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Research Article

Evaluation of GV26 Electrical Acupuncture Stimulation on Anesthetic Recovery Time of Spur-thighed Tortoise *(Testudo graeca)*

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Abstract: Recovery time from anesthesia can be extended in reptiles, consequently patients undergoing general anesthesia, require prolonged monitoring period which increases the probability of postoperative complications. Therefore, prolonged recovery time following inhalant anesthesia is a common complication in chelonians. Ability to intracardiac shunting and bypassing blood from pulmonary circulation, may contribute to their unpredictable inhalant anesthetic recovery times. The acupuncture point Governing vessel (GV-26) has been demonstrated to reduce anesthetic recovery times from inhalant anesthesia in many species. In this study eight spur-thighed tortoises *(Testudo gracea)* were anesthetized by isoflurane administration for 60 min. The trial was performed in two parts with four weeks washout period. In the first time, once isoflurane administration was discontinued, the tortoises received no therapy in the recovery period and in the second part of the study, tortoises received GV-26 electroacupuncture stimulation. Physiologic variables, anesthetic parameters, time to first movement and time to extubation were recorded. Data were compared with the use of independent sample t tests. Tortoises receiving GV-26 electroacupuncture had a significantly reduced time to return of voluntary movement (P<0.001), and a significantly reduced time to extubation (P<0.001). Furthermore, time from first movement to extubation was significantly shorter (P<0.05). Hence, the use of GV-26 electroacupuncture results in significant reduction of anesthetic recovery time in spur-thighed tortoises which have received inhalant anesthetic.

Keywords: *Anesthesia, Electroacupuncture, GV-26, Recovery, Spur-thighed tortoise, Testudo graeca*

GV26 Elektriksel Akupunktur Stimülasyonunun Mahmuzlu Kaplumbağanın *(Testudo graeca)* Anesteziden Uyanma Süresi Üzerine Değerlendirilmesi

Öz: Sürüngenlerde, genel anestezi altındakilerde, operasyon sonrası komplikasyon olasılığını artıran uzun bir izleme süresine ihtiyaç duyulduğundan, anesteziden uyanma süresi uzayabilir. Bu nedenle, inhalasyon anestezisini takiben uzayan uyanma süresi kaplumbağalarda yaygın bir komplikasyondur. İntrakardiyak şantın azaltılması ve pulmoner dolaşımdan kanın bypass edilmesi, inhalasyon anestezisinden uyanma sürelerine katkıda bulunabilir. Akupunktur noktasını yöneten damarın (GV-26) birçok türde inhalasyon anestezisinde anesteziden uyanma sürelerini azalttığı gösterilmiştir. Bu çalışmada, 8 adet mahmuzlu kaplumbağaya *(Testudo gracea)* izofluran uygulanarak 60 dakika süresince anestezi uygulandı. Anestezi işlemi, dört haftalık arınma periyodunu takiben iki kez uygulandı. İlk uygulamada, izofluran verildikten sonra, kaplumbağalar uyanma döneminde herhangi bir tedavi almadı. Çalışmanın ikinci bölümünde ise kaplumbağalara GV-26 elektroakupunktur stimülasyonu verildi. Fizyolojik değişiklikler, anestezik parametreler, ilk hareketliliğe kadar geçen süre ve ekstübasyona kadar geçen süre kaydedildi. Veriler, bağımsız örneklem t testleri ile karşılaştırıldı. GV-26 elektroakupunktur uygulanan kaplumbağalarda istemli hareketin geri dönme süresi (P<0.001) ve ekstübasyon süresi (P<0.001) önemli ölçüde azaldı. Ayrıca, ilk hareketten ekstübasyona kadar geçen süre anlamlı derecede kısaydı (P<0,05). Dolayısıyla, GV-26 elektroakupunktur uygulaması, inhalasyon anestezisi yapılan mahmuzlu kaplumbağalarda anesteziden uyanma süresini önemli ölçüde azaltmaktadır.

