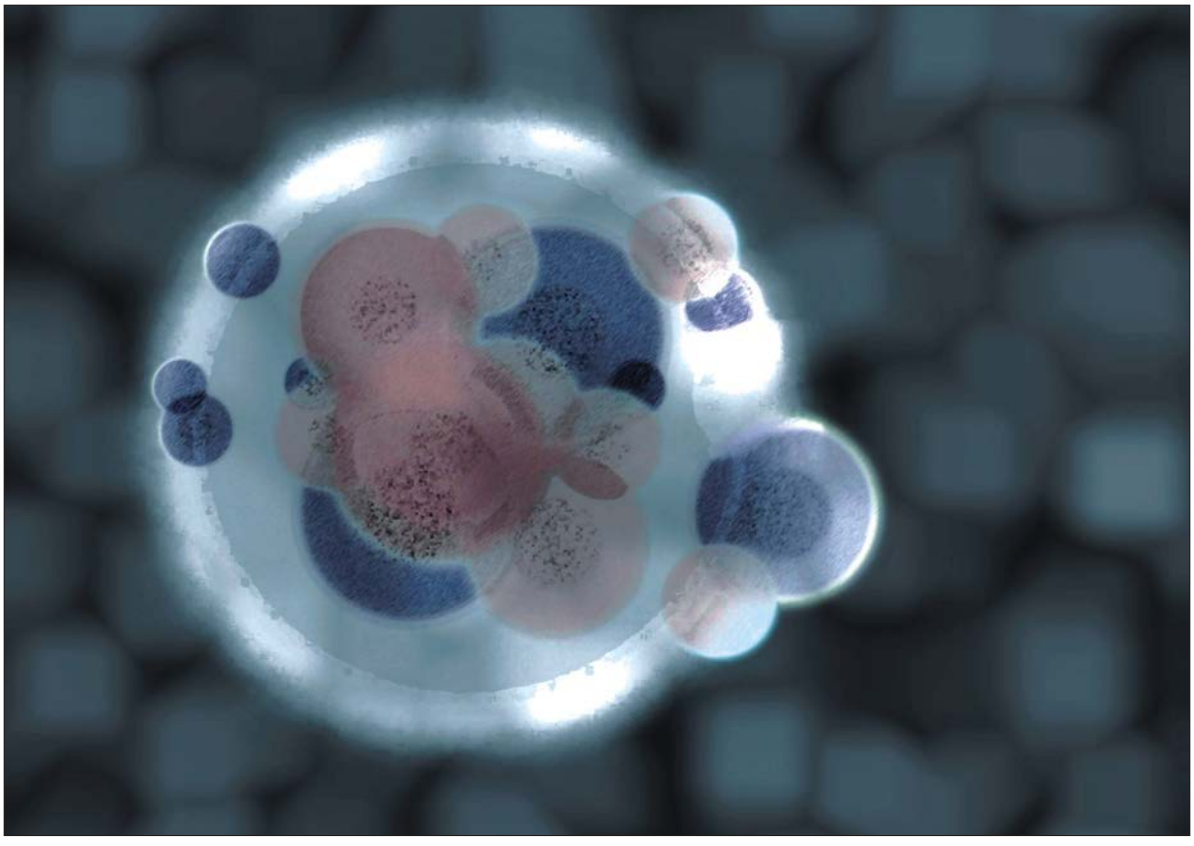

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Melanocyte-Stimulating Hormone Modulates Blood Viscosity in Short-Term Alloxan-Induced Diabetic Rats

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Abstract: The effects of MSH on whole blood viscosity (WBV)and hematocrit (Ht) levels in short-term alloxan-induced diabetic were studied. Male and Female Sprague-Dawley diabetic rats weighing 185-250 g were given intraperitonially (i.p.) a daily injection of 20 mg alloxan solution/100 g of body weight for 10 days. Normal and diabetic rats were given daily injection (i.p.) of alpha-Melanocyte stimulating hormone (MSH) at a dose of 2 μg/100 g b.w for 10 days. Body weight, serum glucose, serum insulin, Ht and WBV were measured. The results indicated that MSH decreased serum glucose levels in diabetic rats in comparison with normal rats. Our study demonstrates that MSH administration significantly lowers blood viscosity of short-term diabetic rats. It proposed that MSH may exert a protective effect on the vascular endothelial cells.

