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Antinociceptive and physiological effects of subcutaneously administration of fentanyl in *Trachemys*sp. (Testudines: Emydidae)

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Abstract**—** *Control of pain in reptiles is a challenge and there is few information about it. This is the first study that evaluate fentanyl on testudines, of genus Trachemys sp. A total of 30 animals were used, 15 T. dorbigni and 15 T. scripta. Two groups composed of ten specimens each were created, as well as two control groups of five animals, for each species. A dose of 0.05 mg/kg of fentanyl was administered to experimental groups, and a 1 mL/kg physiological solution was administered to control, all subcutaneously (SC). The reptiles were monitored in terms of the color of the oral mucosa, cloacal temperature and heart rate, response to the nociceptive stimulus, and myorelaxation. The effects started 10 minutes after the administration and lasted 134 ± 26 minutes and 120 ± 20 minutes for T. dorbigni and T. scripta, respectively. In 80% of the animals, there was a total absence of reaction to a nociceptive stimulus, with an average duration of 39 ± 10 minutes for T. dorbigni and 30 ± 12 minutes for T. scripta. It was concluded that fentanyl 0.05 mg/kg SC is capable of promoting the absence of nociceptive response in Trachemys sp.*

Keywords**— Analgesia, opioid, red-eared slider, Testudines.**

I. INTRODUCTION

The genus *Trachemys* of the Emydidae family has the widest distribution among the semi-aquatic testudines in America [1]. In Brazil, it is naturally represented by *T. dorbigni* species, in the south, and *T. adiutrix*, in the north [2]. The *Trachemys scripta elegans* species naturally occurs in North America and is included in the International Union for Conservation of Nature (IUCN) list of 100 high-potential invasive exotic animals [3].

Popularly known as red-eared slider, specimens of *Trachemys*adapt to a wide variety of habitats, diets, and conditions, and have high growth and reproduction rate [4]. The population interest for trading and keeping these animals as pets, coupled with the lack of knowledge about the biology of *T. scripta elegans*, represents an even greater impact on the global distribution chain of these turtles as an invasive species, which is worsened by their indiscriminate release in unnatural habitats [5].

For centuries, philosophers and scientists have debated the perception of pain by animals. There is controversy regarding the correspondence of structures present in the central and peripheral nervous system among mammals and animals of other orders. Although data is scarce, recent studies prove the ability of reptiles to detect and process nociceptive stimuli, and pain is now recognized as one of the vital signs and an integral part of the entire patient assessment [6].

Reptiles have the brain structures required for perception of nociceptive stimuli in the neocortex and, morphologically, there are direct spinal connections with the

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brainstem and dorsal thalamus of the mesencephalon, as well as endogenous opioid receptors [7]. The mu, kappa, and delta-opioid receptors have been described in the central nervous system of reptiles [8]. However, pain information is still lacking in these animals and protocols are often extrapolated from domestic mammalian medicine, which is one thing that must be thoughtfully undertaken [7].

Different studies have evaluated the efficacy of reptile anesthesia and chemical containment protocols, and most available literature indicates the use of μ-opioid receptor agonists as the best option for producing analgesia.[8] Fentanyl is a μ-opioid nearly 75 to 100 times more powerful than morphine, it has short latency and period of action when administered via intramuscular, subcutaneous or intravenous bolus [9].

The efficacy of fentanyl citrate in reptiles has not been well determined; however, in a study of *Python regius* specimens, plasma concentrations reached 1 ng/mL after 4 hours of application of transdermal fentanyl (12.5 ng/h). The active ingredient was detected for 4 to 6 hours, with a significant reduction in respiratory rate in animals by 23% and 41% after 24 and 48 hours of patch application, respectively, but there was no change in response to nociceptive thermal stimulus, which indicated μ-dependent antinociception resistance in this species [10].

In another study on a *Coruciazebrata* lizard species, fentanyl plasma concentration was detected after 4 to 6 hours of application of a 25 μg/h patch over 10% of the animals' body surface, and lasted for up to 72 hours [11]. Given the lack of statistical data and the variability in species-specific response, the biological significance for fentanyl plasma concentrations is not yet clear, so the antinociceptive action of the drug requires further study [12].

