

# Effects of Vitamin C on Oxidative Stress Due to Anesthesia and Surgical Trauma in Dogs

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Abstract: The study evaluated the antioxidant effects of ascorbic acid (AA) against oxidative stress during laparotomy in dogs under general anesthesia using isoflurane. Twelve dogs were randomly assigned to either the ascorbic acid group (AAG) or the sham group (SG). AAG received intravenous dosage of 100 mg/animal of standard AA 10 minutes before anesthesia. Plasma levels of cortisol, glucose, total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI) were measured. Cortisol level increased significantly with time in both groups (p < 0.05). The change of glucose levels was not significantly different between both groups. TOS and OSI increased significantly with time in the SG (p < 0.05), whereas it did not significantly change with time in the AAG. The TOS and OSI of the AAG were significantly lower than in the SG (p < 0.05). However, TAS did not significantly change between both groups. The results suggest that the decrease of TOS in the AAG might be related to the conversion of antioxidants to oxidants. The decrease of OSI indicates that the decrease of reactive oxygen species (i.e., oxidative stress) produced at the site of injury is able to decrease surgical side effects of abnormal blood circulation, organ failure, and inflammation. Therefore, AA can be used to protect a surgical patient from oxidative stress in canine laparotomy.

Key words: laparotomy, ascorbic acid, antioxidant, cortisol, dog.

#### Introduction

Most surgery can lead to tissue injury and organ dysfunction by lipid, protein, and DNA damage of direct cellular injury (7,8,26,31). Moreover, cellular injuries result in oxidative stress. Therefore, oxidative stress probably plays a key role in the development of organ failure (31).

Oxidative stress is defined as the offensive phenomenon of reactive oxygen species (ROS, also designated as free radicals, free radical oxygen or noxious oxygen) that produces cellular and organ breakdown (7). ROS results from exposure to a variety of factors including 2% noxious oxygen, stress, ultraviolet radiation, environmental pollution, and abnormal blood circulation (17).

ROS are found in most organ systems and have an important role in various physiological and pathophysiological processes such as inflammation (8,16), various diseases (28), aging, and carcinogenesis. The extracellular fluxion of ROS as a surgical side effect produces inflammation of cell tissue.

There are two strategies to decrease ROS-mediated post-surgical detriments: detoxification and prevention of formation of locally produced oxygen radicals. The ROS detoxification strategy involves the use of antioxidants, while prevention of

ROS formation involves minimizing surgery-mediated oxida-

tive stress (29). In situations of most surgery and common illness, a redistribution of antioxidants occurs to tissues or organs in need. Consequently, the depletion of antioxidant storage can be deleterious in the body when oxidative stress is prolonged (4,31). Antioxidants can be supplied to reduce the production of ROS arising from tissue damage.

As an antioxidant, ascorbic acid (AA) is one of the most efficient scavengers (5,7,31). It is transformed into dehydroascorbic acid by removal of the hydrogen from the enediol group of AA by the hydrogen transfer system. However, there are no reports on the effects of AA on antioxidant activities in canine laparotomy.

Therefore, the aim of this study was to evaluate the antioxidant effects of AA against oxidative stress during laparotomy in dogs.

#### Materials and Methods

# Animals

Twelve clinically healthy beagles with a mean weight of 8.7 kg (8-10 kg) were used in the experiments. Dogs were randomly assigned to the sham control group (SG, n = 6) and ascorbic acid treated group (AAG, n = 6). They underwent physical, blood and radiographic examinations to ensure their health. Food, a standard commercial dry canine food (Science Diet® Adult, Hill's Pet Nutrition Inc., USA) was supplied twice daily. Water was supplied as desired. The dogs were fasted for

<sup>1</sup>Corresponding author. E-mail: mckim@cnu.ac.kr 12 hours prior to surgery to prevent any possible adverse effects associated with anesthesia. These experimental and housing protocols were approved by the Chungnam National University Animal Care and Use Committee (Approval No. CNU-00043).

