining with an antibody against factor VIII-related antigen helped identify rBMECs in at least 95% of the cell population [21]. The cells used in the present study were from passage numbers 2 or 3.

The uptake and efflux of prazosin by rBMECs

The amount of uptake of prazosin (National Institute of Control Pharmaceutical and Biological Products, Beijing, China), a typical substrate of BCRP [18, 29], was used to assess the functional activity of BCRP in rBMECs. The cells were cultured on a monolayer in 24-well plates and pre-incubated with 1 ml of Hanks' balanced salt solution (HBSS, pH = 7.4) per well at 37°C for 15 min. The solution was then removed, and 1 ml of HBSS containing prazosin was added to each well and incubated for a designated period of time. The uptake reaction was terminated by the addition of 1 ml of cold HBSS, and the cells were then washed 3 times with 1 ml of ice-cold HBSS. The cells were then frozen in 0.3 ml of purified water and melted repeatedly 3 times to break down the cells.

Time- and temperature-dependent experiments were performed at pre-designated times (5, 15, 30, 60, 90 and 120 min) using 500 nM prazosin at 37°C and 4°C, respectively. Concentration-dependent studies were performed similarly to the above described method, but different concentrations of prazosin (250 nM to 1000 nM) were applied at 37°C for 120 min. The effect of BCRP inhibitors on the steady state uptake of prazosin by rBMECs was investigated by assessing the efflux of prazosin from cells after cotreatment with prazosin (500 nM) and BCRP inhibitors [4, 22], novobiocin and imatinib mesylate, at 37°C. Cultures

of rBMEC were pre-incubated in HBSS containing prazosin (500 nM) at 37°C for 120 min. The HBSS solution was then removed, and cells were washed with drug-free HBSS 3 times at 4°C. The cells were then incubated with fresh prazosin-free HBSS containing either novobiocin (50 μM; 142-06673, Wako, Osaka, Japan) or imatinib mesylate (2 μM; Ange Pharmaceutical Co., Nanjing, China) at 37°C for 20 min. The amount of prazosin remaining in the cells was measured.

The effects of diabetic rat sera on BCRP in rBMECs

The effects of diabetic rat sera on BCRP function and expression in rBMECs were also investigated according to the methods described previously [14]. Diabetic rats were generated by injecting animals with STZ (65 mg/kg, *ip*). The sera of diabetic rats were collected after 5 weeks following STZ injection, and insulin levels of the diabetic rats and normal rats were measured to be 33.4 ± 6.0 mU/l and 71.4 ± 14.3 mU/l, respectively, using a rat insulin ELISA kit (Adlitteram Diagnostic Laboratory, Inc., USA). The sera, inactivated by heating at 56°C for 30 min, filtered with a 0.22 μm filter before being used. The monolayer of cells in the 24-well dish was incubated in the sera from diabetic or normal rats for 72 h, without cell culture medium. The amounts of uptake of prazosin by rBMECs incubated in diabetic rat and control rat sera were measured. Fifty mU/l of insulin (Wanbang Pharmaceutical Co., Xuzhou, China) was then added to the diabetic rat sera to investigate whether insulin reverses the alterations in BCRP function induced by the sera of diabetic rats. The cell viability was measured using the MTT assay, and no significant differences between rat sera incubation and negative controls (HBSS incubation) were observed.

The effect of insulin on BCRP in rBMECs

To further investigate the effects of insulin on BCRP function and expression, rBMECs were incubated in normal culture medium containing different levels of insulin (12.5, 25 or 50 mU/l) for 72 h. The amount of uptake of prazosin and western blot analysis were used to assess the functional activity and protein levels of BCRP, respectively. The functional activity and protein levels of BCRP were also measured in rBMECs following a 72-h incubation in medium containing both 1 μM of a polyclonal anti-insulin receptor antibody (Boshide Biotech Co., Wuhan, China) and 50 mU/l of insulin.

