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Evaluation of the Systemic Oxidative Stress Status during Major Orthopedic Surgery in Dogs: A Clinical Study

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Abstract : The present study evaluated the systemic oxidative stress status during major orthopedic surgery in dogs. Sixteen dogs presented with various orthopedic diseases involving fractures or luxation of limbs. All patients underwent orthopedic surgery for treatment of fractures or luxation of limbs. A significant increase in the plasma total oxidant status (TOS) and oxidative stress index (OSI) levels in dogs after surgery was observed. Plasma total antioxidant status (TAS) levels were significantly decreased in dogs after surgery. The results of this study suggested that association or relationship in serum between TOS or TAS levels and redox imbalance were caused by surgical trauma in orthopedic disease conditions.

Key words **:** orthopedic surgery, oxidative stress status, total antioxidant status, total oxidant status, dogs.

Introduction

Reactive oxygen species (ROS) are a part of the normal physiology of the biological system but their subsequent defense undergoes alteration during diseased conditions. The increase in free radical or ROS production or the decrease in antioxidant mechanisms generates a condition called oxidative stress, defined as the imbalance between pro- and antioxidants in favor of the oxidants (5). Over the last several decades, it has become amply evident that oxidative stress, usually in the form of ROS, is a critical pathogenic factor in endothelial cell dysfunction and the development of diseases (5,8). Anesthesia and surgery of any kind, especially in case of orthopedic surgery, are well-recognized as oxidative stress-inducing manipulations and bleeding (2). It is well known that osteocalcin and alkaline phosphatase play significant roles in healing of bone fractures, whereas oxidative stress delays such healing. Therefore, the evaluation of oxidative stress status during surgery is an important concern for good outcome. Numerous studies have been reported oxidative stress in surgical condition in human, but the oxidative stress of surgical condition in animals has not been fully evaluated.

The purpose of this study was to evaluate of the oxidative stress status associated with major orthopedic surgery in dogs.

Material and Methods

Patients and methods

Sixteen dogs (seven intact males, four intact females, four castrated males and one spayed female) of various breeds American Society of Anesthesiologists (ASA) physical status 1 or 2 weigh 7 (2.7-27) kg [median (min-max)] and aged 5 months to 10 years presented with various orthopedic diseases involving fractures or luxation of limbs. Dogs with additional disease or hematologic and serum biochemical abnormalities were excluded. All dogs underwent orthopedic surgery for treatment of fractures or luxation of limbs. Each surgical technique performed for orthopedic conditions is described in Table 1.

Blood samples

Approximately 3 ml blood was collected into a plasma separation tube containing heparin at each designated time for analysis of oxidative stress markers. Blood samples were centrifuged at 3000 rpm for 10 min to separate plasma, and the plasma samples were stored at −80°C until analysis.

Measurement of total antioxidant status (TAS)

The TAS levels of the sera were determined using an automated measurement method based on bleaching of characteristic color of a more s 2,2'-azino-bis [3-ethylbenz-thiazoline-6-sulfonic acid (ABTS)] radical cation caused by antioxidant(3). The results are expressed in mmol Trolox equiv/L.

Measurement of total oxidant status (TOS)

The TOS levels of the sera were determined using a novel automated measurement method (4). Oxidants present in the sample oxidize the ferrous ion-o-dianisidine complexes into ferric ions. The oxidation reaction is enhanced by glycerol molecules that are abundantly present in the reaction medium. The ferric ions form a colored complex with xylenol orange

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Table 1. Clinical features in 16 dogs with orthopedic diseases