#### Anesthesia and drug administrations

The anesthesia was inducted and maintained with 2% isoflurane under pure oxygen using a face mask while the dog was restrained by an assistant. AA (Vitamin inj®, Dai Han Pharm, Korea; 100 mg, IV) was given prior to the induction of anesthesia. The sham control dogs were given 0.9% saline. The gas mixture (100% oxygen and 2% isoflurane) was administered for 1 hour. The dogs were premedicated with anticholinergic atropine sulfate (Atropine sulfate inj®, Je II Pharm. Korea; 0.04 mg/kg, SC), meloxicam (Metacam®, Boehringer Ingelheim, Korea; 0.2 mg/kg, IM), and cefazolin sodium (Cefazolin inj®, Chong Kun Dang Pharm, Korea; 20 mg/kg, IV). The heart rate (HR), respiratory rate (RR), oxygen saturation (SpO<sub>2</sub>), and rectal temperature (RT) were measured and recorded using a Pulscan-Component patient monitor (Scionic, Korea).

# Surgical procedure and postoperative care

Laparotomy was performed by midline incision. During the surgical operation, the dogs were given IV fluid (Hartmann's solution, 10 ml/kg/h). After opening of the abdominal wall, the organs were observed closely and internal organs (viscera, stomach, liver, and kidney) were cautiously touched by the operator in 10 minutes. Suturing was done by a common method. Anesthetic gas supply was stopped and 100% oxygen was given to enable recovery from anesthesia. The oxygen supply was maintained until the end of the procedure. All dogs received postoperative care. Antibiotics (cefazolin, 20 mg/kg, IV, bid) and ranitidine hydrochloride (Ranitidine Sinil inj®, A Ju Pharm; 1 mg/kg, IV, bid) were administrated for 7 days, and antibiotic cream was applied to the middle line area once daily for 13 days.

## Blood and plasma collection

Blood samples (3 ml) were collected from the cephalic vein at each designated time. The samples were centrifuged at 3,000 rpm for 10 minutes to separate plasma and the plasma samples were stored at -80°C until analysis. Glucose, cortisol, total oxidant status (TOS), and total antioxidant status (TAS) were determined at the pre-operation (baseline), end of the operation (0 hour: 0 h) and 1-hour postoperatively (1 h).

#### Glucose and cortisol

Serum sample data containing glucose (GLU) was obtained using a VetTest 8008 blood chemistry analyzer (IDEXX, USA). Cortisol data was obtained using an Immulite 1000 photometric immunological analyzer (Siemens Medical Solutions, USA).

#### TOS, TAS and OSI assays

TOS and TAS levels for the evaluation of oxidative stress were determined using a commercially available kit developed by Erel (Mega Tip; REL Assay Diagnostics, Turkey) (13). The percentage ratio of TOS to TAS gave the OSI, an indicator of the degree of oxidative stress (6,13).

## Statistical analysis

Data are expressed as mean  $\pm$  SD, and 2-way repeated measures analysis (ANOVA) was used as appropriate. A p-value < 0.05 was considered significance. All statistics were performed using a computer statistical package (Statistics Package for the Social Sciences, version 17.0; SPSS, USA).

# Results

# Vital signs

HR, RR, SpO<sub>2</sub>, and RT data are summarized in Table 1. These data were not significantly different between both groups.

HR in both groups was greatly increased as compared to that before the experiment. In the AAG, HR at 5 (p = 0.001), 10

**Table 1.** Vital signs (heart rate, respiratory rate, SpO2, and rectal temperature) after isoflurane anesthesia in dogs treated with ascorbic acid (AA) or saline (sham)