Key words: MSH, blood viscosity, hematocrit, type 2 diabetes mellitus. alloxan induced diabetic rats

INTRODUCTION

Rheological properties of blood (platelet, erythrocyte aggregation, blood viscosity and platelet adhesion) determine its ability to flow through any vessel (Chmiel *et al*., 2005; Ziegler *et al*., 2005). Abnormal blood flow is evident in patients with peripheral vascular disease (PVD) by various mechanisms (Tayebjee *et al*., 2005; Mitchell *et al*., 2005). The flow of blood is altered in PVD, with different flow characteristics compared with healthy individuals (Suzuki *et al*., 2000). With respect to blood itself, quantification of its flow properties can be made by measurement of hemorheological indices, such as plasma viscosity, blood viscosity, hematocrit and hemoglobin (Cabrales *et al*., 2006; Castellini *et al*., 2006). Abnormalities of hemorheology, such as Blood viscosity is an important cardiovascular risk factor that might be related to diabetes complications (Cinara *et al*., 2006; Vigilance and Reid, 2005; de Simone *et al*., 2005).

Recent studies have shown that long term diabetes mellitus is associated with increased whole blood viscosity (Vigilance and Reid, 2005; Kaymaz *et al*., 2005) and decreased hematocrit (Thomas *et al*., 2006; Morsch *et al*., 2006; Saito *et al*., 2005). It has been suggested that these abnormalities in blood rheology may play a causative role in the pathogenesis of diabetic vascular complications (Zhao *et al*., 2006; Kaymaz *et al*., 2005). Alpha-melanocyte-stimulating hormone (alpha-MSH), a pro-opiomelanocortin (POMC) derivative, is a neuropeptide has modulatory effects on the pathogenesis of diabetes mellitus (Costa *et al*., 2006; Abu-Samak *et al*., 2006) with potent anti-obesity (Getting, 2006) and anti inflammatory properties that inhibits tissue injury in a wide array of inflammation models (Forslin Aronsson *et al*., 2006; Hill *et al*., 2006). Yamaoka-Tojo (2006) suggest that alpha-MSH may play an important role in the pathophysiology of congestive heart failure and suppresses the deleterious vascular damage. Although blood rheology is now receiving increasing attention as an important potential contributory factor to diabetic angiopathy (Cinara *et al*., 2006; Szekely *et al*., 2006; Le Devehat *et al*., 2004), there are few studies that connect blood rheology to diabetic angiopathy. Therefore this study aimed to investigate whether alpha-MSH changes blood viscosity and hematocrite levels during early stages of diabetic rats pathogenesis.

MATERIALS AND METHODS

Animals: Seventy male and female Sprague-Dawley were housed in a temperature and light-controlled room in the laboratories of Medical Technology Department, Applied Science University at least 10 days before the experiments. Food and water were available for the animals all the time and without any restrictions. The

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weights of rats were taken on the day of the experiment and only those weighing 185-250 g were used in this study. The animals were divided into four groups, each injected for 10 days intraperitonially (i.p.) with one of the following preparations: Cont group: Control rats daily injected intraperitonially (i.p.) with 1 mL of normal saline. DM group: Alloxan-induced diabetic rats were given (i.p.) a daily injection of 20 mg alloxan solution/100 g of body weight (Sigma Firm, USA). MSH group: Rats injected (i.p) daily with melanocyte stimulating hormone (Sigma Firm, USA) at a dose of 2 μg/100 g of body weight and DMSH: Alloxan-induced diabetic rats injected (i.p.) with both alloxan (20 mg/100 of body weight) and MSH 2 μg/100 g of body weight).

The investigations were carried out, in spring of 2006 were taking into consideration since it is well known that at this time the rats possess a more stable content of hormones. At the end of the of the experiment, all rats were fasted for 12 h before they were sacrificed and blood collected.

Serum insulin and glucose measurement: The concentrations of insulin was measured in serum by radioimmunoassay using radioimmunoassay kit supplied by the (Cea-Jre-Sorin Firm, France). Concentrations were measured by the glucose oxidase method using a spectrophotometer (Cecil ce 1010 England).

Blood analysis: The hematocrit concentrations were measured using a Cobas Micros CT cell counter (Roche Diagnostic Systems, Montpellier, France). Blood viscosity measurements were performed on a viscometer (Viscometer II, Coulter Electronics Ltd., Luton, England).

Statistical analysis: Data were expressed as means±SE and were analyzed with a two-way ANOVA followed by LSD multiple comparison test, using Statistica Software (OK, USA). Differences were considered significant at $p<0.05$.

