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Comparison of oxidative stress under different propofol administration protocols in Thoroughbred racehorses by bOS and bAP assessment

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It is desirable to reduce surgery-induced oxidative stress (OS) because it can cause immune suppression and delayed wound healing. Propofol is known to have antioxidant potential and to reduce OS in humans, but there have been no studies of this issue in horses. This study was conducted to evaluate OS under three different propofol administration protocols in Thoroughbred racehorses undergoing arthroscopic surgery with sevoflurane anesthesia. Blood oxidative stress (bOS) and blood antioxidant power (bAP) were used as OS biomarkers. Both bOS and bAP significantly decreased after surgery in all groups, but no differences in these reductions were found among them. Different propofol administration protocols with sevoflurane anesthesia did not cause a difference in OS in Thoroughbred racehorses that underwent arthroscopic surgery.

Key words: *anesthesia, oxidative stress, Thoroughbred racehorse*

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Oxidative stress (OS) is induced when the generation of reactive oxygen species (ROS) exceeds the antioxidant capacity [10]. The excessive ROS can cause cell-membrane lipid peroxidation and DNA injury and can adversely affect the body in various ways [10]. Studies of the relationship between surgery and OS in humans and dogs have shown that invasive surgery such as laparotomy or organ transplantation, or time-consuming surgery, is likely to induce OS [12, 18, 19]. In horses, surgical castration under inhalation anesthesia with isoflurane causes mild OS [20]. Surgery-induced OS can cause immune suppression and delayed wound healing and therefore needs to be minimized [5, 14]. A variety of studies have been conducted on the impact of anesthetics on OS [2, 7, 13, 19]. The intravenous anesthetic agent propofol has a chemical structure similar to that of the exogenous antioxidant α-tocopherol [1]. Therefore propofol is known to have antioxidant capacity, and there have been many clinical studies of its antioxidant capacity in humans and animals [7, 9, 12, 19]. However, as far as we know, there have been no studies evaluating the impacts of propofol on OS in horses. Here, we compared three different anesthetic protocols to evaluate the impact of propofol on OS in Thoroughbred racehorses undergoing arthroscopic surgery with sevoflurane anesthesia. Blood oxidative stress (bOS) and blood antioxidant power (bAP) were used as OS biomarkers; the use of them has recently been reported in humans [17].

The subjects were 20 Thoroughbred racehorses that had developed chip fractures of the carpal bones during training or racing and had undergone arthroscopic surgery at Miho Training Center, Japan Racing Association (Ibaraki, Japan). All horses received anti-inflammatory treatment to alleviate swelling and pain before surgery. They were fasted for 12 hr before surgery but had free access to water. We compared three groups (pP, pM, and tM), each of which received anesthesia with a different propofol administration protocol (Table 1). Medetomidine (Domitor, Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan) 6.0 μg/kg and midazolam (Dormicum, Astellas Pharma Inc., Tokyo, Japan) 0.02 mg/kg were administered to all horses for sedation. General anesthesia was induced with propofol (Propofol, Nichi-Iko Pharmaceutical Co., Ltd., Toyama, Japan) 1.0 mg/kg and

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Table 1. Anesthesia protocols in the three study groups

Group	pP	pM	tM
Sedation	Medetomidine 6.0 μg/kg + midazolam 0.02 mg/kg		
Induction	Propofol 1.0 mg/kg		Thiopental Na 4.0 mg/kg
	Ketamine 1.0 mg/kg		GGE 100 mg/kg
Inhalation	Sevoflurane		
CRI	Medetomidine 3.0 μg/kg/hr		
	Propofol 3.0 mg/kg/hr	—	

CRI, constant rate infusion; GGE, guaiacol glycerine ether.

ketamine (Ketalar, Daiichi Sankyo Propharma Co., Ltd., Tokyo, Japan) 1.0 mg/kg in the pP and pM groups and with thiopental sodium (Ravonal, Nipro ES Pharma, Osaka, Japan) 4.0 mg/kg and guaiacol glyceryl ether (5% Guaifenesin, Shinyo Pure Chemicals Co., Ltd., Osaka, Japan) 100 mg/kg in the tM group. Anesthesia was maintained with sevoflurane (Sevofrane, Maruishi Pharmaceutical Co., Ltd., Osaka, Japan) combined with 3.0 μg/kg/hr medetomidine under respiratory support with intermittent positive pressure ventilation with pure oxygen. In the pP group, propofol 3.0 mg/kg/hr was concurrently administered. In order to maintain mean arterial blood pressure at 70 mm Hg or higher, dobutamine (Dobutrex, Kyowa Pharmaceutical Industry Co., Ltd., Tokyo, Japan) was administered as needed. The patients were transferred to the recovery room after surgery and were extubated after recovering stable spontaneous breathing. Blood was collected from the jugular vein before anesthesia, at the end of surgery (at entry into the recovery room), and on the day after surgery (16 to 24 hr after surgery). Collected blood was centrifuged to obtain serum, and the serum was cryopreserved at −80°C until analysis. We used the SPOTCHEM i-Pack Oxystress Test (Arkray Inc., Kyoto, Japan) to measure bOS and bAP levels. Statistical analysis was performed by repeated measures ANOVA using the EZR 1.32 software (Saitama Medical Center, Jichi Medical University, Saitama, Japan) and multiple comparison tests by using the Bonferroni method. Differences were considered significant at $P<0.05$.