	Group	Pre	5 min	10 min	20 min	30 min	40 min	50 min	60 min
	Group	110	<i>y</i> mm	10 11111	20 111111	30 mm	40 IIIII	50 mm	00 111111
Heart rate (beats/min)	AAG	$86.2 \pm 6.9$	$151.3 \pm 25.8^*$	$140.7 \pm 29.0^*$	$136.2 \pm 24.1^*$	$147.3 \pm 17.6^*$	$148.2 \pm 12.5^*$	$144.5 \pm 11.3^*$	$145.0 \pm 17.0^*$
	SG	$80.8 \pm 8.2$	$131.7 \pm 13.3^*$	$138.7 \pm 28.5^*$	$134.7 \pm 22.9^*$	$139.0 \pm 27.4^*$	$130.0 \pm 22.7^*$	$120.7 \pm 19.1$	$118.3 \pm 17.9$
Respiratory rate (beats/min)	AAG	$21.0 \pm 3.29$	$10.3 \pm 2.07^*$	$10.0 \pm 3.52^*$	$10.7 \pm 4.50^*$	$17.7 \pm 11.88$	$11.5 \pm 2.43$	$12.5 \pm 3.51$	$13.3 \pm 6.56$
	SG	$21.3 \pm 4.682$	$20.7 \pm 14.95$	$18.3 \pm 11.08$	$18.8\pm10.63$	$12.8 \pm 9.13$	$23.8 \pm 21.74$	$22.6 \pm 18.15$	$20.3 \pm 12.21$
$\mathrm{SpO}_2$	AAG	$94.8 \pm 4.71$	$96.5\pm2.88$	$95.8 \pm 2.04$	$97.0 \pm 1.55$	$96.0 \pm 2.83$	$96.3 \pm 2.94$	$95.5 \pm 1.05$	$95.0 \pm 2.53$
	SG	$96.5 \pm 4.28$	$97.7 \pm 2.88$	$98.3 \pm 1.37$	$96.5\pm1.87$	$98.0 \pm 1.90$	$97.2\pm1.83$	$97.5 \pm 2.74$	$97.5 \pm 2.74$
Rectal temperature (°C)	AAG	$38.3 \pm 0.26$	N.E	N.E	$37.2 \pm 0.59^*$	N.E	$36.5\pm0.68^*$	N.E	$36.3 \pm 0.23^*$
	SG	$38.0 \pm 0.97$	N.E	N.E	$36.9 \pm 0.84$	N.E	$36.1 \pm 1.33$	N.E	$35.7 \pm 1.28^{\ast}$

Data are expressed as mean  $\pm$  SD (n = 6). SG: Sham group, AAG: Ascorbic acid group.

\*Significantly different (p < 0.05) from the baseline.

N.E: Not examined.

(p = 0.006), 20 (p = 0.017), 30 (p = 0.001), 40 (p = 0.001), 50 (p = 0.003), and 60 minutes (p = 0.002) was significantly different in comparison with that before the experiment. In the SG, HR was significantly different from baseline at 5 (p = 0.034), 10 (p = 0.008), 20 (p = 0.018), 30 (p = 0.008), and 40 minutes (p = 0.042).

RR decreased after the administration of AA in AAG, and it was significantly lower than the baseline at 5 (p = 0.017), 10 (p = 0.012), and 20 minutes (p = 0.024). RR showed the normal range in the SG. SpO<sub>2</sub> was not significantly different in both groups.

RT decreased after administration of AA or saline in both groups, and was significantly lower than the baseline at 20 (p = 0.006), 40 (p = 0.004), and 60 minutes (p = 0.039) in AAG, and at 60 minutes (p = 0.020) in SG. Mean operation time was 30 minutes.

#### Cortisol and glucose

The mean cortisol levels did not change significantly in both groups, but were significantly increased in both groups compared with pre-operation (baseline values) at the end of post-

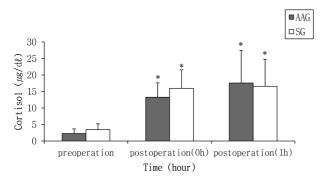


Fig 1. Cortisol values before and after surgery in dogs treated with ascorbic acid (AA) or saline (sham).

Data are expressed as mean  $\pm$  SD (n = 6).

SG: Sham group, AAG: Ascorbic acid group.

\*Significantly different (p < 0.05) from the baseline.

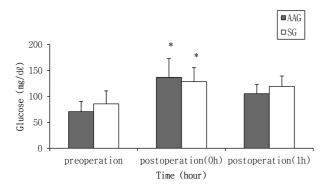


Fig 2. Glucose values before and after surgery in dogs treated with ascorbic acid (AA) or saline (sham).

Data are expressed as mean  $\pm$  SD (n = 6).

SG: Sham group, AAG: Ascorbic acid group.

Significantly different (p < 0.05) from the baseline.

operation (0 h) and postoperatively (1 h) (SG, p = 0.007 and 0.005; AAG, p = 0.027 and 0.003, respectively) (Fig 1).