Western blot assays

Protein levels of BCRP were measured using western blot analysis. Briefly, various treatments were applied to the primary cultures of rBMECs. Cells were pelleted by centrifugation and lysed by ultrasonication in ice-cold cell lysis buffer (10 mM Tris–HCl [pH 7.5], 1 mM EGTA, 1 mM $MgCl_2$, 1 mM mercaptoethanol, 1% glycerol, and protease inhibitor cocktail [1 mM dithiothreitol and, 2 mM phenylmethylsulfonyl fluoride; Sigma Chemical Co., St. Louis, MO, USA]). Proteins were separated by electrophoresis on a 10% SDS-polyacrylamide gel, and the proteins were electrophoretically transferred to a PVDF membrane. The membrane was blocked in Tris-buffered saline containing 0.1% Tween-20 and 5% nonfat dried milk at room temperature for 30 min and washed three times for 15 min in phosphate-buffered saline containing 0.1% Tween-20 (PBST). The membrane was probed with a goat polyclonal anti-ABCG2 antibody (D-20, sc-25156, Santa Cruz, USA) in PBST at 4°C overnight. The membrane was washed with PBST, incubated in the appropriate HRP-conjugated mouse anti-goat secondary antibody (Jackson, USA) at room temperature for 1 h, and washed with PBST (4 × 10 min). The blots were incubated with ECL substrate solution (Lingqi Co., Wuxi, China) for 1 min according to the manufacturer's instructions and visualized with autoradiography. All blots were stripped and probed with polyclonal anti-β-actin antibody (Santa Cruz, USA) to ascertain that equal amounts of protein were loaded into the gel.

Drug assays and statistical analysis

The concentration of prazosin in rBMECs was measured by HPLC [16]. The sensitivity of the assay was 3.9 ng/ml, and an optimal linear curve was obtained from 3.9 to 125 ng/ml. The protein content was measured by the Bradford method [3] using BSA as the standard. The net uptake, expressed as the concentration ratio (ng/μg protein), was obtained by dividing the amount of uptake of prazosin by the protein content (μg protein/ml).

All data were expressed as the means ± standard deviation (SD). Analysis of variance (ANOVA) was

