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Oxidative stress markers in Thoroughbred horses after castration surgery under inhalation anesthesia

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J. Equine Sci.
Vol. 27, No. 2
pp. 77–79, 2016

Oxidative stress has been reported to occur during surgery. It is important to reduce intraoperative oxidative stress to improve the postoperative prognosis. However, there are no reports regarding oxidative stress related to surgery in horses. In the present study, we measured pre and postsurgical diacron-reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP); the oxidative stress index (OSI) was then calculated (OSI=d-ROMs/BAP × 100). d-ROMs were not significantly different between the pre and postsurgical periods. However, BAP significantly decreased after surgery (P=0.02), and OSI significantly increased after surgery (P=0.02). Based on these results, it suggested that castration surgery under inhalation anesthesia decreases the antioxidant potential and causes oxidative stress in horses.

Key words: *oxidative stress, surgery, Thoroughbred*

Oxidative stress is defined as the state in which the concentration of reactive oxygen species (ROS) exceeds that of antioxidants [6, 11]. Although ROS are removed from the body by antioxidants, complete removal is not possible under conditions of excessive ROS production. The remaining ROS causes cell damage and affects various tissues. Oxidative stress has been reported to occur during surgery [11]. In humans, many factors, such as extensive invasion, bleeding, and time-consuming surgeries, have been reported to cause intraoperative oxidative stress [11]. Oxidative stress has been reported to cause depression of the immune system, prolonged wound healing, and other organ (such as the kidney and liver) damage [2, 4, 9, 11]. Therefore, it is important to reduce oxidative stress related to surgery to improve the postoperative prognosis. However, there are no reports regarding oxidative stress related to surgery in horses.

Therefore, in the present study, we evaluated pre and postsurgical oxidative stress in Thoroughbred horses and investigated whether oxidative stress was affected by surgery.

Five male Thoroughbred horses [age, 4.5 (4–5) years; weight, 476 (422–507) kg; median (range)] that underwent castration surgery at the Medical Center of Obihiro University of Agriculture and Veterinary Medicine were used in this study. All horses were riding horses, and were kept at the same farm with almost same exercise routines. The care and use of the horses complied with the local animal welfare laws, guidelines, and policies. Castration surgery was performed by the semi-closed procedure. Horses were intravenously premedicated with 5 μg/kg medetomidine hydrochloride (Domitor, Nippon Zenyaku Kogyo, Fukushima, Japan). Five mins later, 0.03 mg/kg of diazepam (Horizon, Astellas Pharma, Tokyo, Japan) was intravenously administered, followed by rapid infusions of guaifenesin (25 mg/kg; Guaifenesin, ALPS Pharmaceutical Industries, Gifu, Japan) until the horses became ataxic. After guaifenesin injections, 4 mg/kg thiamylal (Isozole, Nichiiko Pharmaceutical, Toyama, Japan) was administered. Thereafter, tracheas were intubated, and the horses were subsequently

Received: October 5, 2015
Accepted: April 6, 2016
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anesthetized by inhalation of isoflurane (Isoflu, Sumitomo Dainippon Pharma, Osaka, Japan) in oxygen. Testes were excised after 10 m*l* of lidocaine (Xylocaine Injection 2%, AstraZeneca, Osaka, Japan) was injected into the spermatic cord. Intermittent positive pressure ventilation (IPPV) was used for respiratory management during anesthesia. Serum samples were collected before inducing anesthesia and immediately before extubation after surgery. Serum samples were stored at −80°C until using assays. Management of serum samples was in accordance with the guidelines at the Miyazaki University Veterinary Teaching Hospital. After collecting all samples, we measured the diacron-reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP) in the serum samples using a free radical analyzer (Free Carpe Diem, Diacron International, Grosseto, Italy) in accordance with a previous report [3]. We calculated the oxidative stress index (OSI) from the measured d-ROMs and BAP (OSI=d-ROMs/BAP × 100). Summarized data for d-ROMs, BAP, and OSI are expressed as medians and ranges. All data were analyzed using Statcel2 (OMS Publishing Inc., Saitama, Japan). Differences between the pre and postsurgical periods were determined using the one-sided Wilcoxon signed-ranks test. The significance level was set at $P<0.05$ for all analyses.