Despite the use of preventive analgesia to avoid pain chain sensitization in pre-, trans- and post-anesthetic phases, little is known about the nociceptive mechanisms, pharmacological efficacy and adverse effects of analgesics in reptiles. Among the potent analgesic drugs used in veterinary medicine, opioids act on the modulation of peripheral, medullary and supraspinal central nervous system (CNS) nociception [13]. Given the above, the present study aimed to evaluate the effects of subcutaneous fentanyl citrate (SC) in *Trachemys dorbigni* and *Trachemys scripta elegans*.

II. MATERIAL AND METHODS

Thirty healthy adults of both genders of the genus *Trachemys* were used, with body weight between 750g and 1800g, being half of the species *T. dorbigni* (DG) and the remaining *T. scripta elegans* (SG). For each species, two random groups were created with five specimens for the control group and ten specimens for the treated groups.

The animals were weighed on a digital scale (Balmak, model ELP-6/15/30, with a capacity of 30kg and precision of 2 g, Campinas, SP, Brazil) and marked on the carapace with tape and pen. To monitor the ambient temperature, a digital thermo hygrometer (Incoterm, Porto Alegre, RS, Brazil) was used and the research was conducted in October and November, with an ambient temperature between 25 and 29°C.

The administration of fentanyl citrate (Fentanest®, 0.05 mg/mL, Laboratorial Cristalia Chemicals Pharmaceuticals Ltda., Itapira, SP, Brazil) at a dose of 0.05 mg/kg subcutaneously (SC) was evaluated. The drug was applied to the left thoracic limb region[14] by previous antisepsis with 70° GL alcohol. Control groups were given water in the volume of 1mL/kg, SC.

The effects of nociceptive stimulus-response, heart rate, cloacal temperature, and mucosal color were observed. For the evaluation of the first parameter, the von Frey pressure test[15] was performed with the aid of a 16cm Kelly hemostatic forceps with the latex coated serrations [16], [17]. Sufficient pressure was applied to produce nociceptive stimulation in interdigital tissue (superficial pain), phalanges and tail end (deep pain).

The nociceptive stimulus was performed before the drug application, signed as time zero (T0) and, after every ten minutes, until the reaction return equivalent to T0. It was considered the reaction to the painful stimulus movements of the head to the side of the pinched limb and the retraction of the stimulated limb, neglecting movements produced by touch or fright to approach. This parameter was classified into four scores: 0 for response equivalent to T0, 1 for response reduction, 2 for intense response delay, with slight reaction to the stimulus, and score 3 for no response to clamping.

Heart rate was monitored using a vascular doppler (MEDPEJ. Ribeirão Preto - SP. Brazil)[18] with a probe positioned between the thoracic limb and neck, counting for one minute. This evaluation was performed at T0 and thereafter every 20 minutes until normal reactions returned. The cloacal temperature was also monitored with the aid of a digital thermometer(Incoterm, Porto Alegre, RS, Brazil) with degrees Celsius scale from -50ºC to 300ºC, inserted up to 2 cm inside the cloaca.

As respiratory movements were masked by physiological apnea and voluntary movement, it was decided

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not to perform respiratory rate monitoring. However, oral mucosa staining was observed every 10 minutes to assess peripheral tissue oxygenation [19].

Data on the onset of drug action, duration of no response to clamping (score 3) and return to nociceptive response equivalent to T0 were also recorded. The collected data was analyzed through the BioEstat 5.3 program [20]. Data distribution patterns were analyzed and the average among T0 and the other times within each group were compared, as well as the difference in latency time, deep sedation duration and total recovery between the control and treated groups.