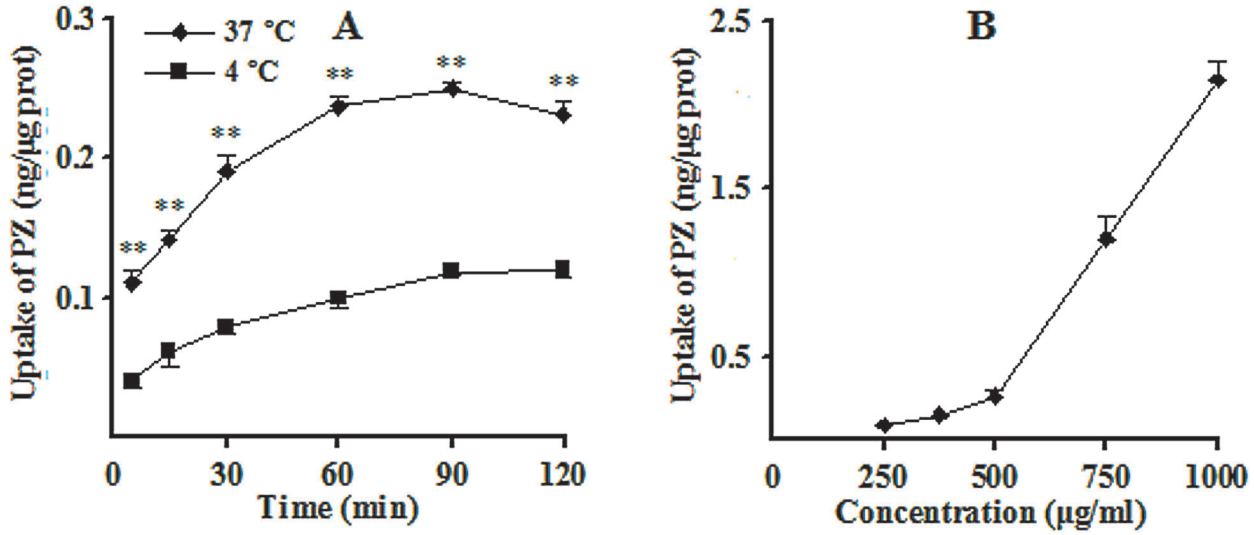

Fig. 1. (**A**) Time- and temperature-dependent uptakes of prazosin (PZ) by rBMECs. The cells were incubated in HBSS containing 500 nM PZ at 37°C and 4°C. (**B**) Concentration-dependent uptake of PZ by rBMECs. The cells were incubated in HBSS containing different concentrations of PZ (250, 375, 500, 750 and 1000 nM) at 37°C for 120 min (the mean ± SD; n = 4; ** $p < 0.01$ *vs.* 4°C)

used to determine if the differences between the experimental groups were statistically significant. The 0.05 level of probability was used as the criteria for significance.

Results and Discussion

The present study explored whether BCRP was expressed in primary cultures of rBMECs. Because previous reports have shown the expression of ABCG2 mRNA in rBMECs [26], our western blots were probed with anti-ABCG2 antibodies, which resulted in a band at 72 kDa that corresponded to BCRP (Fig. 4a). These results verified the existence of BCRP proteins in primary cultured rBMECs and indicated that the cells may be used as an *in vitro* model for assaying BCRP function. The functional activity of BCRP in rBMECs was determined by measuring the time-, temperature-, and concentration-dependent changes in the amount of prazosin uptake by these cells. We show that the uptake of prazosin by rBMECs was time- and temperature-dependent (Fig. 1A). Lowering the temperature from 37°C to 4°C significantly decreased this uptake by 52.3% at 120 min ($p < 0.01$), and the accumulation reached a plateau between 60 and 120 min at 37°C. Accordingly, the uptake of prazosin at 120 min was used for experiments that evaluated the effects of various treatments on the transport characteristics of prazosin. Concentration-dependent experiments consisted of a 120-min incubation in the presence of prazosin at concentrations ranging from 250 to 1,000 nM (Fig. 1B). The results showed that the

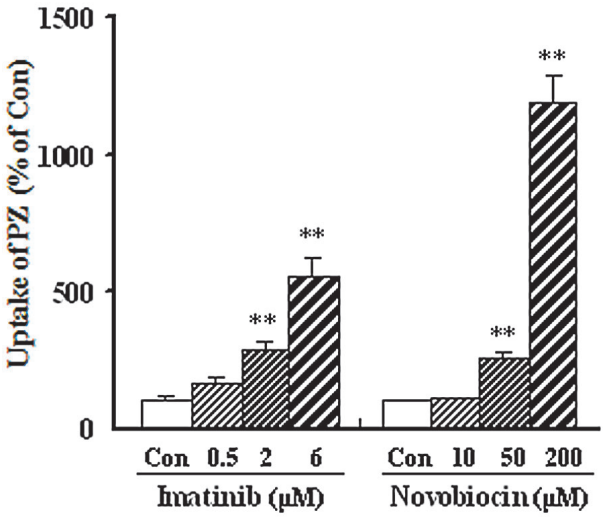

Fig. 2. The effect of BCRP inhibitors imatinib mesylate (Imatinib) and novobiocin on the uptake of prazosin (PZ). The cells were incubated in HBSS containing PZ (500 nM) and different concentrations of imatinib mesylate (0.5, 2 and 6 μM) or novobiocin (10, 50 and 200 μM) at 37°C for 120 min. The results are represented as % of that without inhibitor (Con; the mean ± SD; n = 4; * $p < 0.05$; ** $p < 0.01$ *vs.* control)

cellular accumulation of prazosin was only slightly enhanced with lower concentrations of prazosin (250 to 500 nM); however, a rapid increase in cellular accumulation was observed at the higher concentrations (500 to 1,000 nM). Because the net amount of prazosin in the cell is the difference between the amount influxed and the amount effluxed, the saturable transport of BCRP out of the cell may help explain these findings. BCRP activity could be readily saturated at higher concentrations of prazosin; hence, the role of BCRP in the transport of prazosin across the BBB would become quantitatively less significant.