Anahtar sözcükler: *Anestezi, Elektroakupunktur, GV-26, Uyanma, Mahmuzlu kaplumbağa, Testudo graeca*

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Introduction

Chelonians may be anesthetized for diagnostic, therapeutic and research purposes. Inhalant anesthesia is safe and easy to administrate in veterinary medicine, therefore using these agents for reptile anesthesia are common. Although inhalant anesthesia regularly used, prolonged recovery times from anesthesia is a common complication in many reptiles such as chelonians [1,2]. Non-crocodilian reptiles as chelonians have a three-chambered heart including one ventricle and two atria. Also, a septum-like structure named muscular ridge divide the ventricle into two chambers. Muscular ridge is not complete anatomic separation and lack of complete separation enables intracardiac shunting. Different pressure between the pulmonary and systemic circulations regulates the size and direction of the shunts. The pressure differences are due to vascular resistance which could be controlled by cholinergic and adrenergic factors [1,3]. Right-to-left intra-cardiac shunting which occurs during anesthesia and is documented in chelonians is one theory for prolonged anesthetic recovery times. These shunts affect systemic arterial oxygen content, and also the uptake and elimination of anesthetic agent. Inhalant anesthetics in these species undergoes little metabolism prior to being eliminated from the body and the primary route of inhalant anesthetic elimination is exhalation via the respiratory tract. The circulatory system of chelonians could effectively act as an anesthetic storage site when blood is shunted away from pulmonary circulation during anesthesia [1,4]. Delayed anesthesia recovery time leads to extended monitoring period. Prolonged anesthesia monitoring can result in increased personal time, labor cost, morbidity and mortality. Apnea is the most common post-operative complication of reptile anesthesia and difficulties in monitoring is a frequent problem encountered during post anesthetic period. Consequently, establishing a safe and effective method that reduces right-to-left intracardiac shunting would decrease anesthetic recovery time [5,6]. Therefore, techniques such as acupuncture which is documented in many species, would be advantageous for patients, staff and clinicians [7,8]. The acupuncture Governing vessel 26 (GV-26), also known as Renzhong, is one of the most used emergency acupoints in humans and animals. GV-26 is used commonly as an adjunctive therapy in the treatment of shock, cardiopulmonary arrest and apnea. This point is generally located on midline below the nares [7]. An anatomic location for GV-26 has been illustrated in eastern box turtle *(Terrapene carolina carolina)* [4]. The aim of this study was to evaluate the efficacy of electrostimulation of the GV-26 point on reducing inhalant anesthesia recovery time in the spur-thighed tortoise *(Testudo graeca)*. The hypothesis was that time to extubation would be significantly faster following GV-26 electrostimulation in spur-thighed tortoises.

Material and Methods

Ethical Approval

All procedures were in accordance with relevant guidelines of the animal experimental ethics committee of IR.IAU.REC.1399.248.

Animals

Eight adult female spur-thighed tortoises *(Testudo graeca)* with a body mass ranging of 2.25±0.3 kg (Mean±SD) were obtained from wildlife rehabilitation center (Chamran wild animals park, Karaj, Alborz, Iran).

Study Design

They were housed individually in 100x80x80 cm containers with basking area and UV lamps (UVB 160W, JBL, China). The room humidity was maintained at 40-50%, and photoperiod kept under 12:12 light: dark cycle. The temperature gradient and humidity level of the enclosure were monitored twice daily (at 7:00 am and 7:00 pm) [9]. The animals had free access to water at all times and were fed with green leaf base (75%), vegetable (15%) and fruits (10%) once a day, five days a week. Food was withheld 12 h prior to anesthesia [10]. Tortoises were in good general health and condition based on a physical examination performed prior to experiment [11]. This study was carried out using the same tortoises with a washout period of four weeks. Thus, each tortoise was anesthetized twice, first time in control group they received only inhalant isoflurane with no additional treatment during recovery, and one month later, the same tortoises received GV-26 electroacupuncture immediately following discontinuation of isoflurane as the treatment group. Tortoises were manually restrained and face mask was used with 5% concentration of isoflurane with a flow rate of 1 L/min (Piramal Critical Care, USA) to induce with anesthesia machine (Leon plus Bad Ems, Germany). The anesthesia induction was continued until they reached a sufficient level of unconsciousness for intubation. The animals were intubated with a 2 mm (internal diameter) un-cuffed endotracheal tube. Following intubation, tortoises were connected to rebreathing anesthetic circuit and administered isoflurane 5% in 100% oxygen (1 L/min) to achieve a deep plane of anesthesia, then isoflurane level was reduced and maintained at 3% up to 60 min. Administration of anesthetic flow were discontinued 60 min from the intubation time. Each tortoise received similar anesthetic protocol in each of the two processes of study. In this study some sedation parameters such as palpebral, corneal and withdrawal reflexes and response to limb extension, in addition to vital parameters including heart rate (HR), respiratory rate (RR), saturation of peripheral oxygen (SPO_2), end-tidal CO_2 ($ETCO_2$) and cloacal body temperature were evaluated every 5 min up to 90 min, thereafter evaluated every 10

