

Reduction of Total Antioxidant Capacity after Femoral Fracture

Snížení totální antioxidační kapacity po zlomenině femuru

D. KESKIN¹, A. KIZILTUNC²

¹ Department of Orthopaedics and Traumatology, Ondokuz Mayıs University, Faculty of Medicine, Samsun, Turkey

² Department of Biochemistry, Ataturk University, Faculty of Medicine, Erzurum, Turkey

ABSTRACT

PURPOSE OF THE STUDY

The aim of this study was to determine the changes in total antioxidant capacity (TAC) during the fracture healing process.

MATERIAL AND METHODS

Twenty patients with isolated closed femoral fracture, between the ages 18 and 60 years, were included in the study. The control group was formed with healthy volunteers. Venous blood was drawn from the healthy volunteers once, and from the patients five times during 14 days after fracture. TAC was measured in the sera of these samples.

RESULTS

In the patient group, the serum TAC was the highest in the first 6 hours, whereas there was a decreasing trend on the 3rd, 7th and 14th days, and an increasing trend on the 5th day. The mean serum TAC in all measurements of the patient group were lower than those in the control group.

CONCLUSION

The present study suggests that TAC may be decreased in considerable amounts during the first 2 weeks of fracture healing.

Key words: human bone, fracture, blood, total antioxidant capacity.

INTRODUCTION

Oxygen-derived free radicals (ODFRs) formed in different tissues after ischemia-reperfusion injury are frequently cited for their contribution to tissue injury in the recent years (3, 8, 11, 18). ODFRs cause disintegration of cell membrane and damage DNA (13, 14, 17). Detrimental effects of ODFRs are counteracted by antioxidative mechanisms. Antioxidant molecules of either endogenous or exogenous origin prevent formation of ODFRs or deactivate the formed free radicals (5, 25). Different antioxidant molecules can be measured separately or total antioxidants in one sample can be measured to determine the total antioxidant capacity (TAC), (5, 16). In some diseases, balance between oxidant and antioxidant molecules is disturbed in favor of oxidant molecules causing oxidative injury in the tissues (3, 6, 10, 12). Ischemia-reperfusion mechanism may cause an oxidative stress injury in a fractured extremity and negatively affects the fracture healing (2, 7, 20, 21, 24). But is there any change in TAC of the organism during fracture healing? Our literature search revealed few studies

on this subject. One of these studies reported significant increase in antioxidant levels of blood on the 7th and 14th days after a long bone fracture (21). In our clinical prospective study, TAC in serum of the patients with isolated musculoskeletal injury and femoral fracture was periodically measured for 2 weeks and any change in antioxidant capacity of the organism during fracture healing was investigated.

MATERIAL AND METHODS

Patients

After the institutional Ethic Council had approved the study, 20 patients with isolated musculoskeletal system injury and closed femoral fracture who had not undergone the operative intervention in the 2 weeks following trauma were included in the study. In these patients, surgery had been delayed because of the causes such as the problems of health insurance. Open fractures were not included in the study. Other exclusion criteria were de-

defined as follows: pregnancy, diabetes mellitus or any metabolic disease, vascular dysfunctions and organ dysfunctions, administration of major intravenous volume resuscitation. The control group was formed with healthy volunteers. Each group was formed by 20 subjects, 6 of which were females (30%) and 14 were males (70%) between the ages 18 and 60 years.

Methods

Venous blood samples were taken from healthy volunteers only once at room temperature, and from traumatic patients during the first 6 hours after the trauma and then on 3, 5, 7 and 14th days. Venous blood samples were drawn into vacutainer tubes without an anticoagulant to obtain serum. Blood samples were centrifuged immediately at 2000 × g for 10 minutes and the serum samples obtained were stored at -80 °C until assayed. Within the first 3 day of storage, TAC of serum samples was measured according to the method of Erel (5). Briefly, 225 µl reagent 1 (xylenol orange 150 µM, NaCl 140 mM and glycerol 1.35 M in 25 mM H₂SO₄ solution, pH 1.75) and 11 µl reagent 2 (ferrous ion 5 mM and 0-dianisidine 10 mM in 25 mM H₂SO₄ solution) are added to 35 µl of serum. The resulting chromogen is determined at 560 nm. The reaction rate is calibrated with Trolox, which is widely used as a traditional standard for TAC measurements assays, and the assay results are expressed in mmol Trolox equivalent/l.