RESULTS

All Short-term DM, MSH and DMSH animals had a slight body weight loss during the experimental period in compression with control rats (Table 1). After 10 days of alloxan DM induction, Plasma glucose levels were significantly increased in all DM groups, being 2 folds higher than in the controls. (Cont: 95.4±4.5 mg $dL^{\bullet 1}$, DM: 199±12.3 mg $dL^{\bullet 1}$, $p<0.01$) and serum insulin was significantly reduced in DM rats, (Cont: 2.06±0.32 μIU $L^{\bullet 1}$, DM: 0.65±0.06 μIU $L^{\bullet 1}$, $p<0.01$)

Table 1: Changes of body weights (per gram) in normal and diabetic rat groups under effect of Melanocyte stimulating hormone 2 micro/100 g of body weight

Rat body wight	Control	MSH	DM	DM/MSH
Initial body weight ($g^{\bullet 1}$) at 1st day	202.5±8.24	236.5±11.2	226.4±13.3	192.66±7.66
After 3 days	209.3±9.73	237.0±9.6	220.7±12.84	196.66±9.38
Final body weight ($g^{\bullet 1}$) at 10th day	216.5±9.88	228.5±0.8	208.9±13.1	177.6±6.28

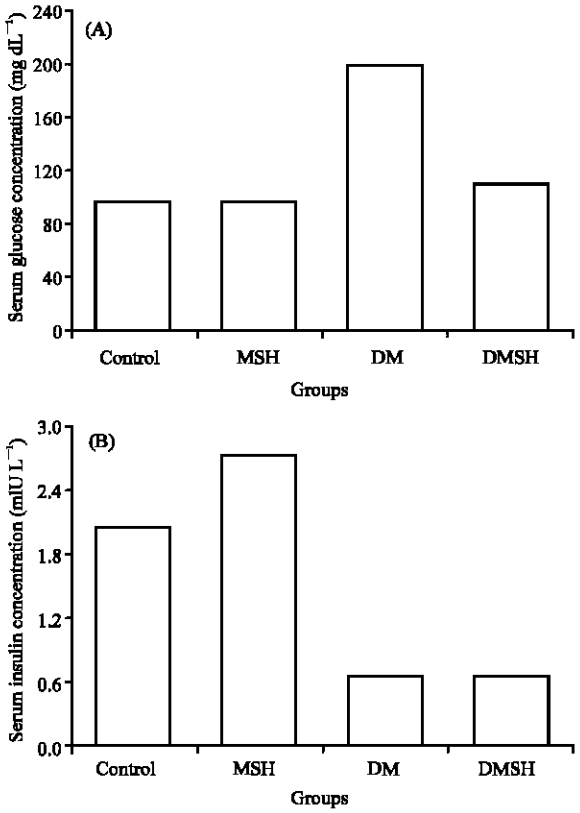

Fig. 1: The effects of • -MSH (2 μg/100 g of body weight), on serum levels of (A) glucose and (B) insulin after short-term (10 days) of alloxan-induced diabetes

(Fig. 1). Although there was no significant difference in serum insulin levels between DM and DMSH injected rats in short-term DM (DM: 0.65±0.06 μIU $L^{\bullet 1}$, DMSH: 0.66±0.10 μIU $L^{\bullet 1}$) serum glucose levels were significantly decreased in DMSH rats to 108.72±11.7, $p<0.01$) in comparison with DM rats (199±12.3 mg $dL^{\bullet 1}$) (Fig. 1).

At the end of experiments, Hematocrit (Ht) of diabetic rats: (34.73±3.67) was uncorrelated to Whole blood viscosity (WBV): 2.96±0.28) in compression with control rats. (Ht: 39.58±3.2, WBV: 3.71±0.43) (Fig. 2). MSH administration significantly decreased blood viscosity in MSH: (2.21±0.17, $p = 0.0017$) and DMSH:

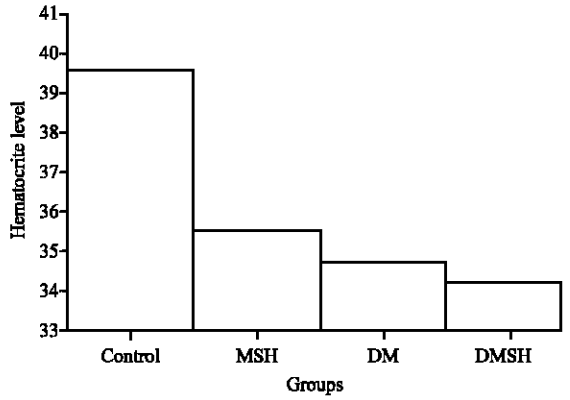

Fig. 2: The effects of • -MSH (2 μg/100 g of body weight), on hematocrite levels after short-term (10 days) of alloxan-induced diabetes

