DOI: 10.4149/BLL_2014_0079

Bratisl Lek Listy 2014; 115 (7)
400 – 404

EXPERIMENTAL STUDY

Investigation of the effects of propofol and vitamin C administration on erythrocyte deformability in rats with streptozotocin-induced diabetes mellitus

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Abstract: *Purpose:* In the current study we aim to investigate the effects of vitamin C and profol on red blood cell deformability in diabetic rats
Materials and methods: Twenty- eight Wistar Albino rats were included in the study after streptozocin (60 mg/kg) treatment for 4 weeks of observation for diabetes presence. Twenty-eight rats were allocated to 4 groups. In group DP (n = 7) 150 mg.kg^{-1} of propofol was injected intraperitoneally. In group DP-vit C (n = 7) rats 100 mg/kg of vitamin C (Ascorbic acid, *Redoxon*® 1000 mg/5 mL - *Roche*) were applied one hour before administrating 150 mg.kg^{-1} of propofol, while rats in control group (n = 7), and diabetic control group (n = 7) received intraperitoneally physiological saline. Deformability measurements were achieved by using erythrocyte suspensions with hematocrit level of 5 % in PBS buffer.
Results: Erythrocyte deformability was significantly higher in diabetic control group than in control and vitamin C plus propofol groups (p = 0.00, p = 0.025, respectively). Erythrocyte deformability indexes were found similar in control group and vitamin C plus propofol group (p = 0.949). Relative resistance was increased in diabetic rat model.
Conclusions: Erythrocyte deformability was damaged in rats with diabetes. This injury might lead to further problems in microcirculation. Application of propofol did not alter red cell deformability in diabetic rats. Vitamin C supplementation seems to reverse those negative effects and variations in erythrocyte deformability *(Fig. 2, Ref. 57)*. Text in PDF *www.elis.sk.*
Key words: erythrocyte deformability, propofol experimental diabet, vitamin C, rat.

In the recent two or three decades, the prevalence of diabetes mellitus (DM) has rapidly increased throughout the world, the estimation being that it will increase by 200 % in the next several decades (1–5). As a result, physicians will be encountered with an increasing population of diabetic patients undergoing anesthesia and surgery with serious complications, such as hypertension, ischemic heart disease in association with significant increases in length of stay and mortality rates in hospital, as well as nephropathy, and autonomic neuropathy (1–3).

In vitro and in vivo studies suggest that lipid peroxidation is associated with diabetic complications and that this plays an important role in the pathogenesis of diabetic complications. So it is very important to control the lipid peroxydation. A variety of endogenoous and exogenous antioxidant mechanisms serve to control this peroxidative process (6–11).

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Ascorbic acid plays a role in reducing compounds such as cytochrome a and c, nitrate, molecular oxygen and it is a water soluble molecule that has the ability of reacting with free radicals in aqeous medium (12).

Several studies showed decreased basal vitamin C levels in diabetic patients compared with healty volunteers (13). Hemorheological parameters which include hematocrit, plasma proteins, erythrocyte aggregation, and erythrocyte deformability in DM, are often disturbed (14).

General anesthesia agents are known to affect cardiovascular functions and microcirculation dynamics (15). However, whether these agents change plasma rheology and/or anesthesia may result in deterioration of tissue perfusion remains controversial. Changes in plasma viscosity have been listed among the factors associated with anesthesia procedures responsible for deterioration of tissue and organ perfusion (16, 17). After surgical procedures performed using general anesthesia, erythrocyte deformability and increased aggregation may be observed (17).

Propofol (2,6-diisopropylphenol) is an intravenous anesthetic agent that is commonly used in daily clinical practice for sedation and general anaesthesia which can scavenge free radicals because it has a chemical structure similar to antioxidants (18). Its cardiovascular side effects have been described in various studies. It can decrease the peripheral vascular resistance (19), decrease

jugular venous oxygen saturation (20), induce haemolysis (21), and cause the so-called propofol infusion syndrome, an often fatal cardiac failure (22).

