Arch. Pharm. Res. 11(2), 145-148(1988)

Effects of Brazilin on Blood Viscosity and Erythrocyte Deformability in Alloxan Induced Diabetic Rats

Chang-Kiu Moon, Jin-Ho Chung, Seong-Soo Choi, Soo-Hwan Lee, Gwi-Seo Hwang, Kwang-Sik Park, Myung-Soo Mock, Seong-Gon Kim, and Ji-Young Kim

College of Pharmacy, Seoul National University, Seoul 151-742, Korea
(Received May 28, 1988)

Abstract □ Effects of brazilin on the blood viscosity and erythrocyte deformability were investigated in alloxan induced diabetic rats. By the treatment of brazilin to alloxan induced diabetic rats, enhancement of erythrocyte deformability was observed. In the *in vitro* study on the erythrocyte deformability, brazilin showed statistically significant improving effects on the erythrocyte deformability. At the concentrations of 10^{-3}M of brazilin, erythrocyte deformability was compared with those of hematoxylin, pentoxifylline and prednisolone.

Keywords □ blood viscosity, erythrocyte deformability, alloxan induced diabetes, brazilin, hematoxylin

The rheological properties of blood play the important roles in governing flow dynamics and circulatory transport in healthy and disease states[1-3]. They are based mainly on rheological terms such as blood viscosity, erythrocyte deformability and erythrocyte aggregation[4]. Blood viscosity is mainly influenced by the specific factors such as the blood flow velocity, hematocrit, degree of platelet and erythrocyte aggregation, plasma viscosity, intrinsic viscosity of the erythrocytes, and flexibility of the erythrocyte membrane[5]. Impaired erythrocyte deformability is especially considered to play an important role in microcirculatory disturbances[3,7,8]. An enhanced blood viscosity and a reduced erythrocyte deformability are generally recognized as microrheological complications in diabetic subjects. In diabetic state, blood viscosity and erythrocyte deformability have been reported to be changed when stimulated by a variety of pharmacological agents *in vivo* and *in vitro*. In our previous works brazilin has been proved to have some positive biologial activities for the prevention and treatment of diabetic complications, such as lens aldose reductase inhibitory effects, hypolipidemic effects and anti-lipidperoxidation effect[9,10]. In this experiment we started to investigate the possible effects of brazilin on the blood viscosity and erythrocyte deformability in normal and diabetic states.

EXPERIMENTAL METHODS

Induction of diabetes

Diabetes was induced in male Spraque-Dawley rats (180-3250 gm) fasted previously for 12 hours by the intraperitoneal injection of alloxan (175 mg/kg body weight). Animals with fasting blood glucose levels of about 350 mg/100m*l* blood were used in this experiment.

Blood viscosity changes by the treatment of brazilin in vivo

Animals were divided in four groups such as normal control group, normal brazilin treated group, diabetic control group and diabetic brazilin treated group. Dose of brazilin was 100 mg/kg body weight. All animals were injected intraperitoneally for 10 days. Blood was obtained from heart with heparinized syringe. The time required for a heparinized blood sample to pass between two points of 2 m*l* volumetric pipette glass bulb was compared with that for distilled water at 37 ± 0.5 °C.

Erythrocyte deformability changes by the treatment of brazilin in vivo

Experimental animals were grouped and administered as described in the determination of blood viscosity. And blood was collected by cardiac puncture with heparinized syringe. The number of erythrocyte was counted in a hemocytometer of known dimensions by the conventional method. Each blood sample was diluted 10 fold with Tris-buffer solution (in NaCl, pH 7.4, 0.25% albumin). Erythrocyte deformability was measured using blood suspension by the filtration technique[11]. Membrane filter (Nucleopore Corp.) from a single batch (Lot; 54F3B27) were used for all filtration operations. Erythrocyte deformability was expressed as whele blood-filtration rate.

Time course of erythrocyte deformability changes in vitro by the treatment of brazilin

Relationship between erythrocyte deformability and incubation time was examined by the following procedure. Blood samples were divided in three groups such as normal group, diabetic control, and diabetic brazilin treated group (7.2×10^{-5} mmol/ml blood). Each blood sample was incubated at 37 ± 0.5 °C. After 5, 45, 90 and 180 minutes' incubations, blood samples were diluted 10 fold with tris-buffer solution. Erythrocyte deformability was measured using blood suspension by filtration technique[11].

Dose-response of erythrocyte deformability by the treatment of brazilin

Blood samples were divided in seven groups such as normal group, diabetic control group, and diabetic treated groups (from 10^{-3} to 10^{-7} mmol/ml of blood, respectively) for the determination of dose dependency of brazilin on erythrocyte deformability. Each group was incubated for 5 minutes at 37 ± 0.5 °C. After the incubation all groups were diluted 10 fold with tris-buffer solution and erythrocyte deformability was measured by the filtration technique.

Comparison of effects of brazilin and hematoxylin on erythrocyte deformability with those of pentoxifylline and prednisolone in vitro

Each blood samples were incubated with the substances tested of 10 mmol/ml blood for 5 minutes at 37 ± 0.5 °C. After the incubation, blood samples were diluted 10 fold with tris-buffer solution and erythrocyte deformability was determined using blood suspension by the filtration technique.