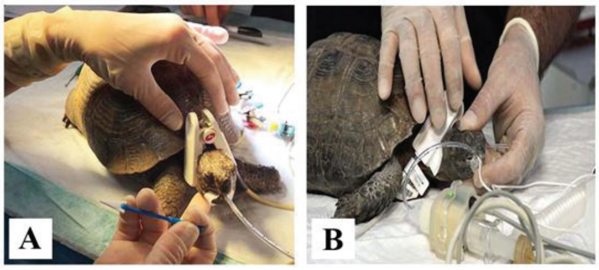

Fig 1. Anesthesia monitoring in spur-thighed tortoise anesthetized with an inhalant anesthetic. A. Evaluation of reflexes, B. GV-26 electroacupuncture needle insertion

min until extubation *(Fig. 1-A)*. In the first experiment, tortoises were received no treatment after they were disconnected from the anesthetic machine, and data were considered as control group. Animal were allowed to washout for 4 weeks before the next experiment. In the second experiment and as treatment group, animals were received electrical stimulation of the GV-26 point with electroacupuncture machine (Hwato SDZ-11, China) immediately after inhalant anesthetic was discontinued. GV-26 stimulation was performed by individual, trained by a certified acupuncturist. The acupoint (GV-26), which is located at midpoint of the ventral margin of the nares, was stimulated with 36-gauge stainless-steel disposable acupuncture needle (Suzhou, China) and a pair of wire ad joint to needle *(Fig. 1-B)*. Electrical stimulation was performed at a continuous 10-Hz frequency, until the time of recovery. Extubation was carried out after a tortoise showed spontaneous respiration and voluntary movement, and it was characterized as recovery.

Statistical Analysis

Data were analyzed using SPSS, version 26. Independent sample t-test was used to compare recovery time and anesthetic parameters for the control and treatment groups. Data were presented as mean ± SD and P<0.05 was considered statistically significant.

Results

No morbidity, mortality or complication were observed in any of the tortoise during the study period. All tortoises were returned to rehabilitation center and few months later they released into wild. Spur-thighed tortoise received GV-26 electroacupuncture had significantly faster times to return of voluntary movement than tortoises in the control group and also extubation was significantly faster following electroacupuncture stimulation of GV-26. The results and P values are displayed *(Table 1) (Fig. 2)*. Mean time from first movement to extubation was significantly faster following GV-26 electrical stimulation in the treatment group. Time from first movement to extubation for both groups is reported *(Table 2)*. Return of spontaneous respiration time recorded in the control and

Table 1. Anesthetic recovery parameters for spur-thighed tortoises

Recovery Parameter	Control Group M±SD	Treatment Group M±SD	P Value
Extubation (min)	105.50±8.35	82.87±8.36	P<0.001
First voluntary movement (min)	121.50±3.96	88.63±12.88	P<0.001

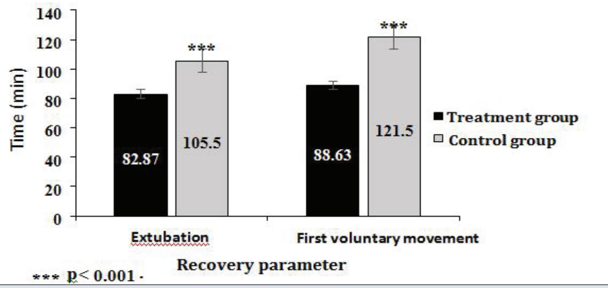

Fig 2. Comparison of mean extubation time and mean first voluntary movement time

treatment groups. Mean time to return of spontaneous respiration were compared and it was not significantly different between groups (P=0.751) *(Fig. 3)*. Return of corneal reflex, palpebral reflex, withdrawal reflex and response to limb extension were compared between the two groups. No significance was found when comparing the time to return of corneal and palpebral reflexes. Tortoises in the treatment group had significantly reduced time to return of withdrawal reflexes and response to limb extension. Result and P value are displayed *(Table 3) (Fig. 4)*. Heart rate at 5 min, 30 min, 60 min and 70 min were not significantly different between groups (P>0.05). Although