Statistical analysis

Independent-samples t test was used to determine the statistical significance among the patient and control groups. Total antioxidant level differences at different times were tested with paired-samples t-test. SPSS software, version 11.5, was used in order to perform statistical evaluations (SPSS, Chicago, Ill., USA). Data were presented as mean ± SD, and a p-value < 0.05 was considered significant, while p < 0.01 was regarded as very significant and p > 0.05 nonsignificant.

RESULTS

The mean ages in the patient and the control groups were 35.35±12.19 and 36.05±13.02 years, respectively. At all times, the mean serum total antioxidant levels in the patient group were significantly lower than those of the control group (Table 1). The total antioxidant level difference between the patient and the control group was statistically significant (p < 0.05) in the first 6 hours, and very significant (p < 0.01) at the other times. In the patient group, whereas the mean serum total antioxidant level was highest in the first 6 hours, it was decreased on the 3rd day and increased on the 5th day. Subsequently, the level was decreased to the lowest value on the 7th day, and thereafter remained almost stable. Compared with the first 6 hours value, the decreasing percentages of the mean serum total antioxidant level on the 3rd, 5th, 7th and 14th days were 45.3%, 30.2%, 47.2% and 49.1%, respectively. Whereas the difference between the total antioxidant levels of the 7th and 14th days was not statis-

tically significant (p > 0.05), the difference was significant (p < 0.05) between the levels of the 3rd-7th and 3rd-14th days. The other level differences were very significant (p < 0.01).

DISCUSSION

Ischemia after a fracture may be due to soft tissue contusions, lacerations, vascular injuries or compartment syndrome secondary to trauma; and subsequent local hypoperfusion caused by all of these factors (14). One of the most reliable indicator of oxidative stress is level of malonyldialdehyde (MDA) either in local tissue or in systemic circulation (2, 7, 20, 21). MDA levels together with other indicators of oxidative stress have been shown to increase in bones of animals with experimental fractures and in serum samples of humans with bone fracture (12, 21, 24). These studies have also shown that oxidative stress is particularly increased on the 7th and 14th day after fracture. Common assumption of these studies was as follows: first few days of fracture healing is the ischemia period during which no oxidative stress injury is formed. But afterwards; during callus formation approximately corresponding to 2nd and 3rd weeks, formation of new capillary beds increase the amount of oxygen in the surrounding, thus leading to formation of ODFRs. These free radicals cause oxidative injury on the bones and hinder fracture healing.

Deleterious effects of ODFRs are counteracted by antioxidative mechanisms. Antioxidant molecules prevent formation of ODFRs or deactivate the formed free radicals (5, 25). Some researchers, have stated that endogenous antioxidant mechanisms become insufficient in the first month of fracture healing and recommended antioxidant treatment for patients with fracture (2, 24). In addition, some studies have showed that administration of exogenous antioxidants exerted positive effects on fracture healing (1, 4, 9, 15, 19, 20, 22, 23). But these studies have not examined the changes in antioxidant capacity during fracture healing. Our literature search revealed few studies on changes of antioxidant capacity in patients with fracture. In one of these studies, Prasad et al., have determined that endogenous antioxidant levels

Table 1. Serum total antioxidant levels in the control and patient groups in the first 6 hours and on 3, 5, 7, 14 days after injury

	Time	Total antioxidant levels (mmol Trolox equiv./l)		
		minimum	maximum	mean ± SD
Patient group	within 6 hours	0.30	1.40	0.71 ± 0.37
	day 3	0.20	0.40	0.33 ± 0.08
	day 5	0.30	0.80	0.46 ± 0.16
	day 7	0.20	0.40	0.31 ± 0.09
	day 14	0.20	0.40	0.29 ± 0.09
Control group		0.50	1.90	0.99 ± 0.37

p < 0.01, very significant; p < 0.05, significant; p > 0.05, non-significant. The differences between mean total antioxidant levels of the patient and control groups were significant in the first 6 hours, and very significant at the other times.

in bloods of individuals with long bone fracture were significantly increased on the 7th and 14th day after fracture compared to control group and they considered this increase as the response of organism to counteract the oxidant molecules (21).