Dog No.	Breed	Gender	Age	Orthopedic condition	Surgical techniques performed
1	Poodle	FS	10y	Rt. distal femoral physeal fracture	Rush pin fixation
2	Jin do dog	F	6m	Rt. Tibial oblique fracture	Intramedullary pining
3	Dalmatian	F	5y	Rt. Proximal femoral physeal fracture	Lag screw fixation
4	Yorkshire terrier	F	9m	Lt. proximal femoral fracture	Femoral head and neck ostectomy
5	Mixed breed	M	9y	Lt. lateral luxation of the elbow	Open reduction of the elbow
6	Golden Retriever	MC	7m	Rt. Coxofemoral luxation	Femoral head and neck ostectomy
7	Mixed breed	M	3y	Rt. Patellar luxation	Transposition of the tibial tuberosity
8	Yorkshire terrier	M	7y	Rt. Patellar luxation	Recession sulcoplasty
9	Yorkshire terrier	MC	7y	Rt.& Lt. Patellar luxation	Recession sulcoplasty
10	Maltease	MC	1y	Rt. Patellar luxation	Trochlea sulcoplasty
11	Maltease	M	5m	Rt. Distal humeral fracture	Intramedullary pining
12	Cocker Spaniel	M	3y	Lt. cranial cruciate ligament rupture	Modified retinacular imbrications technique
13	Poodle	F	10y	Medial luxation of the Lt. shoulder	Surgical repair medial luxation of the Lt. shoulder
14	Maltease	M	5y	Rt. Fractures of Rt. Tibia & fibula	Intramedullary pining
15	Maltease	MC	4y	Lt. proximal femoral fracture	Lag screw fixation
16	Mixed breed	M	6y	Rt. Patellar luxation	Recession sulcoplasty

Abbreviations: FS, female spayed; F, female; M, male; MC, male castrated; y, years; m, months; Rt, right; Lt, left

in an acidic medium. Therefore, the color intensity, measured spectrophotometrically, is related to the total number of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equiv/L).

Oxidative stress index (OSI)

The ratio of TOS to TAS provided the oxidative stress index (OSI), an indicator of the degree of oxidative stress.

Statistical analysis

Data were expressed as mean ± SD, and Mann-Whitney U-test was used as appropriate. A p-value of < 0.05 was considered as significant. All statistics were performed using a computer statistical package (Statistics Package for the Social Sciences, version 19.0; SPSS Inc., IL, USA).

Results

The study presented here contained 16 patients who underwent a surgical procedure to treat repair of orthopedic conditions. Mean operation times for surgery was 98.9 ± 40.1 min. During the surgery there was no significant worsening in hemodynamic changes in all dogs. The plasma TOS, TAS and OSI are shown in Fig 1. A significant increase in the plasma TOS and OSI levels in dogs after surgery was observed. Plasma TAS levels were significantly decreased in dogs after surgery.

Discussion

The major finding of the present study was that oxidative stress parameters was significantly higher at the end of surgery compared to before induction of anesthesia in dogs. A lower TAS level and higher TOS and OSI levels were observed under surgical condition, compared with non-surgical conditions.

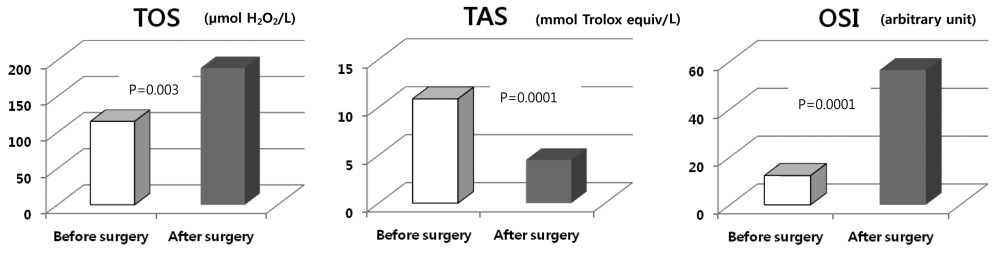

Fig 1. Oxidative stress parameters in dogs undergoing orthopedic surgery. TOS, total oxidant staus; TAS, total antioxidant staus; OSI, oxidative stress index.

