

# Vitamin C pre-medication enhances the anaesthetic effect of ketamine-xylazine combination in the rat

Alireza Najafpour<sup>1</sup>, Goudarz Sadeghi-Hashjin<sup>2</sup>

<sup>1</sup>Department of Clinical Studies, Faculty of Veterinary Medicine, Islamic Azad University Urmia Branch, Urmia, Iran

<sup>2</sup>Division of Pharmacology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

**Submitted:** 9 June 2007

**Accepted:** 15 November 2007

Arch Med Sci 2007; 3, 4: 340-343

Copyright © 2007 Termedia & Banach

**Corresponding author:**

Dr. Alireza Najafpour

Faculty of Veterinary Medicine

Islamic Azad University Urmia

Branch

P.O. Box 969, Urmia 57153, Iran

Phone: +98 914 4415097

Fax: +98 441 3460980

E-mail: anajafpour@iaurmia.ac.ir

## Abstract

**Introduction:** This experimental study was designed to study the effects of ascorbic acid (vitamin C = VitC) for premedication on ketamine/xylazine anaesthesia in the rat.

**Material and methods:** Fifty albino laboratory rats (Sprague-Dawley) of either sex were divided into five groups (A, B, C, D and E) of ten rats in each. Rats in groups C, D and E were pre-treated with 50, 100 and 150 mg/kg, respectively, of VitC. The animals in group A (control) were treated neither with VitC nor ketamine/xylazine. A combination of ketamine 5% (40 mg/kg) and xylazine 2% (5 mg/kg) was administered intraperitoneally (I.P.) to all groups except group A. The onset and duration of hypnosis as well as vital signs were observed and recorded.

**Results:** There was a significant ( $p < 0.01$ ) decrease in the onset and increased duration of anaesthesia in the animals treated with medium (group D) and high (group E) doses of VitC. Heart and respiratory rates were decreased in all anaesthetized animals, irrespective of the presence or absence of the pre-treatment and dose of vitamin C.

**Conclusions:** VitC at 150 mg/kg administration prior to ketamine/xylazine treatment could be used to decrease the time needed to induce and to increase the duration of anaesthesia in rats. Application of VitC as a premedication agent in clinical practice, therefore, might be of interest in human and animal patients to use a lower dose of anaesthetics and, therefore, to minimize their side effects.

**Key word:** ascorbic acid, vitamin C, premedication, ketamine, xylazine, rat, general anaesthesia.

## Introduction

Ascorbic acid (vitamin C = VitC) is known to be highly concentrated in the brain [1, 2]. In spite of several studies, however, the actual physiological role of VitC in the normal function of the CNS remains unclear. It exerts a modulating influence on the CNS either physiologically or pharmacologically. High levels of VitC may have properties similar to those of amphetamine in CNS depression. Impairment of CNS functions has been reported to occur after deficiency of this water-soluble vitamin [1-4]. High doses of VitC have been reported to induce sleep disturbances, headache and gut upset [1-3].

The rat is widely used as a laboratory animal for experimental surgery. This animal species is prone to be easily stressed by improper pre-operative handling and/or induction of anaesthesia, especially with volatile anaesthetics.

Combined effects of stress and anaesthesia can result in cardio-respiratory arrests [5-7]. A prolonged loss of appetite leads frequently to postoperative complications such as GI disturbances in the rat [8]. One example is the combination of an  $\alpha_2$ -adrenoceptor agonist (such as xylazine) with an anaesthetic agent (such as ketamine) [5, 6, 9, 10]. Xylazine is characterized by sedative and muscle relaxant effects and short-time analgesia. In higher doses, ketamine induces perfect immobilization [6, 8]. When ketamine is used as a sole anaesthetic agent, it tends to cause hypertonus, poor muscle relaxation, persistent pain reflex responses and violent recovery from anaesthesia. Xylazine may counteract these undesirable side effects and the combination has been reported to produce a desirable anaesthesia in the rat, although with some mortality [5, 6].

The role of VitC in affecting general anaesthesia is not completely understood. A very old article in Russian, written by Ivanovskaia [11], suggested a role for this chemical in diethyl ether-induced anaesthesia. Since then, with some exceptions, this work has been ignored and no real attempts have been carried out to investigate its interaction with more modern anaesthetic agents. Based on what has been mentioned, this study was designed to determine the effects of VitC, as a modulator of CNS functions, on ketamine/xylazine anaesthesia in rats. We hypothesized that a VitC-induced disruption of CNS function could influence the neuroleptanaesthesia by ketamine/xylazine combination. In this case, the idea could be extended to clinical conditions, where a decreased dose of anaesthetic is of great importance to the health of a patient.