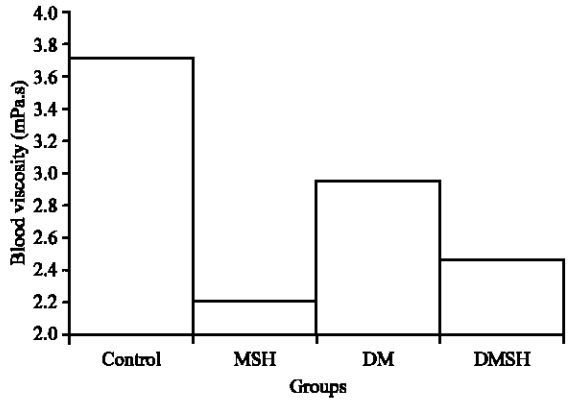

Fig. 3: The effects of • -MSH (2 μg/100 g of body weight), on whole blood viscosity after short-term (10 days) of alloxan-induced diabetes

2.47±0.11, p = 0.011) rats in compression with Cont (3.71±0.43 and DM rats: 2.96±0.10, p<0.001) (Fig. 3).

DISCUSSION

The major finding of the present study was the modulatory role of MSH on blood viscosity values during early stages of induced diabetic rats. The significance of blood viscosity in the microcirculatory flow is an important parameter in the diagnosis of different diseases (diabetes, hypertension, cardiovascular diseases) and a simple and accurate evaluation of hemorheological properties could be an important challenge in clinical practice (Travagli *et al*., 2006; Szapary *et al*., 2003). Although rheological changes in type 2 diabetes in clinical and long term experimental studies is well recognized (Cinara *et al*., 2006; Nukada *et al*., 1993) littlie studies are focused on these changes during early stages of diabetes mellitus, therefore we practiced short-term DM model because in which low multi-doses of alloxan were effective in inducing DM as shown by early stages of DM type 2 (Nascimento-Saba *et al*., 1997).

Blood viscosity and hematocrit are correlated in mammals (Bogar *et al*., 2006; Feher *et al*., 2006) and a major determinant of whole blood viscosity is the hematocrit of the blood. Several studies mentioned to relation between these hematological parameters and insulin resistance (Ellinger *et al*., 2006; Aloulou *et al*., 2006) with dichotomy responses to induced diabetes (Rosse *et al*., 2000; Nukada *et al*., 1993; Kaymaz *et al*., 2005; Zidek *et al*., 1999; Memeh, 1993). In agreement with our results, Brun *et al*. (2004) noted that hematocrit levels were not significantly changed in diabetics. These results suggest that blood viscosity changes via its effects on the determination of flow characteristics of blood (Velcheva *et al*., 2006; Travagali *et al*., 2006) may play a causative role and contributory factor in the pathogenesis of diabetic vascular complications (Kaymaz *et al*., 2005; Le Devehat *et al*., 2004), such as increase high blood pressure (Salaza-Vazquez *et al*., 2006; Brun *et al*., 2004). With increased viscosity, the flow is diminished and the diminution increases as the diameter decreases, this increases the tendency to thrombosis, probably due to the slowed rate of circulation (Rosse *et al*., 2000).

Recent results have reported that alpha-MSH significantly suppressed the deleterious vascular damage and may have a potential in the treatment of stroke or other neurodegenerative diseases (Forslin Anderson *et al*., 2006; Yamaoka-Tojo, 2006; Scholzen *et al*., 2003) via modulatory effects during early stages of diabetic rats (Costa *et al*., 2006; Abu-Samak *et al*., 2006) and its anti inflammatory properties that inhibits tissue injury (Forslin Aronsson *et al*., 2006; Hill *et al*., 2006) or by stimulatory effects on endothelial cells to release vasodilators such as nitric oxide (Vemulapalli *et al*., 2001), therefore we believe that our study highlights the lack of power on some rheological changes under short term administration of MSH during early stages of diabetic diabetes mellitus (DM). Scholzen *et al*. (2003) hyposethized that MSH prevents lipopolysaccharide-induced vasculitis by down-regulating endothelial cell adhesion molecule expression. Therefore we suggest that MSH may participate in this mechanism where it lowers blood viscosity, decreases red blood cells aggregation and decreases friction between RBCs and vessel walls to increase microvascular blood flow and decrease cell injury.

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