The effect of propofol on insulin secretion is not known. It is well known that diabetic patients have a reduced ability to clear lipids from the circulation (23). Alterations in the pharmacokinetics or pharmacodynamics of propofol in rats with DM were reported in an experimental study (24). There is no data showing whether administration of propofol can alter lipid clearance from the circulation. An experimental study showed that propofol impairs diastolic left ventricular filling in experimental models and produces negative lusitropic effects in diabetic cardiomyocytes (25).

In earlier studies with various methodologies, propofol has been shown to cause OS (oxidative stress), not to affect OS, or to have antioxidant effect (26–29). In *in vitro* studies, it has been shown to inhibit lipid peroxidation induced by oxidative stress in the liver microsomes, mitochondria, and brain synaptosomes (30). The products that arise due to lipid peroxidation associated with increased oxidative stress significantly affect membrane permeability and microviscosity, thus diminishing the deformability capacity and survival of the erythrocytes (31).

We hypothesized that propofol and vitamin C and/or the emulsifier might do this through a direct action on the biophysical or functional properties of the blood constituents such as the diabetic rat red blood cell (RBC) deformability.

In this study, the effects of propofol anesthesia and administration of vitamin C before application of propofol on the red blood cell deformability of diabetes male rats were evaluated.

Materıals and methods

Animals and experimental protocol

This study was conducted in the Physiology Laboratory of Kirikkale University upon the consent of the Experimental Animals Ethics Committee of Kirikkale University. All of the procedures were performed according to the accepted standards of the Guide for the Care and Use of Laboratory Animals.

In the study, 28 male Wistar Albino rats weighing between 250 and 300 g, raised under the same environmental conditions, were used. The rats were kept under 20–21 °C at cycles of 12-hour daylight and 12-hour darkness and had free access to food until 2 hours before the anesthesia procedure. The animals were randomly separated into four groups, each containing 7 rats.

Diabetes was induced by a single intraperitoneal injection of streptozotocin (Sigma Chemical, St. Louis, MO, USA) at a dose of 60 $mg.kg^{-1}$ body weight. The blood glucose levels were measured at 72 h following this injection. Rats were classified as diabetic if their fasting blood glucose (FBG) levels exceeded 250 $mg.dl^{-1}$, and only animals with FBGs of > 250 $mg.dl^{-1}$ were included in the diabetic groups (diabetes only, diabetes plus propofol and diabetes plus vitamin C after propofol). The rats were kept alive 4 weeks after streptozotocin injection to allow development of chronic diabetes before they were exposed to propofol (32).

Rats were anesthetized with intraperitoneal ketamine 100 $mg.kg^{-1}$. The chest and abdomen were shaved and each animal was fixed in a supine position on the operating table. The abdomen was cleaned with 1 % polyvinyl iodine and when dry, the operating field was covered with a sterile drape and median laparotomy was performed. Twenty-six rats were allocated to 4 groups. In group DP (n = 6) 150 $mg.kg^{-1}$ of propofol (Propofol 1 % Fresenius 20 mL) was injected intraperitoneally. In group DP plus vitamin C (n = 7) rats were given 100 $mg.kg^{-1}$ of vitamin C (Ascorbic acid, Redoxon® 1000 mg/5 mL, Roche) 30 minutes before administrating 150 $mg.kg^{-1}$ of propofol, while rats in control (n = 7) and diabetic control groups (n = 6) received intraperitoneally physiological saline.

Thirty minutes after propofol administration, all rats received ketamin 100 $mg.kg^{-1}$ intraperitoneally and were euthanized to collect blood samples from vessels in the abdominal cavity. Heparinized total blood samples were used to prepare erythrocyte packs. Deformability measurements were done by erythrocyte suspensions with 5 % htc in phosphate buffered saline buffer.

Deformability measurements

Blood samples were taken very crefully and measurement process was as fast as possible to avoid hemolysis of erythrocytes. The collected blood was centrifuged at 1000 rpm for ten minutes. Serum and buffy coat on erythrocytes were removed. Isotonic PBS buffer was added to collapsing erythrocytes and this was centrifuged at 1000 rpm for ten minutes. Liquid on the upper surface was removed. Finally, pure red cell packs were obtained from the washing process which was repeated three times. Erythrocytes packs were mixed with PBS buffer to generate a suspension with the value of 5 % Htc. Those erythrocyte suspensions were used for the measurement of deformability. Collection and deformability measurements of erythrocytes were done at 22 °C.