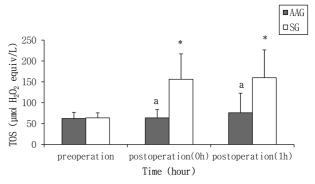
The change of mean glucose levels did not represent a significant difference between both groups. But, it was significantly increased respectively in both groups compared with the preoperation levels at 0 h (SG, p = 0.026; AAG, p = 0.002) (Fig 2).

#### TOS, TAS and OSI

TOS levels in serum were increased significantly compared with the pre-operation levels in SG at 0 h and 1 h (p = 0.027 and 0.021, respectively). But, in AAG, no significant difference was evident. TOS between both groups was significantly different at 0 h and 1 h (p = 0.012 and p = 0.028, respectively) (Fig 3).

TAS levels in serum were decreased from pre-operation levels in SG, but were not significantly different. No significant difference was evident between both groups (Fig 4).

OSI was increased from the pre-operation levels in both groups. In SG, OSI represented a significant difference com-



**Fig 3.** Total oxidant status (TOS) before and after surgery in dogs treated with ascorbic acid (AA) or saline (sham).

Data are expressed as mean  $\pm$  SD (n = 6).

SG: Sham group, AAG: Ascorbic acid group.

<sup>a</sup>Significantly different (p < 0.05) from the control group.

\*Significantly different (p < 0.05) from the baseline.

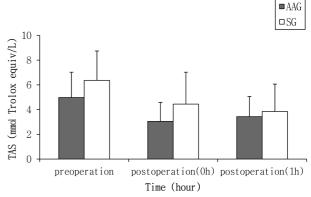
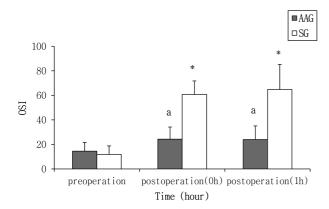


Fig 4. Total antioxidant status (TAS) before and after surgery in dogs treated with ascorbic acid (AA) or saline (sham). Data are expressed as mean  $\pm$  SD (n = 6).

SG: Sham group, AAG: Ascorbic acid group.



**Fig 5.** Oxidative stress index (OSI) before and after surgery in dogs treated with ascorbic acid (AA) or saline (sham). Data are expressed as mean  $\pm$  SD (n = 6). SG: Sham group, AAG: Ascorbic acid group. aSignificantly different (p < 0.05) from the control group.

\*Significantly different (p < 0.05) from the baseline.

pared with pre-operation levels at 0 h and 1 h (SG, p = 0.012 and 0.013, respectively). The OSI was significantly different between both groups at 0 h and 1 h (AAG, p = 0.031 and p = 0.041, respectively) (Fig 5).

#### Discussion

Laparotomy leads to tissue injury and organ dysfunction by lipid, protein, and DNA damage because of direct cellular injury as a result of incision and handling. The use of antioxidants is a means to reduce oxidative stress in laparotomy.

We used ascorbic acid (AA) as an antioxidant to reduce oxidative stress in this study. The use of AA has been reported in several studies (9,14). The effects of vitamin C among antioxidants in renal transplant recipients have been reported (9,20), as was the use of AA in heart transplant recipients (3,25). Other studies of AA used as protective agents also reported about treatment of reperfusion injury in the gastrointestinal tract (15,27).

In this study, a dosage of AA of 100 mg per animal was used for the treatment of oxidative stress, because of documented use of 100-500 mg per animal for adjunctive treatment of some diseases in dogs (23).

In this study, HR, RR, SpO<sub>2</sub>, and RT as general vital signs did not vary significantly regardless of vitamin treatment. The decrease of RT during surgery especially leads to a decrease of blood circulation and an increase of tissue damage, and can be fatal. Studies concerning the variation of body temperature by vitamin C dose in rabbits reported that vitamin C can prevent hypothermia in anesthesia (11,12). But, the RT decrease could not be lessened by AA treatment in dogs.