Table 2. Anesthetic recovery parameters for spur-thighed tortoises

Recovery Parameter	Control Group M±SD	Treatment Group M±SD	P Value
Time from first movement to extubation (min)	16.00±6.19	5.75±7.79	P<0.011

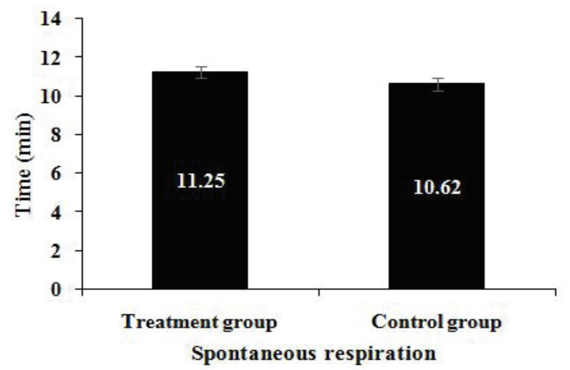

Fig 3. Comparison of return of spontaneous respiration

Table 3. Comparison of return of reflexes

Return of Reflexes	Control Group M (min)±SD	Treatment Group M (min)±SD	P Value
Corneal reflex	78.12±26.31	68.75±12.17	0.376
Palpebral reflex	86.87±21.87	73.75±9.54	0.152
Withdrawal reflex	98.75±12.46	80.00±9.64	0.005
Response to limb extension	107.50±4.63	83.12±11.00	<0.001

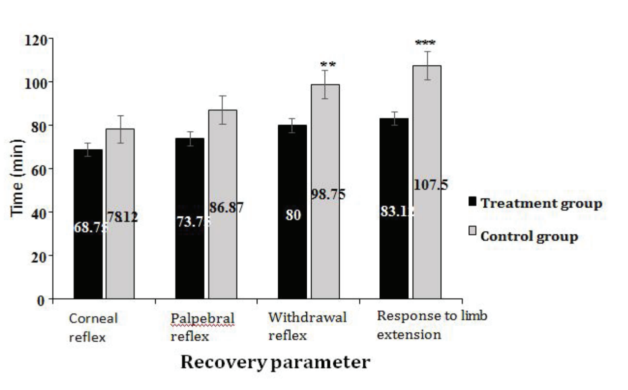

Fig 4. Comparison of mean time to return of reflexes (**P<0.01, ***P<0.001)

within 10 min following GV-26 electrostimulation, heart rate increased relative to control group, but this was not statistically significant (P=0.099) *(Fig. 5)*. Mean end-tidal CO_2 at 5 min, 30 min, 60 min and 70 min were not significantly different between control and treatment groups (P>0.05). Mean end-tidal CO_2 was 18.3± 8.99 and 16.6±18.75 just prior to extubation in treatment and control groups respectively. There was no significant difference between the two groups (P=0.792) *(Fig. 6)*. Mean respiratory rate compared between the control and treatment groups. Despite the fact that mean respiratory rate in treatment group increase faster following GV-26 electrical stimulation, the change of mean respiratory rate from 60th to 70th min was not significant (P=0.125). Furthermore, at time points of 5 min, 30 min, 60 min and 70 min, significant differences were not observed between groups (P>0.05) *(Fig. 7)*. SPO_2, and body temperature