The constant-current filtrometre system was used for measurement of erythrocytes deformability. Samples to be measured were prepared as 10 ml of erythrocytes suspension and PBS buffer. The flow rate was held constant at 1.5 ml/min with an infusion pump. A 28 mm nucleoporin polycabonate fitler with a 5 µm pore diameter was prefered. Consisting pressure changes while the erythroctes were passing through from the filter were detected by the pressure transducer and the data was transferred to computer with the help of MP 30 data equation systems (Biopac Systems Inc, Commat, USA). The necessary calculations were performed with related computer programs by measuring the pressure changes at various times. Pressure calibration of the system was performed each time before measuring the samples. First the buffer(P_T) and then the erythrocytes (P_E) were passed through from the filtration system and the changes in pressure were measured. The relative refractory period value(Rrel) was calculated by relating the pressure value of erythroctes suspension to pressure value of buffer. Increasing in Rrel as the deformability index was interpreted as adversely affecting the ability of erythrocytes deformability (33, 34).

Statistical analysis

Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) 17.0 program was used for statistical analysis. Variations in blood glucose level and erythrocyte deformability were assessed by

using Kruskal-Wallis test. Bonferroni adjusted Mann-Whitney U test was used after significant Kruskal-Wallis to determine which group differs from the other. Results were expressed as mean± standard deviation (Mean ± SD). Statistical significance was set at a p value <0.05.

Results

During the study period 2 of the diabetic rats died (1 in Group DC, 1 in Group DP).

Blood glucose measurements were 83.7 ± 9.1, 374.5± 68.6, 375.3 ± 86.7, 368.5 ± 71.3 mg /dL for Group C, DC, DP and DP plus vitamin C, respectively (Fig. 1). Serum glucose was detected to be significantly lower in Group C when compared to Groups DC, DP and DP plus vitamin C (p = 0.001, p = 0.002, p = 0.001, respectively).

Erythrocyte deformability was significantly higher in diabetic control group when compared with the control group and group propofol plus vitamin C (p = 0.002, p = 0.025 respectively). Erythrocyte deformability index was similar in control group and group propofol plus vitamin C (p = 0.949). Also erythrocyte deformability index was found similar in diabetic control group and diabetic propofol group (p = 0.650). However, relative resistance was increased in diabetic group (Fig. 2).

Conclusion

Hemorheological parameters, such as; hematocrit, plasma proteins, erythrocyte aggregation, and erythrocyte deformability are often disturbed in DM (35). Altered erythrocyte deformability not only changes the oxygen delivery capacity of the erythrocytes but also the survival of the circulating erythrocytes (36–38).

Additionally, it has been suggested that the impaired perfusion at the tissue level observed as a complication of diabetes mellitus is primarily due to the reduced erythrocyte deformability (39,40). Besides, metabolic changes and tissue perfusion due to cardiovascular problems may lead to inadequate recovery in plasma viscosity (41).

Barnes et al (42) showed that erythrocyte deformability was lower in the 14 diabetes patients with the most extensive microangiopathy than in 22 diabetes patients with slight or no complications or in controls. They suggested that hyperviscosity and reduced erythrocyte deformability might be important and potentially treatable factors in the etiology or progression of microcirculatory disease in diabetes. Similar to the previous studies, we also found that erythrocyte deformability was decreased in diabetes induced rats.

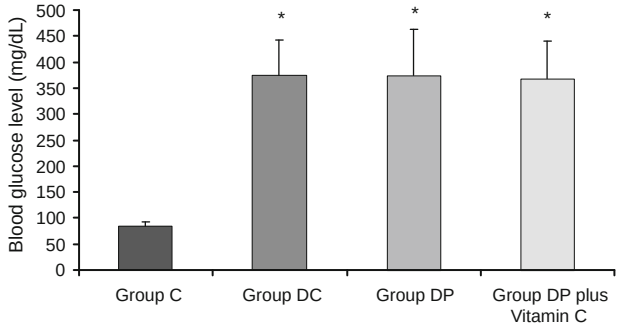

Fig. 1. Blood glucose levels in the groups. Each bar represents mean ± SD. * p < 0.05 compared to Group C.