Surgery time was defined as the time from skin incision to the end of testes extraction. Anesthesia time was defined as the time until termination of inhalation of isoflurane from induction of anesthesia. The median surgery time was 45 (40–49) min in this study. The anesthesia time was 60 min in all of horses in this study. The d-ROMs, BAP, and OSI values for the pre and postsurgical periods are presented in Table 1. There were no significant differences in d-ROMs between the pre and postsurgical periods. However, after surgery, the BAP values were significantly lower ($P=0.02$) than before surgery, and the OSI values were significantly higher ($P=0.02$) than before surgery.

High d-ROMs values indicate higher production of ROS, and high BAP values indicate higher antioxidant capacity. Both d-ROMs and BAP have been reported to be precise and reliable methods of assessing oxidative stress in horses [3, 8, 10]. Therefore, we investigated d-ROMs and BAP as oxidative stress markers in this study. Oxidative stress occurs when the balance of ROS and antioxidants favors ROS. For this reason, it is also important to evaluate the grade of oxidative stress using the OSI, which indicates the balance of d-ROMs and BAP [8]. High OSI values indicate higher oxidative stress. In this study, d-ROMs were not different between the pre and postsurgical periods. However, after surgery, BAP significantly decreased and OSI significantly increased. Based on these results, it appeared that castration surgery under inhalation anesthesia induced oxidative stress in the study horses.

Table 1. Oxidative stress markers before and after surgery

		Before surgery	After surgery
d-ROMs (U.CARR)	Median	202	199
	Range	134–245	149–238
BAP (μmol/*l*)	Median	3,509.4	2,934.4 *
	Range	3,329.2–4,174.1	2,537.4–2,990.2
OSI	Median	5.9	6.7 *
	Range	4.0–6.1	5.7–8.1

*$P<0.05$.

In this study, there was no change in d-ROMs between before and after surgery. The reason why the concentration of d-ROMs was unchanged was that only a small amount of ROS was generated during surgery. The surgery was performed in a relatively short period in this study. Thus, it is presumed that an increase in ROS production occurred during this short period and that the total amount of produced ROS was small. Indeed, Tsuchiya *et al.* reported that the small amount of ROS generated during surgery is quickly eliminated from the body and does not increase ROS; it only reduces the antioxidant capacity [11]. This is likely why the concentration of d-ROMs was unchanged and why only a reduction of BAP was confirmed in this study. In time-consuming surgeries, further depletion of antioxidants is most likely to occur. As a result, ROS that cannot be removed by the remaining antioxidants are considered to be the generated ROS, and an increase in d-ROMs may presumably occur at that stage. Moreover, in humans, other factors such as extensive invasion and bleeding have been reported to increase intraoperative oxidative stress [11]. In this study, the amounts of invasion and bleeding were relatively small. Therefore, additional studies involving time-consuming surgeries with extensive invasion and bleeding are required.

The present study involved castration surgery under inhalation anesthesia with isoflurane even though there are some reports of oxidative stress caused by inhalation anesthesia [1, 7]. Breathing management was performed via IPPV in this study. Ishizuka *et al.* reported that significant PaO_2 elevation was observed in horses during IPPV with administration of intravenous anesthesia as compared with that observed during spontaneous ventilation [5]. In the case of IPPV, the value of PaO_2 during anesthesia showed a higher value after 40 min than the baseline value [5]. Therefore, more oxygen is administered to horses via IPPV breathing management. An increased amount of oxygen in the body is thought to likely increase the production of ROS. Based on the results of this study, oxidative stress may have been caused by inhalation anesthesia in conjunction with IPPV. Further studies evaluating the effects of anesthesia on oxidative stress are necessary in the future.

This study demonstrated the possibility that surgery caused oxidative stress even with a short surgery time. It is important to increase the antioxidant capacity to decrease the oxidative stress during surgery. Administration of antioxidants, such as vitamin C, before surgery, application of procedures to improve antioxidant capacity, such as major ozonated autohemotherapy [12], and use of anesthetics with antioxidant capacity, such as propofol [1, 11], might be effective to reduce intraoperative oxidative stress.

This study was performed using healthy horses with no underlying disease. The oxidative stress of horses with underlying diseases would be higher than that of healthy ones. Therefore, the effects of oxidative stress during surgery in horses with an underlying disease (such as plastic surgery and laparotomy) are assumed to be larger.

In conclusion, castration surgery under inhalation anesthesia appears to decrease antioxidant potential, and cause oxidative stress in horses. Additional studies are necessary to evaluate surgery for other medical conditions and the impact of the oxidative stress on prognosis.

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