RESULTS AND DISCUSSION

In the diabetic subjects, platelet-aggregation, -adhesion to vessel and -turn over rate, and the viscosity of plasma are increased and erythrocyte-deformability is decreased. These haematological abnormalities may be directly involved in the pathogenesis of small vessel disease. Microvascular disease is one of the important complications in diabetes mellitus. Altered blood viscosity and erythorocyte deformability have been regarded as very important negative factors for the microcirculation. In search of the effective substances to improve haematological abnormalities, we already examined brazilin, an active princle of *Caesalpinia sappan* for its effects on the platelet aggregation and MDA-formation in platelet, which resulted in positive responses. As an continuing study on brazilin we started to investigate the effects of brazilin on blood viscosity and erythrocyte deformability in streptozotocin induced diabetic rats.

Effects of brazilin on blood viscosity and erythrocyte deformability in vivo

In order to assess the effect of brazilin on haematological disturbances, blood viscosity and filtration rate were determined *in vivo*. As shown in Table I, blood viscosity was significantly increased ($p < 0.01$) in diabetic control group compared to that of normal control group. Although no statistical difference of blood viscosity was found between normal control and normal brazilin treated group, blood viscosity-lowering tendency was observed in diabetic rats treated with the dose of 100 mg brazilin/kg body weight for 10 days but it was not statistically significant under the experimental condition tested. Another parameter, blood filtration rate, was usually used to evaluate the erythrocyte deformability. Blood filtration rate in

Table I. Changes in blood viscosity and filtration rate of normal and diabetic rats treated with brazilin

Group	Blood viscosity (centi poise)	Blood filtration rate (ml/min.)
Normal control	1.45 ± 0.08	22.9 ± 0.3
Normal brazilin treated	1.40 ± 0.06	23.1 ± 0.3
Diabetic control	1.62 ± 0.09[a)]	17.4 ± 0.1[a)]
Diabetic brazilin treated	1.52 ± 0.07	19.6 ± 0.4[b)]

Normal and diabetic rats were administered brazilin (100 mg/kg) intraperitoneally for 10 days. Values represent the mean + S.D. of six measurements; a) $p < 0.01$ vs. normal control, b) $p < 0.01$ vs. diabetic control

diabetic animal was decreased significantly compared to those of normal animal. However, brazilin-treatment increased the blood filtration rate significantly in diabetic animal. It was, therefore, suggested that brazilin might have an improving effect on blood viscosity and filtration rate of erythroyte as well.

Time coures observation of erythrocyte deformability by the treatment of brazilin.

Erythrocyte deformabilty was examined *in vitro* by filtration method (using blood suspension) at 5, 45, 90 and 180 minutes of the incubation. As shown in figure 1, all three groups basically showed the similiar pattern depending upon incubation time. Although no significant differences in filtration rate of erythrocyte were observed until 45 minutes incubation, the filtration rate was decreased significantly thereafter. Nevertheless, the extent of reduction in filtration rate throughout the incubation time appears to be minimal. These data suggested that 5 minutes incubation might be adequate enought to perform the following experiments.

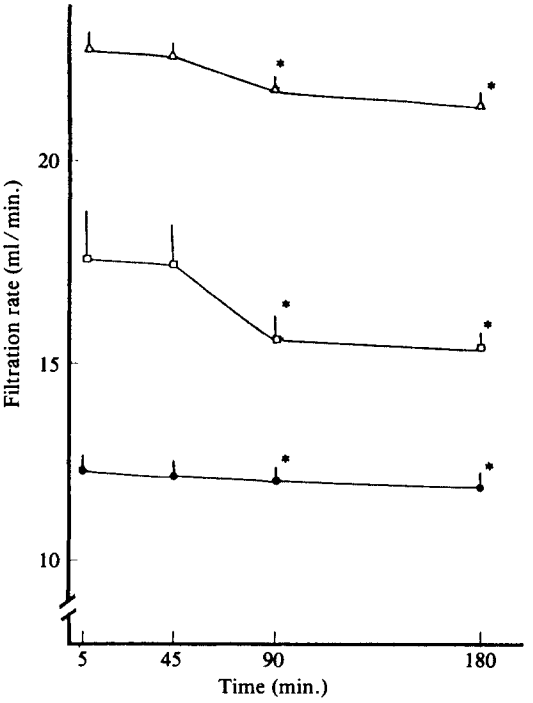

Fig. 1. Time-course effects of filtration rate of erythrocytes by treatment of brazilin.
Blood from diabetic rats were incubated with brazilin (7.2×10^{-5} mmol/ml) at 37 °C for 5, 45, 90 and 180 minutes. Groups are normal control (△----△), diabetic control (●---●), and diabetic animals with brazilin-treated (□---□).
*represent significant differences from the filtration rates at 5 minutes ($p < 0.05$).