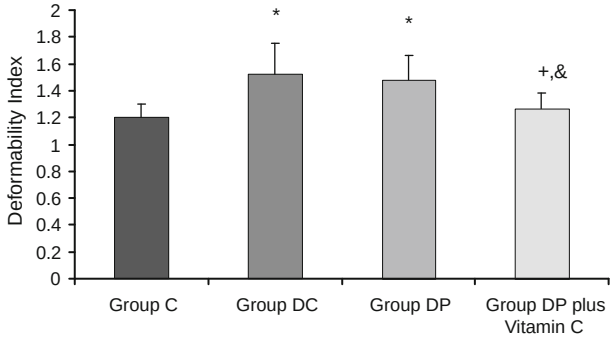

Fig. 2. Erythrocyte deformability values in the groups. Each bar represents mean ± SD.
*** p < 0.05 compared to Group C; + p < 0.05 compared to Group DC; & p < 0.05 compared to Group DP.**

There are various procedures to measure erythrocyte deformability. The two of the prominent techniques for this measurement are measuring either change in optical diffraction pattern (ektacytometry) of erythrocytes or erythrocyte filtration through membrane. In the first technique the diffraction pattern of erythrocytes changes from circular to elliptic form during stationary flow conditions in rheoscope or microchannel (43). The erythrocyte filtration through membrane technique is based on the measurement of passage time of erythrocyte suspension through microscope membrane, which is reciprocal to the erythrocyte deformability (43). For better correlation of this measurement the applied pressure should be comparable to that in microcirculation, below 10 Pa. As erythrocytes flowing under low pressure may block the membrane pores, a low hematocrit (less than 10 %) is preferable. The initial flow method, which minimizes the influence of gravitational field by operating within the specified range of applied pressure, has been used to measure erythrocyte deformability under varied conditions (43, 44). The deformability is also measured from the change in erythrocyte count before and after filtration through a membrane under gravitational field (44). Another historical measurement is determining the volume of RBCs (VRBC) filtered per minute through approximately 5 μm pore-size filters. The VRBC was found to be significantly reduced in diabetes patients compared with healthy controls (14).

In our study we used constant flow filtration technique for determining erythrocyte deformability. The filtration technique measurement shows that the erythrocyte deformability is significantly decreased (44–47). Similar decrease in deformability by ektacytometry (48) and transparent microchannels were observed (49).

Erythrocytes are very sensitive to oxidative injury (50). To defend themselves against oxidative stress (OS), erythrocytes are equipped with an effective and complex antioxidant system,

including protective enzymes and biological antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, glutathione, vitamin C, and vitamin E (51).

Among the potential antioxidants in foods, vitamin C and vitamin E are the principal dietary antioxidants that protect erythrocytes from damage caused by reactive oxygen species. Non-enzymatic antioxidants such as vitamin E, vitamin C and L-carnitine act towards reducing the OS (52).

Inhalation and intravenous anesthetic agents are known to affect cardiovascular functions and microcirculation and ongoing studies are investigating the issue. Yesilkaya et al (53) have found that halothane and pentobarbital impair erythrocyte deformability.

Yerer et al (54) investigated the effects of desflurane on erythrocyte deformability and found that it impaired the deformability in young and old rats. Aydogan et al (55) showed negative effects of sevoflurane on the deformability of the old rats.

Kim et al (56) showed that verapamil and ascorbic acid have protective effect against *tert*-butyl hydroperoxide induced oxidative stress. They found that ascorbic acid reverses the effects of tert-butyl hydroperoxide and improves deformability of erythrocytes to the values of non *tert*-butyl hydroperoxide treated groups'. In addition our previous studies results showed that high dose of Dexmedetomidine impaired erythrocyte deformability and administration of vitamin C given 1 hour before dexmedetomidine reversed these negative effects and improved erythrocyte deformability (57).

Our results showed that diabetes mellitus impairs erythrocyte deformability and administration of vitamin C given 30 minutes before propofol reverses this negative effects and improves erythrocyte deformability.