The effects of BCRP inhibitors on the function of BCRP in rBMECs were also studied. We found that both novobiocin and imatinib mesylate increased the amount of uptake of prazosin by rBMECs in a concentration-dependent manner (Fig. 2). The addition of 10, 50 and 200 μM of novobiocin led to an increased uptake of prazosin by 1.1-, 2.6- and 11.9 fold, respectively, compared to controls. The addition of 0.5, 2 and 6 μM of imatinib mesylate also resulted in a significant increase of prazosin uptake by 1.6-, 2.8- and 5.4 fold, respectively, compared to controls. The co-administration of 50 μM of novobiocin or 2 μM of imatinib mesylate with prazosin significantly decreased the efflux of prazosin from the cells, resulting in an increased amount of prazosin remaining in the rBMECs (18.9 % and 18.8 % of the controls, respectively; $p < 0.01$) at the 20 min timepoint. These data demonstrated the existence of functional BCRP and BCRP expression in rBMECs.

Our previous studies have shown that the diabetic state may impair the function and expression of BCRP [17] and P-GP [15] in the brains of STZ-induced diabetic rats, and insulin treatment may attenuate this impairment. Other studies have shown that low levels of insulin in the sera of diabetic rats may be an important factor contributing to the impairment of P-GP function and expression at the BBB [14, 15]. In the present study, the effects of the sera from diabetic rats on BCRP function were observed in rBMECs. In contrast to the findings in both P-GP [15] and *in vivo* studies [17], a 72-h incubation in sera from diabetic rats significantly ($p < 0.01$) decreased the accumulation of prazosin in rBMECs (39.8%) to levels similar to control cells that were incubated with the sera from normal rats, indicating that the sera from diabetic rats increased the function of BCRP in rBMECs. Insulin treatment (50 mU/l) reversed the decrease in prazosin accumulation in cells incubated

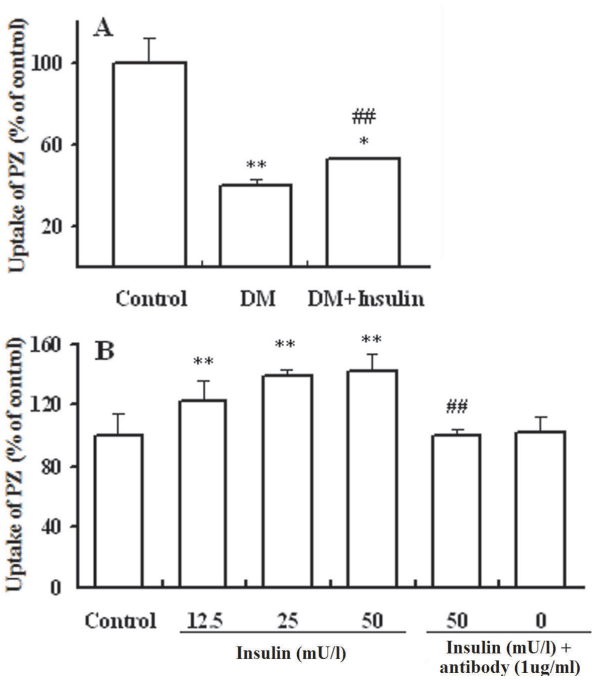

Fig. 3. (**A**) The effects of sera from diabetic rats on the uptake of PZ. The rBMEC cells were incubated in diabetic rat sera (DM), with or without 50 mU/l of insulin, for 72 h. The results are represented as % of the control rat sera (the mean ± SD; n = 4; ** $p < 0.01$ *vs.* control; ## $p < 0.01$ *vs.* DM). (**B**) The effects of insulin on the uptake of PZ by rBMECs. The rBMECs were incubated in normal culture media with increasing concentrations of insulin and with/without insulin receptor antibody (antibody) for 72 h. The results are represented as % of the control (HBSS; the mean ± SD; n = 5; * $p < 0.05$; ** $p < 0.01$ *vs.* control; ## $p < 0.01$ *vs.* 50 mU/l insulin)

with sera from diabetic rats, resulting in a 1.3-fold increase compared with cells incubated with sera from diabetic rats without insulin. These results indicated that insulin suppressed the function of BCRP in rBMECs (Fig. 3A).