The clinical characteristics of the patients are shown in Table 2. No significant differences were found in horse age, gender, body weight, and duration of anesthesia among the three groups. However, the average end-tidal concentration of sevoflurane during surgery was significantly lower in the pP group (1.8 ± 0.1%) than in the pM and tM groups (2.5 ± 0.1% and 2.3 ± 0.2%, respectively). The average flow rate of dobutamine was significantly lower in the pP group (0.17 ± 0.05 μg/kg/min) than in the pM group (0.61 ± 0.15 μg/kg/min).

The average bOS level at the end of surgery was 10.9 ± 1.2 mg/d*l* in the pP group, 10.7 ± 1.6 mg/d*l* in the pM group, and 10.9 ± 1.5 mg/d*l* in the tM group. In all groups, the values were significantly lower than those before anesthesia and on the day after surgery ($P<0.05$) (Table 3). There were no significant among-group differences in bOS levels at any time point. The average bAP level at the end of surgery was 2,582.9 ± 218.3 μmol/*l* in the pP group, 2,527.6 ± 236.4 μmol/*l* in the pM group, and 2,739.9 ± 185.7 μmol/*l* in the tM group. In all groups, the average bAP levels were significantly lower at the end of surgery than those before anesthesia and on the day after surgery ($P<0.05$). There were no significant among-group differences in bAP levels at any time point, as was the case with bOS.

We used bOS and bAP as OS biomarkers. bOS quantifies the metabolism of ROS, and bAP quantifies antioxidant capacity. These two biomarkers have recently been reported in anti-aging medical checkups in humans [17]. On the other hand, in horses, derivatives of reactive oxygen metabolites (d-ROM) and biological antioxidant power (BAP) have recently been used as OS biomarkers [11, 20]. bOS and bAP are strongly correlated with d-ROM and BAP respectively [17]; therefore, bOS and bAP were regarded as reliable OS biomarkers in this study.

It had been expected that bOS levels at the end of surgery would differ among the three groups because different propofol administration protocols were applied to the patients in the different groups. However, bOS levels significantly decreased after surgery in all groups, and no differences in bOS reductions were found. This study therefore did not reveal that different propofol administration protocols caused a difference in OS in Thoroughbred racehorses that underwent arthroscopic surgery. The pM and tM groups, which received less propofol or no propofol, also showed decreases in bOS levels that were equivalent to those in the pP group. This result suggested that anesthetics other than propofol were also likely to contribute to the decrease in bOS levels in these groups.

The average end-tidal concentration of sevoflurane was significantly higher in the pM and tM groups than that in the pP group. It has recently been reported that sevoflurane has antioxidant capacity and reduces OS and that it does not

Table 2. Patient characteristics

Group	pP	pM	tM
No. of horses	7	7	6
Age (years)	3.0 ± 1.0	3.2 ± 0.5	2.7 ± 0.7
Gender (male/female/gelding)	5/2/0	4/2/1	4/2/0
Weight (kg)	463.6 ± 10.7	458.6 ± 30.9	439.3 ± 16.6
Duration of anesthesia (min)	63.9 ± 7.8	63.1 ± 12.4	70.0 ± 12.3
Sevoflurane (%)[1]	1.8 ± 0.1[a]	2.5 ± 0.1	2.3 ± 0.2
Dobutamine (μg/kg/min)[2]	0.17 ± 0.05[b]	0.61 ± 0.15	0.38 ± 0.34

Values are expressed as means ± standard deviation. [a]P<0.05 compared with the pM and tM groups; [b]P<0.05 compared with the pM group. [1]Average end-tidal concentration; [2]Average flow rate.