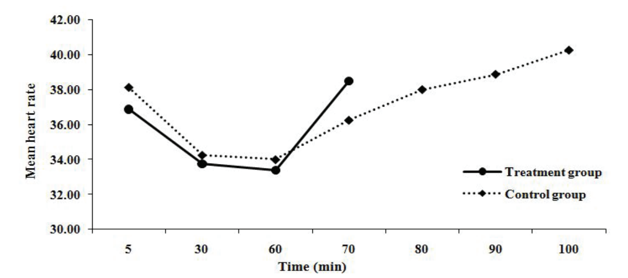

Fig 5. Distribution of heart rate over time

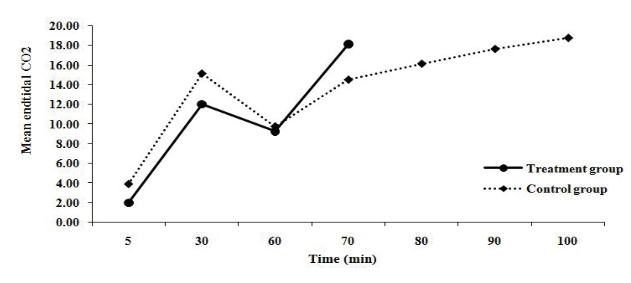

Fig 6. Distribution of Mean end-tidal CO_2 over time

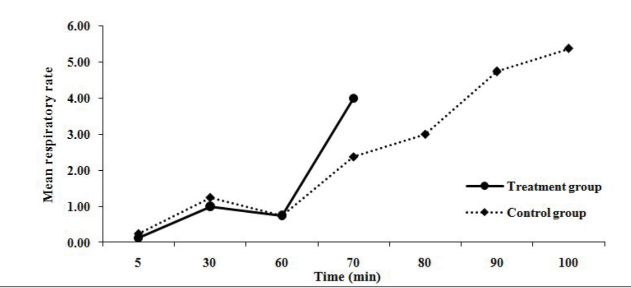

Fig 7. Distribution of Mean respiratory rate over time

were compared between the control and treatment groups at time points of 5 min, 30 min, 60 min and 70 min. These variables were not significantly different between groups at mentioned time points (P>0.05).