To further study the role of insulin in the regulation of the function and expression of BCRP in rBMECs, we measured the functional activity and protein levels of BCRP in rBMECs following a 72-h incubation in normal culturing media containing 12.5, 25 and 50 mU/l of insulin. Our results showed that insulin enhanced the uptake of prazosin by the cells (Fig. 3B) and decreased the levels of BCRP protein in rBMECs in a concentration-dependent manner (Fig. 4). The co-administration of 1 μg/ml of insulin receptor antibody almost abolished the insulin-induced (50 mU/l insulin) alterations in BCRP activity and expression. These results demonstrated that insulin suppressed the functional activity and expression of BCRP in rBMECs, indicating that the effects of insulin on the

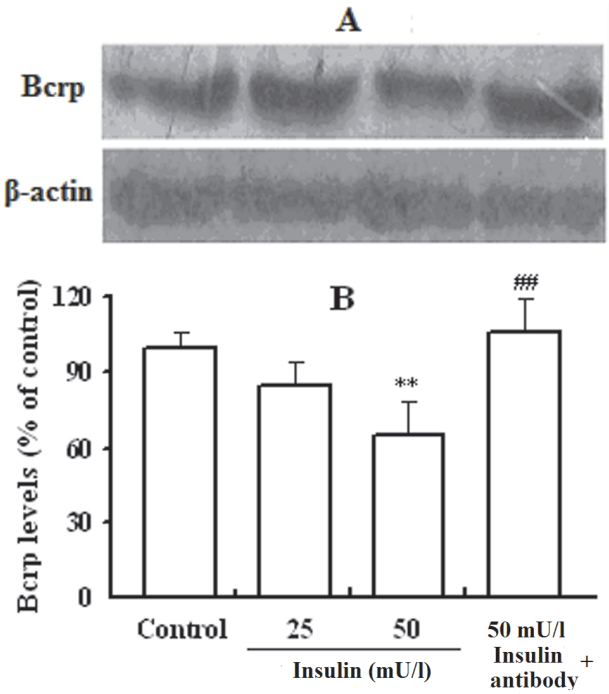

Fig. 4. The effects of insulin on BCRP levels in rBMECs. (**A**) Representative western blots of BCRP and (**B**) BCRP levels in the cells are shown. The rBMECs were incubated in normal culture media with increasing concentrations of insulin and with or without insulin receptor antibody (antibody) for 72 h (the mean ± SD; n = 3; * $p < 0.05$; ** $p < 0.01$ *vs.* control; ## $p < 0.01$ *vs.* 50 mU/l of insulin)

regulation of BCRP function and expression were different from those of P-GP; however, the mechanism by which this occurs remains unclear.

