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# Attenuated function and expression of P-glycoprotein at blood–brain barrier and increased brain distribution of phenobarbital in streptozotocin-induced diabetic mice

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**Abstract**

The aim of the study was to investigate whether diabetes mellitus modulated the function and expression of P-glycoprotein and the distribution of phenobarbital in the brain of 3-week streptozotocin-induced diabetic mice. P-glycoprotein function in blood–brain barrier was assessed by measuring the brain-to-plasma concentration ratios of rodamine123, a well-known P-glycoprotein substrate, in non-diabetic mice and diabetic mice. P-glycoprotein expression in the brain cortex was evaluated with western blot. Whether diabetes mellitus changed the distribution of phenobarbital (60 mg/kg, i.v.) in brain of mice was measured, and whether the changed distribution caused the difference of phenobarbital (80 and 100 mg/kg) -induced loss of the righting reflex in non-diabetic and diabetic mice were also investigated. The results showed that the brain-to-plasma concentration ratio value of rodamine123 in diabetic mice was significantly higher than that of non-diabetic mice, western blot suggested that the protein level of P-glycoprotein in the brain of 3-week diabetic mice was significantly lower than that of non-diabetic mice, and insulin treatment restored the impairment of P-glycoprotein. The exposure of phenobarbital in brain of diabetic mice was 1.30-fold of that of non-diabetic mice, while in plasma the fold was 1.09. The increased distribution of phenobarbital in the brain of diabetic mice significantly increased the duration of phenobarbital-induced loss of the righting reflex and reduced the latency time of loss of the righting reflex. All the results suggested that the function and expression of P-glycoprotein might be impaired and the brain distribution of phenobarbital was increased in brain of streptozotocin-induced diabetic mice.


*Keywords:* P-glycoprotein; Diabetes mellitus; Blood–brain barrier; Rodamine123; Phenobarbital; (Mice)

## 1. Introduction

Diabetes mellitus is a systematic metabolic disease. Diabetes mellitus develops a number of well-recognized vascular complications. More recently, it has become apparent that the blood vessels of the brain (especially the brain microvessels) are not spared from the effects of diabetic pathology with evidence of alterations in cerebral blood flow and blood–brain transport, both of which may result from a dysfunctional endothelium (Fouyas et al., 2003). The possible causes of central nervous system (CNS) dysfunction in diabetes have been reviewed in previous publication (Mooradian, 1997), one of the potential causes is the alteration of blood–brain barrier, and one of the targets is P-glycoprotein.

P-glycoprotein, the 170-kD protein product of the multidrug resistance-1 gene, is expressed not only in anticancer drug resistant cells, but also in various normal tissues. In the brain, P-glycoprotein is mainly expressed in the luminal membrane of the microvessel endothelium and in the apical membrane of the choroids plexus epithelium (Sun et al., 2003; Hiroyuki and Yuichi, 2001). P-glycoprotein plays an important role in the integrity of blood–brain barrier and protects the brain from many

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exogenous toxins. Absence of functional P-glycoprotein in blood–brain barrier leads to highly increased brain penetration of a number of important drugs, resulting in dramatically increased neurotoxicity, or fundamentally altered pharmacological effects of the drugs on the CNS (Schinkel, 1999).

In our previous study, we found P-glycoprotein expression level in the rat brain cortex was significantly decreased by diabetes mellitus, as compared to that found in non-diabetic rats (Liu and Liu, 2006). Recently, Van Waarde reported that hepatic gene and protein expression of multidrug-resistant type 2 was modulated by diabetes (Van Waarde et al., 2005). Kamei reported P-glycoprotein expression levels in the whole brain were significantly decreased in diabetic mice, as compared to those found in non-diabetic mice (Kamei et al., 2005). Thus, it is possible that the function of P-glycoprotein at the blood–brain barrier be modulated by diabetes, and this modulation may contribute to the alteration of the penetration of the P-glycoprotein substrates into the CNS.

Phenobarbital, one of the antiepileptic drugs, has recently been shown to be a substrate for P-glycoprotein (Löscher and Potschka, 2002; Seegers et al., 2002; Potschka et al., 2002). Thus, the reduced expression of P-glycoprotein in the blood–brain barrier of diabetic animals is likely to contribute to increasing penetration of phenobarbital into the parenchyma and thereby increasing the pharmacological effects or toxicological effects.