Dikmen et al (29) have reported that at clinical doses, propofol andremifentanil have no effect on oxidative stress, and sevoflurane can protect erythrocytes against oxidative stress.

In our previous study (33), propofol was found to impair the erythrocyte deformability in both genders, but it was more pronounced in the male rats. This may be accounted for to general protective effects of estrogen in female rats.

Erythrocyte deformability was impaired in diabetic rats. These impairments may cause further problems in microcirculations. Additionally, administration of propofol in diabetic rats was observed to have no protective effect on eiythrocyte deformability index. It was observed that administration of vitamin C reversed the negative effect on eiythrocyte deformability However, our early results should be confirmed by further detailed clinical and experimental studies on the issue.

References

1. Robertshaw HJ, Hall GM. Diabetes mellitus: anaesthetic management. Anaesthesia 2006; 61: 1187–1190.

2. McAnulty GR, Robertshaw HJ, Hall GM. Anaesthetic management of patients with diabetes mellitus. Br J Anesth 2000; 85: 80–90.

3. McAnulty GR, Hall GM. Anaesthesia for the diabetic patient. Br J Anesth 2003; 88: 428–430.

4. Gu W, Pagel PS, Warltier DC, Kersten JR. Modifying cardiovascular risks in diabetes mellitus. Anesthesiology 2003; 98: 774–779.

5. Kadoi Y. Anesthetic considerations in diabetic patients. Part I: preoperative considerations of patients with diabetes mellitus. J Anesth 2010; 24: 739–747

6. Vincent AM, Russell JW, Low P, Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. Endocrine Reviews 2004; 25: 612–628.

7. Memisogullari R, Taysi S, Bakan E, Capoglu I. Antioxidant status and lipid peroxidation in Type II Diabetes Mellitus. Cell Biochem Func 2003; 21: 291–296.

8. Sacks DB. Diabetes Mellitus. In: Burtis CA, Ashwood ER, (Ed). Tietz Texbook of clinical chemistry. Philadelphia: WB Saunders Co: 1999; 766–776.

9. Cherubini A, Ruggiero C, Polidori MC, Mecocci C. Potential markers of oxidative stress in stroke. Free Radic Biol Med 2005; 39: 841–852.

10. Memisogullari R, Bakan E. Levels of ceruloplasmin, transferrin, and lipid peroxidation in the serum of patients with Type 2 diabetes mellitus. J Diabetes Complications 2004; 18: 193–197.

11. Masella R, Benedetto RD, Varý R, Filesi C, Giovannini C. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathionerelated enzymes. J Nutr Biochem 2005; 16: 577–586.

12. Memisogullari R. The role of free radicals and the effect of antioxidants in diabetes. Düzce Tip Fakültesi Dergisi 2005; 3: 30–39.

13. Will JC, Byers T. Does diabetes mellitus increase the requirement for vitamin C? Nutr Rev 1996; 54: 193–202.

14. Cho YI, Mooney MP, Cho DJ. Hemorheological disorders in diabetes mellitus. J Diabetes Sci Technol 2008; 2: 1130–1138.

15. Erdogan C, Erdem A, Akinci SB, Dikmenoglu N, Basgül E, Balkanci D et al. The effects of midazolam on erythrocyte deformability and plasma viscosity in rats. Anestezi Dergisi. 2005; 13: 205–208.

16. Muller R, Musikic P. Hemorheology in surgery: a review. Angiology. 1987; 38: 581–592.

17. Dormandy JA. Effects of anaesthesia and surgery on the flow properties of blood. Microcirc Endothelium Lymphatics. 1984; 1: 151–168.

18. Kofke WA. Anesthetic management of the patient with epilepsy or prior seizures. Curr Opin Anesthesiol 2010; 23: 391–399.

19. Boer F, Ros P, Bovill JG, van Brummelen P, van der Krogt J. Effect of propofol on peripheral vascular resistance during cardiopulmonary bypass. Br J Anaesth 1990; 65: 184–189.