The surgical/anesthesia trauma is associated with an increased production of ROS. This enhanced oxidative stress leads to cell damage resulting in various complications such as sepsis and increased mortality (8). Damage from oxygen-free radicals after reperfusion has been documented in many different tissues, including skeletal muscle. Orthopedic surgery is a good patient model for excessive production of oxidants (2). Ischemia in the extremities is associated with protein or lipid peroxidation, an autocatalytic mechanism leading to oxidative destruction of cell membranes and the production of toxic reactive metabolites and cell death. Cellular oxidative damage is a well-established general mechanism of cell and tissue injury that is primarily caused by free radicals and ROS. Low levels of ROS are indispensable in many biochemical processes (11); however, overproduction and/or inadequate removal of ROS can result in oxidative stress, which is characterized as an imbalance between the formation of active oxygen metabolites and the rate at which they are scavenged by enzymatic and non-enzymatic antioxidants. Oxidative stress can participate in the pathogenesis and complications of many diseases (11). Therefore, the evaluation of oxidative stress can indirectly reflect the changes in microcirculation in the surgical area during surgery. Different oxidative stress markers have been investigated, including nitric oxide; levels of tissue and blood malondialdehyde and total antioxidant; and levels of thiobarbituric acid reactive substances to assess lipid peroxidation, protein carbonyl content and protein sulfhydryl. In addition to these methods, lipid peroxidation has been used to reflect oxidative stress and total glutathione has been used to reflect actions against oxidative stress (9). However, the dynamic distribution of different antioxidants in various biological samples and their potential interactions make it difficult to measure each antioxidant separately, and such measurements are also unlikely to represent the overall antioxidant substances in the body. Similarly, to assess the oxidant status *in vivo*, it is essential to measure the levels of overall oxidants.

In previous studies concerning the role of antioxidants in diseases, most reports measured only one or several antioxidant substances separately (1,6,7,10), such that the results did not reflect the total antioxidant levels in patients. In the current study, we recommended measuring the total oxidant/antioxidant status of an organism. Owing to the various interactions among antioxidants and oxidants, we believe that by measuring the total effects of the oxidants/antioxidants present in an organism, we can obtain more valuable results. In our study, in addition to the TAS, we measured serum TOS levels and calculated OSI values. The limitations of this study are clear that conditions of fracture or luxation without surgical treatment can also cause oxidative injury, and this study we were unable to determine the pre-conditioning data from dogs. Furthermore, it is difficult to evaluate the appropriate reference range. However, the changes of oxidative stress parameters in blood sample reflect the systemic stress status in patients. The results of this study suggest that association or relationship in serum between TOS or TAS levels and redox imbalance were caused by surgical trauma in orthopedic diseases conditions. Therefore, increased levels of oxidants and decreased levels of antioxidants may provide evidence for patients who were exposed to potent oxidative stress.

In conclusion, the results of this study show that oxidative stress induced by orthopedic surgery in dogs. In addition, TOS, TAS and OSI could be useful biomarker in detection of systemic oxidative stress during major orthopedic surgery. However, further studies are needed to confirm the exact mechanism of oxidative stress status in surgical condition in dogs.

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개에서 정형외과 수술에 따른 전신 산화스트레스 상태의 평가

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요 약 : 본 연구는 개에서 주요 정형외과 수술에 따른 전신 산화 스트레스 상태를 평가하였다. 16 마리의 골절이나 사지의 탈구 질환을 포함하는 환축을 대상으로 연구를 실시하였고 모든 환축은 골절이나 탈구의 치료를 위해 다양한 정형외과 수술을 받았다. 수술 후 혈장 총 산화상태(total oxidant status, TOS) 및 산화스트레스 지수(oxidative stress index, OSI)에서 유의적인 증가가 확인되었다. 수술 후 혈장 총 항산화상태(total antioxidant status, TAS)에서 유의적인 감소를 확인하였다. 본 연구를 통해 정형외과 수술외상에 따른 혈장 내 총 산화 및 항산화 상태가 산화스트레스와 관련 있음을 확인 할 수 있었다.

주요어 : 정형외과 수술, 산화 스트레스, 총 항산화 상태, 총 산화 상태, 개