In this study, we investigated the effect of diabetes mellitus on the function and expression of P-glycoprotein at blood–brain barrier and the brain distribution of phenobarbital in streptozotocin-induced diabetic mice. At the same time, whether the change of brain distribution of phenobarbital resulted in the changed pharmacological and toxicological effect of phenobarbital was also studied. We examined the pharmacokinetics of phenobarbital in brain and blood of the non-diabetic and diabetic mice respectively. Phenobarbital-induced loss of righting reflex, and the latency time of loss of the righting reflex were used as indexes of the CNS-depressant effect.

## 2. Materials and methods

### 2.1. Reagents

Streptozotocin and rhodamine 123 were purchased from Sigma Chemical Co. (St. Louis, MO, USA), phenobarbital was obtained from National Institute for the Pharmaceutical and Biological Products (Beijing, China), evans blue was purchased from Shanghai Reagent Co.(Shanghai, China). All other reagents were commercially available and were of analytical grade.

P-glycoprotein monoclonal antibody C219 was purchased from Calbiochem-Novabiochem (Seattle, WA, USA), IRDyeTM800 conjugated affinity purified anti-mouse IgG was purchased from Rockland Inc (Rockland, Ontario, Canada), Blueranger prestained protein molecular weight maker mix was purchased from Pierce (Rockford, Illinois, USA), and polyclonal anti-β-actin antibody was purchased from Boshide Biotech Co. (Wuhan, China).

### 2.2. Animals

Male ICR mice were supplied by Center of Experimental Animals, China Pharmaceutical University, the mice aged 4 weeks and weighing about 20 g at the beginning of the experiments, were used. They had free access to food and water in an animal room that was maintained at 24±1 °C and 55±5% humidity with a 12 h light-dark cycle (light phase 8:00–20:00). The streptozotocin-induced hyperglycemic state was used as an animal model of type 1 diabetes mellitus (Tarui et al., 1997; Kamei et al., 2005) by an i.p. injection of streptozotocin (190 mg/kg). Age-matched control mice were injected with the vehicle (sodium citrate buffer, pH=4.5). The experiments were conducted 3 weeks after the injection of streptozotocin or vehicle. Mice with blood glucose levels above 11.1 mM were considered diabetic. Blood glucose levels were determined using a glucose kit (Jiancheng, Nanjing, China). Some diabetic mice were treated with insulin (40 U/ml, Wanbang Pharmaceutical Co., XuZhou, China) twice a day, the dose of insulin was 20 U/kg/day administered subcutaneously. Mice whose plasma glucose was decreased below 7 mM were considered to be controlled diabetes mellitus. This study was carried out in accordance with the guide for the care and use of laboratory animals as adopted by the Committee on Care and Use of Laboratory China Pharmaceutical University.

### 2.3. Effect of diabetes mellitus on P-glycoprotein functions in mouse brain cortex

To examine the effect of diabetes mellitus on the brain distribution of rodamine123, the mice were sacrificed under light ether anesthesia at 1 h following i.v. (tail vein) administration of rodamine123 (0.8 mg/kg), and the whole brain cortex and blood samples were taken.

### 2.4. Effect of diabetes mellitus on evans blue extravasation in mouse brain cortex

To examine the effect of diabetes mellitus on the blood–brain barrier integrity, the mice were sacrificed at 1 h following i.v. (tail vein) administration of evans blue (1% in saline, 0.12 ml/10 g), and the whole brain cortex samples were taken.

### 2.5. Effect of diabetes mellitus on P-glycoprotein expression in mouse brain cortex

Diabetic mice and non-diabetic mice were sacrificed under ether anesthesia and the brains were quickly removed. The cortex weighing 0.1 g was homogenized in ice-cold cell lysis containing 10 mM Tris–HCl (pH 7.5), 1 mM EGTA, 1 mM $MgCl_2$, 1 mM mercaptoethanol, 1% glycerol, protease inhibitor cocktail (1 mM dithiothreitol, 2 mM phenylmethylsulfonylfluoride(Sigma Chemical Co., St. Louis, MO, USA)). The homogenate was centrifuged at 1, 3000 ×*g* for 10 min at 4 °C. The soluble fractions were obtained as membrane fractions for western blot. The protein concentration in the solution was measured by the Bio-Rad Protein Assay (Bio-Rad Labs,