20. Nandate K, Vuylsteke A, Ratsep I, Messahel S, Oduro-Dominah A, Menon DK et al. Effects of isoflurane, sevoflurane and propofol anaesthesia on jugular venous oxygen saturation in patients undergoing coronary artery bypass surgery. Br J Anaesth 2000; 84: 631–633.

21. Masuda A, Hirota K, Satone T, Ito Y. Pink urine during propofol anesthesia. Anesth Analg 1996; 83: 666–667.

22. Cremer OL, Moons KG, Bouman EA, Kruijswijk JE, de Smet AM, Kalkman CJ. Long-term propofol infusion and cardiac failure in adult head-injured patients. Lancet 2001; 357 (9250): 117–118.

23. Wicklmyr M, Rett K, Dietz G, Mehnert H. Comparison of metabolic clearance rates of MCT/LCT and LCT emulsions in diabetics. J Parenter Enter Nutr 1988; 12: 68–71.

24. Leal N, Calvo R, Agrad FZ, Lukas JC, de la Fuente L, Suarez E. Altered dose-to-effect of propofol due to pharmacokinetics in rats with experimental diabetes mellitus. J Pharm Pharmacol 2005; 57: 317–325.

25. Wickey PJ, Shiga T, Murray PA, Damron DS. Propofol modulates Na?–Ca2? exchange activity via activation of protein C in diabetic cardiomyocytes. Anesthesiology 2007; 106: 302–311.

26. Runzer TD, Ansley DM, Godin DV, Chambers GK. Tissue antioxidant capacity during anesthesia: propofol enhances *in vivo* red cell and tissue antioxidant capacity in a rat model. Anesth Analg 2002; 94: 89–93.

27. Allaouchiche B, Debon R, Goudable J, Chassard D, Duflo F. Oxidative stress status during exposure to propofol, sevoflurane and desflurane. Anesth Analg 2001; 93: 981–985.

28. De La Cruz JP, Sedeno G, Carmona JA, Sanchez de la Cuesta F. The in vitro effects of propofol on tissular oxidative stress in the rat. Anesth Analg 1998; 87: 1141–1146.

29. Dikmen B, Erk C, Et G, Kös T, Horasanli E, Kilci O et al. The effects of propofol/remifentanil anesthesia and sevoflurane anesthesia on oxidant and antioxidant system in human erythrocytes. Turkiye Klinikleri J Anest Reanim 2005; 3: 15–20.

30. Musacchio E, Rizzoli V, Bianchi M, Bindoli A, Galzigna L. Antioxidant action of propofol on liver microsomes, mitochondria and brain synaptosomes in the rat. Pharmacol Toxicol 1991; 69: 75–77.

31. Sivilotti ML. Oxidant stress and haemolysis of the human erythrocyte. Toxicol Rev 2004; 23: 169–188.

32. Türeci E, Is M, Uzüm G, Akyüz F, Ulu MO, Dösoglu M et al. Alterations in blood-brain barrier after traumatic brain injury in streptozotocin-induced diabetic rats. J Nervous Sys Surg 2009; 2: 79–86.

33. Arslan M, Comu FM, Isik B, Unal Y, Cekmen N, Kurtipek O. Effects of a general anaesthetic agent, propofol, on erythrocyte deformability. Bratisl Med J 2010; 111: 126–128.

34. Tatlican S, Duran FS, Eren C, Eskoglu F, Dikmenoglu N, Oktay B et al. Reduced erythrocyte deformability in active and untreated BehCet's disease patients. Int J Dermatol 2010; 49: 167–171.

35. Barnes A, Willars E. "Diabetes" in Clinical Hemorheology. In: Chien S, Dormandy J, Ernst E, A Matrai A (Eds). Dordrecht: Martinus Nijhoff; 1987: 275–309.

36. Zinchuk VV. Erythrocyte deformability: physiological aspects. Usp Fiziol Nauk 2001; 32: 66–78.

37. Kuypers FA. Red cell membrane damage. J Heart Valve Dis 1998; 7: 387–95.

38. Sivilotti ML. Oxidant stress and haemolysis of the human erythrocyte. Toxicol Rev 2004; 23: 169–188.

39. Le Devehat C, Khodabandehlou T, Vimeux M. Relationship between hemorheological and microcirculatory abnormalities in diabetes mellitus. Diabete Metab 1994; 20: 401–404.