## Material and methods

### Animals

In the trial, 50 adult albino laboratory rats (Sprague-Dawley, 20 males and 30 females) were used. Animals were 10-12 months old with weights ranging from 320 to 460 g. They had free access to tap water and commercial chow. Clinical examination included examination of the size of submandibular and popliteal lymph nodes by palpation and the fur, the colour of the mucosa, auscultation of the heart and lung sounds, and palpation of the abdominal cavity prior to inclusion in the trial and immediately before anaesthesia [8]. The animals were clinically healthy. They were randomly divided into 5 groups of ten rats each (A, B, C, D, and E) and housed in the animal house of the Faculty of Veterinary Medicine, Urmia Branch of Islamic Azad University. The rats were allowed to acclimatize for two weeks before the experiment commenced.

### Experimental protocol

Rats in the test groups of C, D and E were pre-medicated with either 50, 100 or 150 mg/kg VitC,

respectively, via intra-peritoneal (i.p.) injection. Negative controls (group A) and positive controls (group B) received physiological salt solution. Five minutes after premedication, animals in groups B, C, D and E were subjected to a combination anaesthesia. For this, ketamine 5% (Rotexmedica, Trittau, Germany) plus xylazine 2% (Rompun®, Bayer, Leverkusen, Germany) were applied i.p. at the doses of 40 and 5 mg/kg, respectively, according to Flecknell [5]. Group A received saline solution. Vital signs, including body temperature (BT), heart rate (HR) and respiratory rate (RR), were taken and recorded after injection of the anaesthetic cocktail. Rectal BT was obtained by a digital thermometer, HR by an ordinary stethoscope, and RR by observation of breast movements. The onset and duration of hypnosis were also observed and recorded. Onset of anaesthesia was evident by recumbence, decreased respiratory rate, loss of pedal and papillary reflexes and loss of movements [5]. The time between loss and return of skin reflex to superficial pain-inducing stimulus was taken as the duration of anaesthesia.

### Statistical analysis

The results are presented as means  $\pm$  standard deviation (SD). Differences between positive controls (group B) and premedicated animals (groups C, D and E) were first analyzed for statistical significance using one-way ANOVA. When  $p < 0.05$ , Bonferroni's test was performed to analyze differences between group B and other groups one by one. Differences in vital signs (only) observed between negative (A) and positive (B) control groups were evaluated by unpaired Student's t-test. A p value less than 0.05 was considered to reflect statistical significance.

## Results

As shown in Table I, negative control animals (group A) did not show any signs of CNS depression. In the non-premedicated rats (group B) onset and duration of ketamine/xylazine-induced anaesthesia were  $5.35 \pm 0.7$  min and  $80.5 \pm 9.2$  min, respectively. Pre-treatment of rats with 50, 100 and 150 mg/kg of VitC resulted in a significant ( $p < 0.05$ ) decrease in the onset of anaesthesia by 16, 42 and 64%, respectively. The duration of hypnosis was increased by VitC dose-dependently. However, it reached the level of significance only with the highest dose (150 mg/kg).

Administration of VitC prior to ketamine/xylazine injection also significantly decreased the heart rate ( $p < 0.05$ ). This, however, was not dose-dependent. Respiratory rates and body temperature were unaffected after VitC pre-medication (Table I).

## Discussion

The present study shows that pretreatment with VitC decreases the time needed to induce and

**Table I.** Effect of ascorbic acid on ketamine/xylazine (K/X) anaesthesia and vital signs of rats after intra-peritoneal injection of ascorbic acid (AA)

Groups	Drug treatment	Anaesthesia parameter		Vital Signs		
		Onset of action (min)	Duration of hypnosis (min)	Heart rate (bpm)	Respiratory rate (per min)	Rectal temperature (°C)
A	Not treated	–	–	302±24	100±10	38.8±0.3
B	K (40 mg/kg) + X (5 mg/kg)	5.35±0.7	80.5±9.2	292±10	89±7.5	38.9±0.2
C	50 mg/kg AA + (K/X)	4.5±0.4**	83.9±10.1	120±14***	80±7.2	38.7±0.2
D	100 mg/kg AA + (K/X)	3.1±0.2***	91.9±11.2*	215±10***	88±10.2	38.9±0.1
E	150 mg/kg AA + (K/X)	1.9±0.3***	110±5.2***	210±11*	82±15	38.8±0.4

Data are presented as means ± SD. Number of animals in each group =10

\* $p=0.023$ , \*\* $p=0.004$ , \*\*\* $p<0.001$  show significant differences in comparison to the average data obtained from the corresponding positive control group (group B)

increases the duration of anaesthesia by a combination of xylazine/ketamine in the rat in a dose-dependent fashion.

Laurence et al. reported that high doses of VitC may cause some CNS effects, including sleep disturbances, headache and gut upset [4]. In addition, VitC has been shown to induce depression and impairment of the function of CNS when administered at high doses [1]. Our findings appear to be in agreement in part with those reported by Ezenwanne and Anuka [3] on behavioural excitation noticed in rats with low doses (5-8 mg/kg, i.p.) of VitC, while the animals were sedated at higher doses (160-320 mg/kg, i.p.).