Several reports have shown that the mechanisms that are involved in regulating functional activity and expression of BCRP are different from those of P-GP. The pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-6 significantly reduced the mRNA levels and the functional activity of BCRP in human hCMEC/D3 cells, whereas IL-6 treatment only slightly reduced P-GP mRNA expression. Tumor necrosis factor-α (TNF-α) treatment significantly increased P-GP mRNA levels, but it significantly decreased BCRP mRNA levels [20]. In human colon carcinoma cells, TNF-α had the opposite effect on MRP and lung resistance protein (LRP) gene expression [23]. A 6-h treatment of primary rBMECs with dexamethasone significantly induced higher levels of P-GP and BCRP [22]. However, dexamethasone decreased mRNA levels and protein levels of BCRP in a dose-independent and time-dependent manner in MCF-7/MX breast cancer cells [11]. The limited information provided in this study also showed that P-GP expression suppressed BCRP function and expression. SK-MES-1/WT lung cancer cells expressed BCRP; however, BCRP expression was significantly down-regulated in doxorubicin-resistant SK-MES-1/DX1000 cells that over-expressed P-GP [1]. Similarly, the P-GP-deficient mdr1a knockout mice (mdr1a –/–) have increased levels of BCRP mRNA expression at the BBB [7]. Inverse relationships between MRP and P-GP have been reported in several reports [2, 5, 6, 12]. Our previous studies have shown that insulin up-regulated P-GP function and expression [14] in rBMECs, and our present study demonstrated that insulin suppressed BCRP function and expression in rBMECs. Collectively, these results may partly explain the up-regulation of BCRP activity in rBMECs when incubated with the sera from diabetic rats.

In conclusion, the present study demonstrated that primary cultures of rBMECs expressed BCRP and showed functional activity of this protein, which were both suppressed by insulin.

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References:

1. Bark H, Xu HD, Kim SH, Yun J, Choi CH: P-glycoprotein down-regulates expression of breast cancer resistance protein in a drug-free state. FEBS Lett, 2008, 582, 2595–2600.
2. Bordow SB, Haber M, Madafiglio J, Cheung B, Marshall GM, Norris MD: Expression of the multidrug resistance-associated protein (MRP) gene correlates with amplification and overexpression of the N-myc oncogene in childhood neuroblastoma. Cancer Res, 1994, 54, 5036–5040.
3. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 1976, 72, 248–254.
4. Brendel C, Scharenberg C, Dohse M, Robey RW, Bates SE, Shukla S, Ambudkar SV et al.: Imatinib mesylate and nilotinib (AMN107) exhibit high-affinity interaction with ABCG2 on primitive hematopoietic stem cells. Leukemia, 2007, 21, 1267–1275.
5. Brock I, Hipfner DR, Nielsen BS, Jensen PB, Deeley RG, Cole SP, Sehested M: Sequential coexpression of the multidrug resistance genes MRP and mdr1 and their products in VP-16 (etoposide)-selected H69 small cell lung cancer cells. Cancer Res, 1995, 55, 459–462.