Discussion

Isoflurane is currently the inhalation anesthetic choice for reptiles; however, inhalant anesthesia in these species may result in a prolonged time to return of breathing. Intracardiac shunting occurs during anesthesia and these right-to-left shunts can affect the elimination of inhalant agents. Isoflurane undergoes extremely limited renal and hepatic metabolism. Isoflurane is excreted almost exclusively by the lungs and the respiratory excretion has negative influences on the recovery time in these species. Furthermore, because reptiles lack diaphragm, they rely on the thoracic muscles for ventilation. Both inspiration and expiration are active processes, so the respiratory depression associated with anesthesia may be more profound relative to mammalian species where expiration is a passive process. It is imperative that regular spontaneous respiration is in conjunction with movement of the limbs. It is established, re-sedation and apnea may occur, if the animal is not continually stimulated during recovery period [2,12]. Generally, chelonians have relatively lower metabolic rate between all reptile species. They are expected to metabolize and excrete drugs more slowly compared to other species. Furthermore, chelonians are faced with additional respiratory challenges since expansion of the thoracic cavity by movement of the ribs is not possible [1,3]. During apnea, parasympathetic tone leads to increased pulmonary vascular resistance, bradycardia and reduction of pulmonary perfusion. These cardiovascular changes result in the development of right-to-left shunt. In contrast, ventilation is associated with reduction of pulmonary vascular resistance, tachycardia and increase in pulmonary perfusion. Therefore, sudden changes in the direction of the blood can lead to sudden changes in serum concentration of inhalant anesthetics which can markedly decrease recovery times [13]. Several studies have evaluated recovery in reptiles receiving various anesthetic protocols; however, few have evaluated methods specifically focused on shortening the recovery period [5,13,14]. The use of acupuncture in reptiles has been described but not objectively evaluated. Positive effects of GV-26 electrostimulation on resuscitation and anesthetic recovery in human and many animal species, make this acupoint of particular clinical interest. No complication was encountered, and no adverse effects have been noted by the authors [13-15]. In a study, electrical stimulation of GV-26 in common snapping turtles *(Chelydra serpentina)* reduced significant recovery times following inhalant isoflurane anesthesia. Also return of spontaneous ventilation and time to movement were significantly faster following the GV-26 electrostimulation in the turtles [14]. In another study, eastern box turtles *(Terrapene carolina carolina)* received either GV-26 acupuncture and GV-26 electroacupuncture following anesthesia with intramuscular administration of dexmedetomidine. Both of the treatments led to significantly reduced time to return of voluntary movement and a significant reduced time to anesthetic recovery [13]. Our results were parallel to aforementioned studies since stimulation of GV-26 with electroacupuncture was associated with significantly reduction of time to first voluntary movement and significantly reduced time to extubation, thus, significant reduction of anesthetic recovery time in isoflurane-anesthetized spur-thighed tortoises achieved. Furthermore, in the present study, time from first voluntary movement to extubation, time to return of withdrawal reflex and time to return of response to limb extension were significantly shorter. These results were both statistically and clinically significant. Following induction with isoflurane, tortoises became relaxed from cranial to caudal sides and motor function was returned in the opposite direction during the recovery time. Response to limb extension was observed in all animals prior to the return of voluntary movement. Similarly, another study on eastern box turtle indicated that animals received electroacupuncture had significantly reduced time to response to limb extension [4]. In the present study, despite the lack of statistical significance, heart rate and end-tidal CO_2 were increased faster following GV-26 electroacupuncture stimulation. End-tidal CO_2 also increased over time indicating increase of pulmonary blood flow. In another study, GV-26 electroacupuncture had similar effects on heart rate and end-tidal CO_2 in common snapping turtles *(Chelydra serpentina)* [7]. In this study, mean time for spontaneous respiration return was 10 min in the control group and 11 min in the treatment group, so there was no significant difference between the groups. The main reason for this event was probably the reduction of isoflurane concentration from 5% to 3% after few minutes from anesthesia induction. Since the mean breath per minute (BPM) was between zero to one until isoflurane was discontinued, it may consider that some tortoises were apnea in all time points and another reason is that the tortoises had just one breath in every few minutes due to their periodic respirations [2]. Although, the tortoises were maintained at a deep plane of anesthesia in order to encourage maximal pulmonary to systemic shunting. The absence of reflexes indicated the deep plane of anesthesia during the isoflurane administration. This study was considered more stringent than those normally used in a clinical setting but were chosen to maximize patient safety. Given the threatened conservation status of this tortoise, a larger study population was not possible. In eastern box turtle GV-26 acupuncture alone did not have a significantly reduced time to response of limb extension but receiving either GV-1 and GV-26 acupuncture or GV-1 and GV-26 electroacupuncture associated with significant changes. However, needle location is most likely responsible for the reported therapeutic benefits, but electroacupuncture is said to further stimulate the response of acupuncture. The mechanism of action responsible for faster anesthesia recovery with GV-26 stimulation has been attributed to a neurorespiratory or adrenergic mechanism [13,14]. The use of GV-26 electroacupuncture to shorten recovery periods in a clinical setting may result in reduced monitoring time, shorter hospitalization, decreased personal time, reduced client expense and fewer post anesthetic complications. The criteria used to define time to recovery in this study were considered more stringent than those normally used in a clinical setting but were chosen to maximize patient safety. GV-26 electroacupuncture can be used as an adjunctive method to reduce anesthetic recovery time in spur-thighed tortoises that have received inhalant anesthetic. This study represents an early step in investigation of clinical use of electroacupuncture in reptiles. Further neurophysiologic investigations are required to better explain the mechanism of GV-26 stimulation in reptiles and whether such benefits can be ascribed to a neurorespiratory or adrenergic mechanism [5,13,14].

In conclusion, GV-26 electroacupuncture can be used as an adjunctive method to reduce anesthetic recovery time in spur-thighed tortoises that have received inhalant anesthesia. Further investigations need to be performed on other anesthetic protocols and additional research appears warranted in reptilian species which are predisposed to slow anesthetic recoveries.

Availability of Data and Materials

The data that support the finding of this study are available on asking from the corresponding authors. The data are not publicly available due to privacy or ethical restriction.

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All process of research were carried out by own author's budget.

Competing Interest

The Authors declare that there is no conflict of interest. The authors alone are responsible for the content and writing of this article.

Author Contributions

HSN carried out the preparation process and wrote the first draft of the manuscript. All of process of the project was under supervision of HF and BV. MM and AKR advised and co-supervised the project. The final revision of the manuscript was done by HSN, HF and BV. HSN, HF, BV, MM and AKR approved the final version.

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