Ketamine/xylazine and VitC combination caused significant ( $p<0.05$ ) decrease in heart and respiratory rates when compared to the controls. Heart rate was significantly below the baseline value during anaesthesia in ketamine/xylazine groups, which is in agreement with the findings in dogs and cats [12-14]. The decrease in heart rates in the VitC-pretreated rats is worthy of note as ketamine/xylazine combination *per se* did not significantly reduce the heart rate. The observed decrease may have resulted from VitC-induced CNS depression activity [1, 3]. Interestingly, a recent work also suggested that vitamin C at higher doses could potentiate ketamine anaesthesia in rabbits [15]. In addition, a work earlier revealed a potentiatory role for VitC on anaesthesia by pentobarbital [16].

Based on the observation by Nuh, ordinary doses of ketamine/xylazine caused respiratory depression [17]. In the present study, ketamine/xylazine at 40 and 5 mg/kg in rats with or without VitC induced significant respiratory depression, a finding in agreement with those reported by Nuh [17]. Rectal temperature is expected to decline after administration of general anaesthetics (like barbiturates) by reduction of muscular activity and depression of the thermoregulatory centre [5, 6, 8, 9]. Based on our observations, however, ketamine/xylazine combination, with or without

VitC pretreatment, did not significantly reduce the body temperature.

In conclusions, the results of this study clearly suggest that VitC administration prior to ketamine/xylazine anaesthesia could be used to accelerate the onset of action and increase the duration of anaesthesia in rats. Although the exact mechanism has to be verified in detailed studies later, the finding is of a meaningful outcome as VitC administration can be applied to decrease the dose of anaesthetics needed in general anaesthesia and to diminish their side effects on patients.

## References

- Lyhs B. Influence of ascorbic acid deficiency on the function of the central nervous system of the guinea pigs. *Vet Med* 1961; 16: 131-4.
- Sjostrand SE. Pharmacological properties of dehydro-ascorbic acid and ascorbic acid. Effects on the central and peripheral nervous systems in experimental animals. *Acta Physiol Scand Suppl* 1970; 356: 1-79.
- Ezenwanne EB, Anuka JA. The pattern of gross behavioral activities in acute administration of varying dose of ascorbic acid in rats. *Nigerian J Neurosci* 1991; 1: 47-59.
- Laurence D, Bennett PN, Brown MJ. *Clinical Pharmacology*. 8<sup>th</sup> ed. London: Churchill Livingstone; 1997.
- Flecknell PA. *Laboratory Animal Anesthesia. An Introduction for Research Workers and Technicians*. 2<sup>nd</sup> ed. London: Academic Press; 1996.
- Green CJ, Knight J, Precious S, Simpkins S. Ketamine alone and combined with diazepam or xylazine in laboratory animals: a 10 year experience. *Lab Animal* 1981; 15: 163-70.
- Lukasik VM. Premedication and sedation. In: Seymour C, Gleed R (eds). *Manual of Small Animal Anesthesia and Analgesia*. Cheltenham: BSAVA; 1999; 71-85.
- Hauptman K, Jekl V Jr, Knotek Z. Use of medetomidine for sedation in the laboratory rats (*Rattus norvegicus*). *Acta Vet Brno* 2003; 72: 583-91.
- Flecknell PA. Anaesthesia and post-operative care of small mammals. In *Practice* 1991; 9: 180-9.
- Klein LV, Klide AM. Central alfa 2-adrenergic and benzodiazepine agonists and their antagonists. *J Zoo Wildlife Med* 1989; 20: 138-53.

11. Ivanovskaia TV. Effect of VitC and of vitamin B complex on the course of ether anesthesia. *Eksp Khirurgiia* 1956; 1: 50-7.
12. Cullen LK. Medetomidine sedation in dogs and cats: a review of its pharmacology and dose. *Br Vet J* 1996; 152: 519-35.
13. Moens Y, Fargetton X. A comparative study of medetomidine/ketamine and xylazine/ketamine anesthesia in dogs. *Vet Rec* 1990; 127: 567-71.
14. Verstegen J, Fargetton X, Ectors F. Medetomidine/ketamine anesthesia in cats. *Acta Vet Scand Suppl* 1989; 85: 117-23.
15. Elsa A, Ubandawaki S. Ketamine anesthesia following premedication of rabbits with vitamin C. *J Vet Sci* 2005; 6: 239-41.
16. Hollinshead MB, Spillert CR, Flynn EJ, Lazaro EJ. Pharmacologic doses of ascorbic acid prolong the effects of pentobarbital anesthesia. *Res Commun Chem Pathol Pharmacol* 1990; 68: 379-82.
17. Nuh K. A comparison between medetomidine-ketamine and xylazine-ketamine anesthesia in rabbits. *Turk J Vet Anim Sci* 2004; 28: 921-6.