Table 3. Blood oxidative stress (bOS) and blood antioxidant power (bAP) values at each point

Parameter	Group	Preanesthesia	End of surgery	Day after surgery
bOS (mg/d*l*)	pP	12.8 ± 2.1	10.9 ± 1.2*	12.0 ± 1.4
	pM	12.4 ± 1.7	10.7 ± 1.6*	11.8 ± 1.4
	tM	12.9 ± 1.9	10.9 ± 1.5*	12.7 ± 2.0
bAP (μmol/*l*)	pP	2,979.6 ± 229.2	2,582.9 ± 218.3*	2,747.1 ± 232.8
	pM	2,749.6 ± 215.8	2,527.6 ± 236.4*	2,709.0 ± 176.1
	tM	2,870.7 ± 296.5	2,739.9 ± 185.7*	2,904.4 ± 360.4

Values are expressed as means ± standard deviation. *Compared with preanesthesia and day after surgery, P<0.05. bOS, blood oxidative stress; bAP, blood antioxidant power.

affect DNA damage in patients who have undergone minimally invasive surgery [4, 6, 13]. Moreover, there have been some studies comparing propofol and sevoflurane in terms of their antioxidant capacity. Sevoflurane was reported to provide greater protection against myocardial oxidative stress than propofol in human coronary surgery [3], but other studies have reported that the antioxidant capacity of propofol was superior to sevoflurane [2, 19]. Thus, it is still controversial which anesthetic has more antioxidant capacity, but the fact that the pM and tM groups showed decreases in bOS levels that were equivalent to those in the pP group suggested that sevoflurane may have covered the antioxidant potential in these two groups on behalf of propofol.

The average flow rate of dobutamine in the pM group was significantly higher than that in the pP group. A single high-dose subcutaneous injection of dobutamine was reported to increase superoxide dismutase and catalase activities, modulating oxidative stress in diabetic rats [15]. On the other hand, continuous intravenous administration of dobutamine (2.5 μg/kg/min or higher) does not ameliorate cardiovascular oxidative stress in pigs [16]. Although there was a significant difference in the average flow rate of dobutamine between the pP and pM groups, the flow rates were very low (0.17 ± 0.05 μg/kg/min and 0.61 ± 0.15 μg/kg/min, respectively). Therefore dobutamine administration was considered to not affect OS in this study.

Other than the above drugs, there was a difference in the anesthetics administered for induction among the groups. Ketamine and thiopental were reported to have less antioxidant capacity than propofol [9, 12], and there are no studies referring to the antioxidant potential of guaiacol glyceryl ether; therefore, their impacts on OS were not considered in this study.

Considering the above things, the inhalation of sevoflurane was likely to cover the antioxidant potential in the pM and tM groups and result in no differences in bOS after surgery among the three groups with different propofol administration protocols.

The dose rates of medetomidine for sedation and constant rate infusion during maintenance of anesthesia were equal in all horses. The α-2 adrenergic receptor agonist dexmedetomidine, which is used mainly as a sedative in humans, reportedly has antioxidant capacity similar to that of propofol [7]. To our knowledge, there have been no previous studies describing the antioxidant potential of medetomidine, but the antioxidant capacity of dexmedetomidine has thus been demonstrated; therefore, the α-2 adrenergic receptor agonist medetomidine might have exhibited antioxidant capacity. Midazolam was also administered to all horses for sedation. However, midazolam has less antioxidant capacity than propofol and dexmedetomidine [7] and does not affect OS at clinically relevant concentrations [8]; therefore, the impact of midazolam on OS was not considered.

bAP levels also significantly decreased after surgery in all groups, with no among-group differences. A decrease of antioxidant capacity typically indicates the consumption of antioxidants to scavenge ROS; therefore, ROS levels commonly increase or remain at baseline when antioxidant capacity decreases. However, bOS levels also decreased even though bAP decreased in this study. Therefore the decrease in bAP levels was unlikely to have been attributable to the consumption of antioxidants. The reason why bAP decreased after surgery was unclear, and further study is required to clarify this issue.

The results in this study indicated that different propofol administration protocols with sevoflurane anesthesia did not cause a difference in OS in Thoroughbred racehorses that underwent arthroscopic surgery. Further study is needed to identify the impact of anesthetics on OS in horses.