Table II. Dose-response effects on filtration rate of blood from diabetic rats treated with brazilin

Group	Blood filtration rate (ml/min.)
Normal control	22.8 ± 0.4
Diabetic control	16.1 ± 0.8
Diabetic brazilin treated	
(10^{-3}mmol/ml)	21.6 ± 0.4*
(10^{-4}mmol/ml)	20.3 ± 0.6*
(10^{-5}mmol/ml)	17.8 ± 0.4*
(10^{-6}mmol/ml)	16.7 ± 0.3
(10^{-7}mmol/ml)	16.8 ± 0.4

Values are the mean + S.D. of six measurements. *represent significant differences from diabetic control ($p < 0.01$).

Dose-response effects of brazilin on erythrocyte deformability in vitro

Next approach was to determine the dose-response effects of brazilin on filtration rate of blood from diabetic rats *in vitro* (Table II). Blood from diabetic rats reduced the filtration rate significantly compared to normal rats. Treatment with brazilin in diabetic animals increased the filtration rate in dose-dependent fashion. Blood filtration was approached almost those of normal animals at the highest concentrations of brazilin used (10^{-3} mmol/ml). It was concluded that the effects of bra-

Table III. Comparisons of effect of brazilin on blood filtration rate to hematoxylin, pentoxifylline and prednisolon

Group	Blood filtration rate (ml/min.)
Normal control	22.8 ± 0.4
Diabetic control	16.8 ± 0.8
Diabetic brazilin treated	21.6 ± 0.4*
Diabetic hematoxylin treated	18.9 ± 0.5*
Diabetic pentoxifylline treated	20.2 ± 0.2*
Diabetic prednisolone treated	18.4 ± 0.3

Blood from diabetic rats were incubated *in vitro* with brazilin, hematoxylin, pentoxifylline, or prednisolone at the concentration of 10^{-3} mmol/ml for 5 minutes at 37 °C. Filtration rate was determined right after incubation. Values are the mean + S.D. of six measurements. *represent significant differences from diabetic control (p 0.01).

zilin on erythrocyte deformability changed in a dose-dependent manner.

Comparisons of effect of brazilin on erythrocyte deformalility to hematoxylin, pentoxifyllin and prednisolon

In other to compare the effect of brazilin on blood filtration rate, bloods from diabetic animals were incubated with equimolar of brazilin, hematoxylin, pentoxifylline, and prednisolon for 5 minutes at 37 °C. Pentoxifylline and prednisolon would serve as a positive control. Blood filtration rate was significantly improved by treatment of all test substances compared to those of the diabetic control animals. The most significant effect was observed in brazilin treated group. This result suggests a possibility that brazilin could be utilized in improving the diabetic hematorheological complications.

In conclusion, a remarkable improvement of the haematorheological complication could be achieved in alloxan diabetic rats by the treatment of brazilin. Biochemical researches are in progress in our laboratory; such as roles of ATP, 2,3-diphosphoglycerate and Ca^{++} etc. in erythrocyte deformability. The action mechanism of brazillin on the erythrocyte deformability is now under the investigation.

ACKNOWLEDGEMENT

This work was partially supported by the grants from the Education and Research Foundation of Pharmaceutical Sciences attached to College of Pharmacy, Seoul National University.

LITERATURE CITED

1. Chien, shu: Determinants of blood viscosity and red cell deformability. *Scan. J. clin. Lab. Invest.* **40,** Suppl. 156, 7-12 (1981).
2. Boisseau, M.R., Lorient, M.F. and Bricand, H.: Red cell deformability and risk factors in one hundred patients with cerebro-vascular thrombosis. *Throm. and Haemost.* **42,** 107 (1979).
3. Reid, H.L., Dormandy, J.A., Lock, P.J. and Dormandy, T.L.: Impaired red cell deformability in peripheral vascular disease. *Lancet.* **1,** 666 (1976).
4. Skalak, R.: Theoretical models of deformability in blood flow. *Scan. J. clin. Lab. Invest.* **41,** Suppl. 156, 55-58 (1981).
5. Ehrly, A.M.: Therapy of occulusive arterial diseases with Ancrod (Arwin). *Artery* **2,** 98 (1976).
6. Ehrly, A.M. and Achroeder, W.: Zur pathophisiologie derchronischen Arteriellen Verschusser krankung. I. *Mikrozirkulatorische Blutverteilungsstorung in der Skelettmuskulatur Herz Kreisl,* **11,** 275 (1979).
7. Ehrly, A.M. and Kohler, H.J.: Altered deformability of erythrocytes from patients with chronic occlusive arterial disease. *V A S A.* **5,** 319 (1976).
8. Braasch, D.: Red cell deformability and capillary blood flow. *Physiol. Rev.* **51,** 679 (1971).
9. Moon, C.K., Kang, N.Y. and Chung J.H.: Effect of brazilin on plasma lipid levels in spontaneously hypertensive rats. *Fette Seifen Anstrichmittel* **87,** 74-76 (1985).
10. Moon, C.K., Yun, Y.P., Lee, J.H., Wagner, H. and Shin, Y.S.: Inhibition of Lens-aldose reductase activity by brazilin and hematoxylin. *Planta Medica* 66-67 (1985).
11. James, M. Norton and Petter, W. Rand. Decreased deformability of erythrocytes from smokers. *Blood.* **57.** 4 (April), 671-674 (1981).