During surgery, increased plasma concentrations of cortisol were evident within several minutes of the start of surgery. The cortisol and glucose result observed in this study are quite similar to aspect of surgical stress hormone response in a gynecologic surgery study (24), and is also similar in duration of

etomidate-induced adrenocortical suppression during surgery in dogs (10). The cortisol and glucose levels between AAG and SG did not change significantly, and the glucose levels of both groups were increased compared with the pre-operative levels. However, in both groups, cortisol increased significantly compared with the pre-operative levels (p < 0.05). Similar results have been reported concerning neuroendocrine stress response in patients undergoing benign ovarian cyst surgery by laparoscopy, minilaparotomy and laparotomy (21).

Accordingly, surgical stress presently increased the cortisol and glucose. Moreover, glucose levels in AAG was higher than in SG after surgery, because AA is similar in structure to glucose and many animals convert the intermediate gluconic acid to vitamin C (12).

In this study, the increased pattern of TOS in SG was higher than that apparent post-operatively following laparoscopic cholecystectomy and pediatric patients undergoing laparoscopic surgery in previous studies (2,18). The TOS of SG was increased significantly compared with the pre-operative level (p < 0.05). However, it was not increased significantly, in contrast to an earlier study (18). Therefore, the change of TOS may differ according to the surgical operative method.

TOS of AAG was increased insignificantly, but was still much lower than in SG. The difference between the groups was significant (p < 0.05). The low TOS in AAG could mean that the AA supply was used as much as endogenous antioxidant consumption to reduce oxidative stress during surgery.

The decreased TAS of both groups was generally affected by oxidative stress during surgery. Theoretically, the decrease of TAS should be beneficial to eliminate oxidative stress. In proportion to treatment dosage of AA antioxidant, TAS can obtain various outcomes (19,30). In this study, the TAS levels were not significantly decreased between both groups. Immediately after surgery, the change of TAS in AAG was most likely caused by the efficacy of a small dosage of AA.

The antioxidant effect of AA treatment has been reported (1,22,26). The increase of TOS and OSI indicates an increase of oxidative stress throughout laparotomy. As a consequence, the low TOS and OSI apparent in AAG indicated that AA has an antioxidant effect compared with SG in this study. Therefore, AA was able to decrease tissue damage by oxidative stress during surgery. Consequently, AA decreases affected markers of oxidation, inflammatory stress, and operative stress during surgery, supporting its use to minimize surgical side effects.

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# 개 마취와 수술 창상에 따른 산화스트레스에 대한 비타민 C의 효과

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요 약:이 연구는 개에서 isoflurane을 이용한 전신마취 하에 개복술시 아스코르빈산 (AA)의 항산화 효과를 평가하였다. 12마리의 개를 아스코르빈산 그룹 (AAG) 또는 sham 그룹 (SG)으로 무작위 배정하였다. AAG는 마취 10분 전에 표준 아스코르빈산의 100 mg/animal 복용량을 정맥주사하였다. 현장에 있는 코티졸, 포도당, total oxidant status (TOS), total antioxidant status (TAS)와 oxidative stress index (OSI)를 측정하였다. 코티졸 수치는 두 그룹에서 시간이 지남에 따라 유의성있게 증가하였다 (p < 0.05). 포도당 수치의 변화는 두 그룹 사이에 유의성이 없었다. TOS와 OSI는 SG에서 시간이 지남에 따라 유의성 있게 증가하였다 (p < 0.05). 그런데 AAG에서는 유의성있는 변화가 없었다. 그리고, AAG의 TOS와 OSI는 SG에서 보다 더 유의성있게 낮았다 (p < 0.05). 그러나 TAS는 두 그룹 사이에 유의성이 있는 변화가 없었다. 이런 결과는 AAG에서 TOS 감소가 항산화제에서 산화제로의 변환과 관련 있음을 예측해 볼 수 있게 한다. OSI 감소는 손상부위에 발생한 활성산소 (ROS, 즉 산화 스트레스)의 감소가 혈액순환 이상, 기관 부전 및 염증의 수술 부작용을 줄일 수 있다는 것을 의미한다. 그러므로, AA는 개의 개복술에서 산화 스트레스로 부터 수술환자를 보호하기 위하여 사용될 수 있다.

주요어 : 개복술, 아스코르빈산, 항산화제, 코티졸, 개