References

1. Aarts, L., van der Hee, R., Dekker, I., de Jong, J., Langemeijer, H., and Bast, A. 1995. The widely used anesthetic agent propofol can replace α-tocopherol as an antioxidant. *FEBS Lett.* **357**: 83–85. [Medline] [CrossRef]
2. Allaouchiche, B., Debon, R., Goudable, J., Chassard, D., and Duflo, F. 2001. Oxidative stress status during exposure to propofol, sevoflurane and desflurane. *Anesth. Analg.* **93**: 981–985. [Medline] [CrossRef]
3. Ballester, M., Llorens, J., Garcia, A. J., Perez, G. J., Tebar, E., Martinez, L. J., Belda, J., Juez, M. 2011. Myocardial oxidative stress protection by sevoflurane vs. propofol: a randomized controlled study in patients undergoing off-pump coronary artery bypass graft surgery. *Eur. J. Anaesthesiol.* **28**: 874–881. [Medline]
4. Bedirli, N., Demirtas, C.Y., Akkaya, T., Salman, B., Alper, M., Bedirli, A., and Pasaoglu, H. 2012. Volatile anesthetic preconditioning attenuated sepsis induced lung inflammation. *J. Surg. Res.* **178**: e17–e23. [Medline] [CrossRef]
5. Cruzat, V.F., Krause, M., and Newsholme, P. 2014. Amino acid supplementation and impact on immune function in the context of exercise. *J. Int. Soc. Sports Nutr.* **11**: 61. [Medline] [CrossRef]
6. Dal Molin, S.Z., Kruel, C.R., de Fraga, R.S., Alboim, C., de Oliveira, J.R., and Alvares-da-Silva, M.R. 2014. Differential protective effects of anaesthesia with sevoflurane or isoflurane: an animal experimental model simulating liver transplantation. *Eur. J. Anaesthesiol.* **31**: 695–700. [Medline] [CrossRef]
7. Han, C., Ding, W., Jiang, W., Chen, Y.U., Hang, D., Gu, D., Jiang, G., Tan, Y., Ge, Z., and Ma, T. 2015. A comparison of the effects of midazolam, propofol and dexmedetomidine on the antioxidant system: a randomized trial. *Exp. Ther. Med.* **9**: 2293–2298. [Medline] [CrossRef]
8. Hata, M., Kobayashi, K., Yoshino, F., Yoshida, A., Sugiyama, S., Miyamoto, C., Tokutomi, F., Maehata, Y., Wada-Takahashi, S., Takahashi, S.S., Komatsu, T., Yoshida, K., and Lee, M.C. 2011. Direct assessment of the antioxidant properties of midazolam by electron spin resonance spectroscopy. *J. Anesth.* **25**: 765–769. [Medline] [CrossRef]
9. Khoshraftar, E., Ranjbar, A., Kharkhane, B., Tavakol Heidary, S., Gharebaghi, Z., and Zadkhosh, N. 2014. Antioxidative effects of propofol vs. ketamin in individuals undergoing surgery. *Arch. Iran Med.* **17**: 486–489. [Medline]
10. Kirschvink, N., de Moffarts, B., and Lekeux, P. 2008. The oxidant/antioxidant equilibrium in horses. *Vet. J.* **177**: 178–191. [Medline] [CrossRef]
11. Kusano, K., Yamazaki, M., Kiuchi, M., Kaneko, K., and Koyama, K. 2016. Reference range of blood biomarkers for oxidative stress in Thoroughbred racehorses (2–5 years old). *J. Equine Sci.* **27**: 125–129. [Medline] [CrossRef]
12. Lee, J.Y. 2012. Oxidative stress due to anesthesia and surgical trauma and comparison of the effects of propofol and thiopental in dogs. *J. Vet. Med. Sci.* **74**: 663–665. [Medline] [CrossRef]
13. Lee, Y.M., Song, B.C., and Yeum, K.J. 2015. Impact of volatile anesthetics on oxidative stress and inflammation. *BioMed Res. Int.* **2015**: 242709. [Medline]
14. Meephansan, J., Rungjang, A., Yingmema, W., Deenonpoe, R., and Ponnikorn, S. 2017. Effect of astaxanthin on cutaneous wound healing. *Clin. Cosmet. Investig. Dermatol.* **10**: 259–265. [Medline] [CrossRef]
15. Mert, T., Oksuz, H., Tugtag, B., Kilinc, M., Sahin, E., and Altun, I. 2015. Anti-hypernociceptive and anti-oxidative effects of locally treated dobutamine in diabetic rats. *Pharmacol. Rep.* **67**: 1016–1023. [Medline] [CrossRef]
16. Rosário, A.L., Park, M., Brunialti, M.K., Mendes, M., Rapozo, M., Fernandes, D., Salomão, R., Laurindo, F.R., Schettino, G.P., and Azevedo, L.C.P. 2011. SvO(2)-guided resuscitation for experimental septic shock: effects of fluid infusion and dobutamine on hemodynamics, inflammatory response, and cardiovascular oxidative stress. *Shock* **36**: 604–612. [Medline] [CrossRef]
17. Sato, K., Yagi, M., and Yonei, Y. 2015. A new method for measuring oxidative stress using blood samples. *Glycative Stress Res.* **2**: 15–21.
18. Shi, S., and Xue, F. 2016. Current antioxidant treatments in organ transplantation. *Oxid. Med. Cell. Longev.* **2016**: 8678510. [Medline] [CrossRef]
19. Tsuchiya, M., Sato, E.F., Inoue, M., and Asada, A. 2008. Open abdominal surgery increases intraoperative oxidative stress: can it be prevented? *Anesth. Analg.* **107**: 1946–1952. [Medline] [CrossRef]
20. Tsuzuki, N., Sasaki, N., Kusano, K., Endo, Y., and Torisu, S. 2016. Oxidative stress markers in Thoroughbred horses after castration surgery under inhalation anesthesia. *J. Equine Sci.* **27**: 77–79. [Medline] [CrossRef]