In our study, opposite to the results reported by Prasad et al. (21) TAC of patients with long bone fractures was significantly lower within 2 weeks after fracture compared to control group. TAC was highest within 6 hours after the fracture and demonstrated a tendency to decrease except for the 5th day. Antioxidant levels were significantly elevated on the 5th day compared to all other levels excluding the first 6 hours. These findings suggest that long bone fractures negatively affect TAC of the organism. Trauma and resultant fractures create an unpleasant situation for the patient; so they are malnourished for a certain period of time. Exogenous antioxidants supplied by diet may improve the total antioxidant capacity. Malnourishment during the first few days after fracture may be one of the reasons for reduction of TAC. Some of the studies mentioned above have reported significant increase of ODFRs within one month after fracture, particularly in the 2nd and 3rd weeks. Most likely, more antioxidant molecules are consumed to deactivate these free radicals. This way of consumption of antioxidant molecules may be another reason for reduced TAC. We believe that significant increase of TAC in our patients on the 5th day indicates the effort of the organism to struggle with these free radicals. But as antioxidant molecules are rapidly consumed, this effort is short-lived and insufficient.

CONCLUSION

TAC is significantly reduced during the first 2 weeks of fracture healing. Antioxidant treatment administered during the first few weeks after fracture may support fracture healing.