40. Zimny S, Dessel F, Ehren M, Pfohl M, Schatz H. Early detection of microcirculatory impairment in diabetic patients with foot at risk. Diabetes Care 2001; 24: 1810–1814.

41. Muller R, Musikic P. Hemorheology in surgery: a review. Angiology 1987; 38: 581–592.

42. Barnes AJ, Locke P, Scudder PR, Dormandy TL, Dormandy JA, Slack J. Is hyperviscosity a treatable component of diabetic microcirculatory disease?. Lancet 1977; 2 (8042): 789–791.

43. Singh M, Shin S. Changes in erythrocyte aggregation and deformability in diabetes mellitus: a brief review. Indian J Exp Biol 2009; 47: 7–15.

44. Babu N, Singh M. Influence of hyperglycemia on aggregation, deformability and shape parameters of erythrocytes. Clin Hemorheol Microcirc 2004; 31: 273–280.

45. Banerjee R, Nageshwari K, Puniyani RR. The diagnostic relevance of red cell rigidity. Clin Hemorheol Microcirc 1998; 19: 21–24.

46. Béder I, Kittová M, Mataseje A, Câársky J, Országhová Z, Babinská K. Effect of selected substances with antiglycative and antioxidative properties on erythrocytedeformability in diabetic patients. Scripta Medica (BRNO) 2002; 75: 239–244.

47. Bareford D, Jennings PE, Stone PC, Baar S, Barnett AH, Stuart J. Effects of hyperglycaemia and sorbitol accumulation on erythrocyte deformability in diabetes mellitus. J Clin Pathol 1986; 39: 722–727.

48. Schwartz RS, Madsen JW, Rybicki AC, Nagel RL. Oxidation of spectrin and deformability defects in diabetic erythrocytes. Diabetes 1991; 40: 701–708.

49. Tsukada K, Sekizuka E, Oshio C, Minamitani H. Direct measurement of erythrocyte deformability in diabetes mellitus with a transparent microchannel capillary model and high-speed video camera system. Microvasc Res 2001; 61: 231–239.

50. Bernabucci U, Ronchi B, Lacetera N, Nardone A. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. J Dairy Sci 2002; 85: 2173–2179.

51. Vani R, Reddy CS, Asha DS. Oxidative stress in erythrocytes: a study on the effect of antioxidant mixtures during intermittent exposures to high altitude. Int J Biometeorol 2010; 54: 553–562.

52. Asha Devi S, Subramanyam MV, Vani R, Jeevaratnam K. Adaptations of the antioxidant system in erythrocytes of trained adult rats: impact of intermittent hypobaric-hypoxia at two altitudes. Comp Biochem Physiol C Toxicol Pharmacol 2005; 140: 59–67.

53. Yesilkaya A, Ertug Z, Yegin A, Melikoglu M, Baskurt OK. Deformability and oxidant stress in red blood cells under the influence of halothane and isoflurane anesthesia. Gen Pharmacol 1998; 31: 33–36.

54. Yerer MB, Aydogan S, Comu FM, Arslan M, Günes Ekinci I, Kurtipek O et al. The red blood cell deformability alterations under desfluran anesthesia in rats. Clin Hemorheol Microcirc 2006; 35: 213–216.

55. Aydogan S, Yerer MB, Comu FM, Arslan M, Günes Ekinci I, Unal Y et al. The influence of sevoflurane anesthesia on the rat red blood cell deformability. Clin Hemorheol Microcirc 2006; 35: 297–300.

56. Kim DH, Kim YK, Won, DI, Shin S, Suh JS. Assessment of hemorheological deformability of human red cells exposed to *tert*-butyl hydroperoxide, verapamil and ascorbate by ektacytometer. Korean J Lab Med 2008; 28: 325–331.

57. Kurtipek O, Comu FM, Ozturk L, Alkan M, Pampal K, Arslan M. Does Vitamin C Prevent the Effects of High Dose Dexmedetomidine on Rat Erythrocyte Deformability?. Bratisl Med J 2012; 113: 135–138.

Received September 29, 2012.
Accepted March 31, 2013.