글루코오즈 용액에 노출된 적혈구의 유변학적 특성에 대한 In-Vitro연구

신세현*, 염교선*, 구윤희*, Megha Singh**, 서장수***

In-vitro study of hemorheological characteristics of erythrocyte incubated in glucose medium

Sehyun Shin*, Gyoseon Yeom*, Yunhee Ku*, Megha Singh**, Jangsoo Suh***

1. Introduction

Elevated levels of glucose in the medium or blood, which is frequently observed in diabetic mellitus (DM), are known to cause membrane damage and death of red blood cells (RBC) (1). Moreover, ideal glucose level in anticoagulant preservation solutions has been actively searched in order to maximize the maintenance of both RBC viability and function during storage in blood transfusion centers (2). It is not comprehensively understood the biochemical mechanisms how the cell membrane is damaged due to elevated levels of glucose. However, it has been proposed that nonenzymatic glycosylation of certain proteins results in their irreversible cross-linking, which may contribute to the loss of elastic characteristic of cells exposed to hyperglycemia.(3)

After reviewing previous researches, it is found that the glucose level in suspending medium is strongly dependent of erythrocyte hemorheological properties, even though some controversial issues are remained. Therefore, the objective of the present study is to investigate the in-vitro effect of glucose on erythrocyte rheological parameters. Various glucose concentration and incubating durations will be tested on the cell rheological parameters including deformability and aggregation. Erythrocyte deformability was measured using a microfluidic laser-diffractometer and erythrocyte aggregation was measured by rotational aggregometer. In addition, hemoglobin level in erythrocytewas also measured in a phospectrometer.

* 경북대 기계공학부
** Indian Institute of Technology in Madras
*** 경북대 진단검사의학과

2. Materials and Methods

Samples of venous blood were drawn from the antecubital vein and collected into (K3)EDTA containing Vacutainers (BD, Franklin Lakes, NJ).Whole blood was centrifuged at 800 g for 10 min. Plasma and buffy-coat were then removed. The remaining RBCs were washed three times with phosphate buffer saline (PBS: pH 7.4) at 25 °C. Each packet of RBCs was divided into five aliquots. One aliquot was left untreated as a control sample. The other four aliquots were incubated in water bath at 37°C for different glucose concentrations upto two hours. The hematocrit in the incubated media was fixed at 10%. These glucose incubating media were prepared by diluting 5% of glucose solution (Dextrose 5% DW, Choongwae Parma Co,

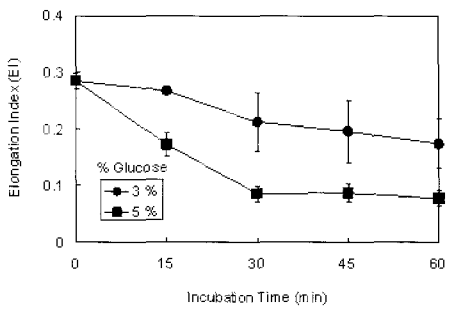

Fig. 1 Effect of incubation time on deformability of erythrocytes incubated in two different glucose concentrations.

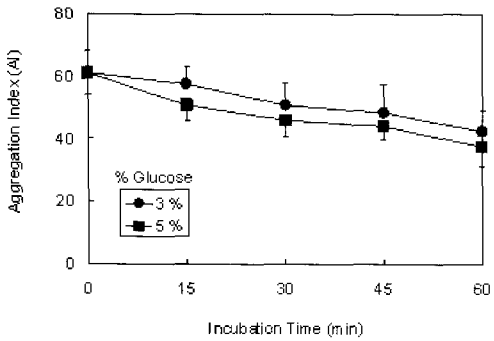

Fig. 2 Effect of incubationtime on aggregation of erythrocytes incubated in two different glucose concentrations.

Seoul, Korea) with 0.9% NaCl solution (DC C hemical with PBS at 25 °C to remove any remnants of glucose. Each aliquot of RBC packet was resuspended in autologous plasma to obtain the required hematocrit. We did not observe the presence of hemolysis during the post-glucose washing with PBS at all glucose concentration except 10 %.

3. Results and Discussion

The deformability of erythrocytes was decreased by the glucose incubation. Figure 1 shows the effect of glucose concentration on erythrocyte deformability. As the glucose concentration increases, the erythrocytedeformability decreases. Beyond 2% of glucose concentration, EI is rapidly decreased.

The aggregation of erythrocytes was also decreased by the glucose incubation. Figure 2 shows the effect of glucose concentration on aggregation index (AI). As the glucose concentration increases, the AI decreases. For 30 min of incubation time, the aggregation index decreases slowly with increasing glucose concentration. However, for 60 min of incubation time, the AI is almost independent of glucose concentration. This facts imply that beyond a certain period of incubation, the reduced aggregation due to glucose incubation reaches a saturated value and there is no effect of glucose oncentration on aggregation alteration.

Glycation associated with hyperglycemia used to increase in HbA1C, which clinical significance is markedly increased in diagnosis of diabetic mellitus(5). In our recent study, a good correlation between the erythrocyte deformability and HbA1C was observed. Thus, these result that the increase of HbA1C as measure of glycation was accompanied with the decrease of erythrocyte deformability are coincede with the present results. Impairment of erythrocyte deformabilityis found to be strongly correlated with diabetic microvascular diseases such as retinopathy and nephropathy (1). These diabetic microangiopathies are accompanied with reduced erythrocyte deformability(1) and elevated blood viscosity. The blood viscosityis strong function of erythrocyte deformability. Thus, impaired erythrocyte deformability due to glycation may be a common factor correlated with diabetic mellitus and its complications.

Acknowledgement

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