References

- ALCANTARA-MARTOS, T., DELGADO-MARTINEZ, A. D., VEGA, M. V., CARRASCAL, M. T., MUNUERA-MARTINEZ, L.: Effect of vitamin C on fracture healing in elderly Osteogenic Disorder Shionogi rats. *J. Bone Jt Surg.*, 89-B: 402–407, 2007.
- CETINUS, E., KILINC, M., UZEL, M., INANC, F., KURUTAS, E. B., BILGIC, E., KARAOGUZ, A.: Does long-term ischemia affect the oxidant status during fracture healing? *Arch. Orthop. Trauma Surg.*, 125: 376–380, 2005.
- DURAK, I., KAVUTCU, M., CIMEN, M. Y. B., AVCI, A., ELGUN, S., OZTURK, H. S.: Oxidant/antioxidant status of erythrocytes from patients with chronic renal failure: Effects of hemodialysis. *Med. Princ. Pract.*, 10: 187–190, 2001.
- ERDEM, M., GULABI, D., ASCI, M., BOSTAN, B., GUNES, T., KOSEOGLU, R. D.: The effects of melatonin and caffeic acid phenethyl ester (CAPE) on fracture healing under ischemic conditions. *Acta Orthop. Traumatol. Turc.*, 48: 339–345, 2014.
- EREL, O.: A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin. Biochem.*, 37: 112–119, 2004.
- FASOLA, F., ADEAPO, K., ANETOR, J., KUTI, M.: Total antioxidants status and some hematological values in sickle cell disease patients in steady state. *J. Natl. Med. Assoc.*, 99: 891–894, 2007.
- GOKTURK, E., TURGUT, A., BAYCU, C., GUNAL, I., SEBER, S., GULBAS, Z.: Oxygen-free radicals impair fracture healing in rats. *Acta Orthop. Scand.*, 66: 473–475, 1995.
- GUZ, G., DEMIROGULLARI, B., ULUSU, N. N., DOGU, C., DEMIRTOLA, A., KAVUTCU, M., OMEROGU, S., STEFEK, M., KARASU, C.: Stobadine protects rat kidney against ischaemia/reperfusion injury. *Clin. Exp. Pharmacol. Physiol.*, 34: 210–216, 2007.
- HALICI, M., ONER, M., GUNEY, A., CANOZ, O., NARIN, F., HALICI, C.: Melatonin promotes fracture healing in the rat model. *Ekleml Hastalik. Cerrahisi*, 21: 172–177, 2010.
- ISIK, A., KOCA, S. S., USTUNDAG, B., SELEK, S.: Decreased total antioxidant response and increased oxidative stress in Behcet's disease. *Tohoku J. Exp. Med.*, 212: 133–141, 2007.
- JEYABALAN, G., TSUNG, A., BILLIAR, T. R.: Linking proximal and downstream signalling events in hepatic ischaemia/reperfusion injury. *Biochem. Soc. Trans.*, 34: 957–959, 2006.
- KARAKOC, M., ALTINDAG, O., KELES, H., SORAN, N., SELEK, S.: Serum oxidative-antioxidative status in patients with ankylosing spondylitis. *Rheumatol. Int.*, 27: 1131–1134, 2007.
- KAZZAZ, J. A., XU, J., PALAIA, T. A., MANTELL, L., FEIN, A. M., HOROWITZ, S.: Cellular oxygen toxicity. Oxidant injury without apoptosis. *J. Biol. Chem.*, 271: 15182–15186, 1996.
- KEEL, M., TRENTZ, O.: Pathophysiology of polytrauma. *Injury*, 36: 691–709, 2005.
- KESKIN, D., KARSAN, O., EZIRMIK, N., CİFTÇİOĞLU, A.: The effect of alpha-tocopherol on fracture healing in rabbits. *Arthroplasty Arthros.*, 10: 207–210, 1999.
- KORACEVIC, D., KORACEVIC, G., DJORDJEVIC, V., ANDREJEVIC, S., COSIC, V.: Method for the measurement of antioxidant activity in human fluids. *J. Clin. Pathol.*, 54: 356–361, 2001.
- KRETZSCHMAR, M., PFEIFFER, L., SCHMIDT, C., SCHIRRMESTER, W.: Plasma levels of glutathione, alpha-tocopherol and lipid peroxides in polytraumatized patients; evidence for a stimulating effect of TNF alpha on glutathione synthesis. *Exp. Toxicol. Pathol.*, 50: 477–483, 1998.
- MALLICK, I. H., WINSLET, M. C., SEIFALIAN, A. M.: Pyrrolidone dithiocarbamate protects the small bowel from warm ischaemia/reperfusion injury of the intestine: the role of haemoxygenase. *Clin. Sci.*, 111: 373–380, 2006.
- MOHAMAD, S., SHUID, A. N., MOHAMED, N., FADZILAH, F. M., MOKHTAR, S. A., ABDULLAH, S., OTHMAN, F., SUHAİMİ, F., MUHAMMAD, N., SOELAIMAN, I. N.: The effects of alpha-tocopherol supplementation on fracture healing in a postmenopausal osteoporotic rat model. *Clinics (Sao Paulo)*, 67: 1077–1085, 2012.
- PETROVICH, Y. A., PODOROZHNYA, R. P., KICHENKO, S. M., KOZLOVA, M. V.: Effects of selenium-containing compounds and their metabolism in intact rats and in animals with bone fractures. *Bull. Exp. Biol. Med.*, 137: 74–77, 2004.
- PRASAD, G., DHILLON, M. S., KHULLAR, M., NAGI, O. N.: Evaluation of oxidative stress after fractures. A preliminary study. *Acta Orthop. Belg.*, 69: 546–551, 2003.
- SANDUKJI, A., AL-SAWAF, H., MOHAMADIN, A., ALRASHIDI, Y., SHEWEITA, S. A.: Oxidative stress and bone markers in plasma of patients with long-bone fixative surgery: role of antioxidants. *Hum. Exp. Toxicol.*, 30: 435–442, 2011.
- TURK, C., HALICI, M., GUNEY, A., AKGUN, H., SAHIN, V., MUHTAROGLU, S.: Promotion of fracture healing by vitamin E in rats. *J. Int. Med. Res.*, 32: 507–512, 2004.
- YELER, H., TAHTABAS, F., CANDAN, F.: Investigation of oxidative stress during fracture healing in the rats. *Cell. Biochem. Funct.*, 23: 137–139, 2005.
- YOUNG, I. S., WOODSIDE, J. V.: Antioxidants in health and disease. *J. Clin. Pathol.*, 54: 176–186, 2001.

Corresponding author:

Prof. Dr. Davut Keskin

Department of Orthopaedics and Traumatology
Ondokuz Mayıs University, Faculty of Medicine
Samsun, Turkey

E-mail: keskind@hotmail.com