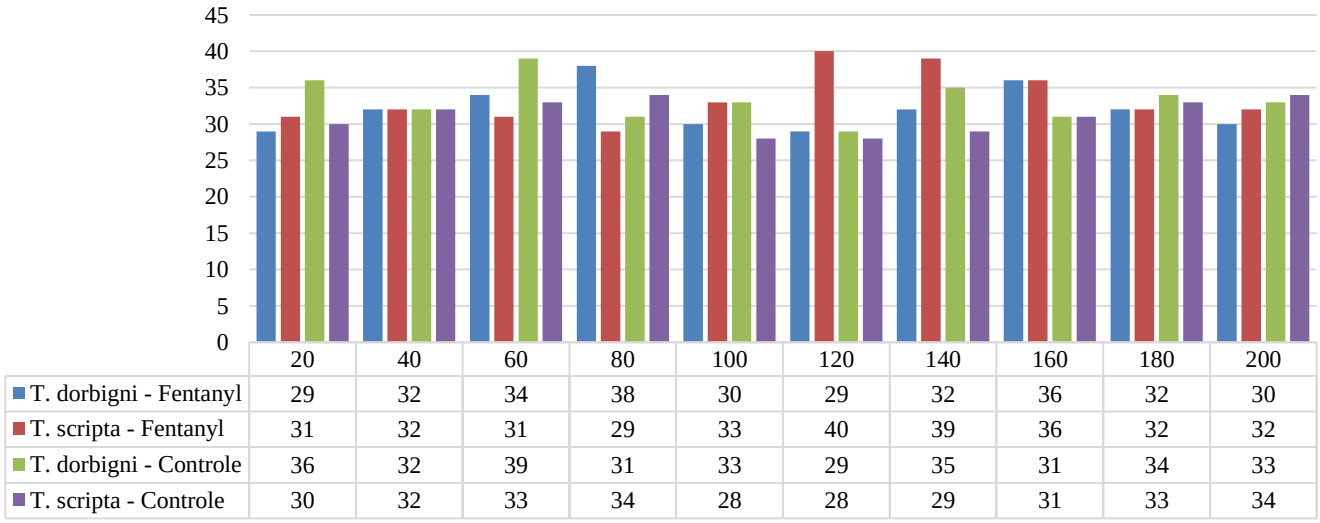
III. RESULTS

In the first evaluation interval after fentanyl citrate application, signs of deletion in the nociceptive response were observed in all (100%) *T. dorbigni* and in nine (90%) *T. scripta elegans*. This last specimen of SG showed signs of analgesia from the second evaluation at 20 minutes. Nociceptive response deletion (score 2) was observed in all (100%) animals treated with the 0.05 mg/kg SC fentanyl citrate protocol and only two individuals (20%) from each group did not achieve the complete absence of response to clamping (score 3).

The heart rate of the two species did not vary statistically when compared with the initial frequencies (T0) and the control group (Graph 1). The DGan average of 32.2 ± 3.01 beats per minute (bpm) and its control group presented 33.3 ± 2.86 bpm (P=0.4139). While SG presented 33.5 ± 3.62 bpm, and its control group showed 31.2 ± 2.34 bpm (P=0.1096).

Regarding the cloacal temperature, it remained between 24 and 28ºC, with averages and standard deviations of 25.2 ± 0.97ºC and 26.3 ± 1.95ºC for *T. dorbigni* and *T. scripta*, respectively. Also, there were no statistical differences (p> 0.05) between the time intervals for the effects of fentanyl citrate on the two species tested (Table 1).

Graph 1. Average in minutes of heart rate of Trachemys dorbigni and Trachemys scripta elegans on subcutaneousfentanyl citrate 0.05 mg/kg



	20	40	60	80	100	120	140	160	180	200
T. dorbigni - Fentanyl	29	32	34	38	30	29	32	36	32	30
T. scripta - Fentanyl	31	32	31	29	33	40	39	36	32	32
T. dorbigni - Controle	36	32	39	31	33	29	35	31	34	33
T. scripta - Controle	30	32	33	34	28	28	29	31	33	34

Table 1. Average and standard deviation, in minutes, for latency, the total absence of nociceptive response (TANR), TANR duration and end of action of subcutaneous fentanyl citrate 0.05 mg/kg in Trachemys dorbigni and Trachemys scripta elegans

	Trachemys dorbigni	*Trachemys scripta elegans*	*p*
Latency	10 ± 0	11 ± 3,16	0,33
TANR	20 ± 11,55	15 ± 10,8	0,33
TANR Duration	45 ± 16,90	36,25 ± 15,98	0,62
End of action	144 ± 25,9	131 ± 19,12	0,21

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IV. DISCUSSION

No loss of proprioception, central nervous system depression and/or loss of consciousness were observed in any of the animals evaluated. These findings confirm observations made by Bouts and Gasthuys[21] when describing reptiles' resistance to opioid depressive effects.

As a high-affinity agonist opioid to μ receptors, fentanyl citrate belongs to the class of opioids best suited to achieve satisfactory analgesia in invasive procedures [8], [22], [23].Waara-Wolleat et al. [24] describe fentanyl as an ultra-rapid, short-acting, and high-potency analgesic. In agreement with the authors, practically all experimental animals showed signs of analgesia within 10 minutes after subcutaneous application, and 80% of them showed a total absence of response to nociceptive stimulus lasting about 15 minutes.