6. Choi CH, Kim HS, Rha HS, Jeong JH, Park YH, Min YD, Kee KH, Lim DY: Drug concentration-dependent expression of multidrug resistance-associated protein and P-glycoprotein in the doxorubicin-resistant acute myelogenous leukemia sublines. Mol Cells, 1999, 9, 314–319.
7. Cisternino S, Mercier C, Bourasset F, Roux F, Scherrmann JM: Expression, up-regulation, and transport activity of the multidrug-resistance protein Abcg2 at the mouse blood-brain barrier. Cancer Res, 2004, 64, 3296–3301.
8. Cooray HC, Blackmore CG, Maskell L, Barrand MA: Localisation of breast cancer resistance protein in microvessel endothelium of human brain. Neuroreport, 2002, 3, 2059–2063.
9. Eisenblätter T, Galla HJ: A new multidrug resistance protein at the blood-brain barrier. Biochem Biophys Res Commun, 2002, 293, 1273–1278.
10. Eisenblätter T, Hüwel S, Galla HJ: Characterisation of the brain multidrug resistance protein (BMDP/ABCG2/BCRP) expressed at the blood-brain barrier. Brain Res, 2003, 971, 221–231.
11. Elahian F, Kalalinia F, Behravan J: Dexamethasone downregulates BCRP mRNA and protein expression in breast cancer cell lines. Oncol Res, 2009, 18, 9–15.
12. Friedrich RE, Punke C, Reymann A: Expression of multi-drug resistance genes (mdr1, mrp1, bcrp) in primary oral squamous cell carcinoma. In Vivo, 2004, 18, 133–147.
13. Hawkins BT, Ocheltree SM, Norwood KM, Egleton RD: Decreased blood-brain barrier permeability to fluorescein in streptozotocin-treated rats. Neurosci Lett, 2007, 411, 1–5.
14. Liu H, Liu X, Jia L, Liu Y, Yang H, Wang G, Xie L: Insulin therapy restores impaired function and expression of P-glycoprotein in blood-brain barrier of experimental diabetes. Biochem Pharmacol, 2008, 75, 1649–1658.
15. Liu H, Xu X, Yang Z, Deng Y, Liu X, Xie L: Impaired function and expression of P-glycoprotein in blood-brain barrier of streptozotocin-induced diabetic rats. Brain Res, 2006, 1123 245–252.
16. Liu XD, Wang DW, Xie L: Correlation between quinolone uptakes by BCECs in vitro and brain-to-plasma concentration ratios in rats. Eur J Drug Metab Pharmacokinet, 2005, 30, 249–254.
17. Liu YC, Liu HY, Yang HW, Wen T, Shang Y, Liu XD, Xie L, Wang GJ: Impaired expression and function of breast cancer resistance protein (Bcrp) in brain cortex of streptozocin-induced diabetic rats. Biochem Pharmacol, 2007, 74, 1766–1772.
18. Milane A, Vautier S, Chacun H, Meininger V, Bensimon G, Farinotti R, Fernandez C: Interactions between riluzole and ABCG2/BCRP transporter. Neurosci Lett, 2009, 452, 12–16.
19. Nowicki MT, Aleksunes LM, Sawant SP, Dnyanmote AV, Mehendale HM, Manautou JE: Renal and hepatic transporter expression in type 2 diabetic rats. Drug Metab Lett, 2008, 2, 11–17.
20. Poller B, Drewe J, Krähenbühl S, Huwyler J, Gutmann H: Regulation of BCRP (ABCG2) and P-glycoprotein (ABCB1) by cytokines in a model of the human blood-brain barrier. Cell Mol Neurobiol, 2010, 30, 63–70.
21. Qian ZY, Huang Q, Zhou LY, Sun ZF: Isolation and long-term cultivation of rat brain microvascular endothelial cells. Chin J Cell Biol, 1999, 21, 42–45.
22. Shiozawa K, Oka M, Soda H, Yoshikawa M, Ikegami Y, Tsurutani J, Nakatomi K et al.: Reversal of breast cancer resistance protein (BCRP/ABCG2)-mediated drug resistance by novobiocin, a coumermycin antibiotic. Int J Cancer, 2004, 108, 146–151.
23. Stein U, Walther W, Laurencot CM, Scheffer GL, Scheper RJ, Shoemaker RH: Tumor necrosis factor-α and expression of the multidrug resistance-associated genes LRP and MRP. J Natl Cancer Inst, 1997, 89, 807–813.
24. Sun JJ, Xie L, Liu XD: Transport of carbamazepine and drug interactions at blood-brain barrier. Acta Pharmacol Sin, 2006, 27, 249–253.
25. Van Waarde WM, Verkade HJ, Wolters H, Havinga R, Baller J, Bloks V, Müller M et al.: Differential effects of streptozotocin-induced diabetes on expression of hepatic ABC-transporters in rats. Gastroenterology, 2002, 122, 1842–1852.
26. Warren MS, Zerangue N, Woodford K, Roberts LM, Tate EH, Feng B, Li C et al.: Comparative gene expression profiles of ABC transporters in brain microvessel endothelial cells and brain in five species including human. Pharmacol Res, 2009, 59, 404–413.
27. Zhang L, Liu XD, Xie L, Wang GJ: P-glycoprotein restricted transport of nimodipine across blood-brain barrier. Acta Pharmacol Sin, 2003, 24, 903–906.
28. 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.
29. Zhang Y, Laterra J, Pomper MG: Hedgehog pathway inhibitor HhAntag691 is a potent inhibitor of ABCG2/BCRP and ABCB1/Pgp. Neoplasia, 2009, 11, 96–101.

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