Analgesia produced by fentanyl citrate at 0.05 mg/kg SC confirmed its analgesic potential. Compared with butorphanol and morphine [13], [25], it has higher analgesic capacity and shorter duration, since the other two drugs remain active in the animal organism for more than 24 hours. In *Salvatormerinae*, between morphine and butorphanol, only morphine promoted antinociception at doses of 5 and 10 mg/kg [26]. Similar to fentanyl, tapentadol, another μ-opioid receptor agonist, has also demonstrated effective analgesia in *T. scripta*after a single intramuscular application at a concentration of 5 mg/kg [27], [28].

Although the subcutaneous route of administration is ineffective in reptiles because it is a poorly vascularized and slowly absorbing region [12], it is adequate for fentanyl administration. Similarly, meperidine administered subcutaneously in *T. scripta* caused 30-minute analgesic effects on the tested specimens [12], [13].

Hawkins et al. [29] recently investigated the pharmacokinetics of subcutaneous application of the opioid hydromorphone in *T. scripta* and *Pogona vitticeps*. High plasma concentrations were observed after 30 minutes of application for both species and five of the six *Pogona vitticeps* showed decreased response to stimuli at 1.0 mg/kg. However, none of the turtles showed signs of clinical sedation at any dosage [29]. In the present study, fentanyl caused a lack of nociceptive response similar to the 30-minute SC hydromorphone peak.

Darrow et al.[30]evaluating the transdermally applied plasma fentanyl concentration in two *Python regius* specimens, noticed a high and continuous plasma concentration of the active principle during the seven days of evaluation, without behavioral changes. Kharbush et al. [31] researched the same pathway in *T. scripta*, and also detected high plasma concentration, however, with no change in thermal antinociception. This may have been due to the resistance of this species to μ agonist opioids or to a variation in response to thermal stimuli in these ectothermic individuals, since in the present study, the pressure stimulus was reduced with the drug. According to Mark & Tully[32], reptiles do not have the same natural reflex to avoid the heat found in vertebrates of other classes, which makes them stay in contact with the thermal source even when trauma occurs. Therefore, there is divergence on the reliability of the use of this type of stimulus in analgesic evaluation.

Fentanyl has excellent fat solubility, which contributes to its distribution and binding to nervous tissue [33]. Besides that, hypotension and bradycardia produced by most μ-receptor agonist opioids are caused by histamine release, but fentanyl citrate does not stimulate such release [34]. This property reflected the absence of change in heart rate of the specimens of the present study and corroborates with Lervik et al. [35] and Williamson et al. [36], which cite the cardiovascular stability provided by fentanyl citrate.

Fentanyl overdoses can lead to intense muscle stiffness in mammals[37] and a drastic decrease in spinal cord sensitivity to carbon dioxide partial pressure ($PaCO_2$), resulting in marked respiratory depression [33], [38]. This fact may elucidate the episodes of apnea observed in the present study, but it is worth noting the advantage of opioid antagonists such as naloxone [39], [40]. Despite the apnea, the reverser was not used in the specimens of *Trachemys* sp., in order not to compromise the duration evaluation. To monitor the absence of tissue hypoxia, mucosal staining was evaluated, which remained stable compared to T0. The worked dose was previously used by Souza[41] in *Trachemys* sp. as pre-anesthetic medication, using propofol as inducer, but without detailing the effects of fentanyl specifically.

Respiratory depression is the main risk factor for opioid use. From the monitoring of mucosal staining and the presence of sporadic breathing movements, it was decided not to promote assisted ventilation indicated by Frye[19]. The animals were still alert during the evaluated period and obtained a safe return with the dose used, without associated deleterious effects. The central nervous system of reptiles has resistance to hypoxia and apnea is a physiological event for Testudines [42], [43]. Additionally, the cloacal temperature in the treated groups followed the values presented by the control groups, according to Hicks and Wang [43], [44], the

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presence of hypoxia can be induced by hypothermia in reptiles.

V. ETHICAL CONSIDERATIONS

This study was approved by the Ethics Committee on Animal Use of the Federal University of Uberlândia (CEUA/UFU), protocol 080/12, and authorized by the Biodiversity Authorization and Information System (SISBio), protocol 63133-1.

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