

Traumatic brain injury. Clinical and pathological parameters in an experimental weight-drop model¹

Lesão cerebral traumática. Parâmetros clínicos e patológicos em um modelo experimental de queda de peso

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ABSTRACT

Purpose: To investigate the function of an experimental cranium trauma model in rats. **Methods:** The equipment, already described in the literature and under discreet adaptations, is composed by a platform that produces closed head impact controlled by weight drop with pre-defined and known energy. 25 Wistar male rats (*Rattus norvegicus albinus*) were divided into five equal groups that received different quantities of cranial impact energy: G1, G2, G3 and G4 with 0,234J, 0,5J, 0,762J and 1J respectively and G5 (Sham). Under intense analgesia, each group was evaluated clinically in a sequence of intervals and had their encephalon removed for pathologic analysis. **Results:** Important clinical alterations (convulsions, bradycardia, bradypnea and abnormal postures) and focal pathologic (hematomas and hemorrhages) kept proportion with the intensity of the impact. No fracture was observed and the group 4 had 80% mortality rate. **Conclusion:** The experimental cranium trauma animal model by weight drop is an alternative of low cost and easy reproduction that allows evaluating clinical and pathological alterations in accordance with studies in experimental surgery aims for new traumatic brain injury approach in rats.

Key words: Models, Animal. Brain Injuries. Parameters. Rats.

RESUMO

Objetivo: Investigar o uso de um modelo de trauma craniano experimental em ratos. **Métodos:** O equipamento, já descrito na literatura e sob discretas adaptações, contou-se de uma plataforma para produção de lesão craniana fechada controlada por queda de peso com energia pré-definida e conhecida. 25 ratos Wistar machos (*Rattus norvegicus albinus*) foram divididos em cinco grupos iguais que receberam níveis diferentes de energia de impacto craniano: G1, G2, G3 e G4 com 0,234J, 0,5J, 0,762J e 1J respectivamente e G5 (Sham). Sob intensa analgesia, cada grupo foi avaliado clinicamente em uma sequência de intervalos e tiveram seus encéfalos removidos para análise patológica. **Resultados:** Alterações clínicas importantes (convulsões, bradicardia, bradipnéia e posturas anormais) e patológicas focais (hematomas e hemorragias) guardaram proporção com a intensidade do impacto. Nenhuma fratura foi observada e o grupo 4 teve 80% de mortalidade. **Conclusão:** O modelo animal para trauma craniano experimental por queda de peso é uma alternativa de baixo custo e fácil reprodução, gerando alterações clínicas e patológicas compatíveis com os objetivos de estudos em cirurgia experimental para abordagem do trauma craniano em ratos.

Descritores: Modelos Animais. Traumatismos Encefálicos. Parâmetros. Ratos.

Introduction

Traumatic brain injury has a complex pathophysiology and happens in phases with several space and time specific mechanisms^{1,2}. Many experimental studies² have been focusing on observing biomechanics and pathology associated to traumatic brain injury with the objective of reproducing them in laboratory and develop new approaches for encephalic lesions related to direct trauma.

Several related models in the literature²⁻⁴ allow producing a varied range of lesions from simple and reproducible mechanic principles, such as the models of weight dropping and direct impact by pneumatic piston. These models are able to produce a controlled cortical impact with increasing energy levels⁴. The model involving weight fall has been used for a long time in order to produce diffuse lesions on encephala of rats for researches on experimental traumatic brain injury^{2,3}. The most diverse outcomes can be reached by varying the energy that the object passes on to the cranium of the animal when achieves it and the cranium of the animal relation with the support that is given to it (if mobile, rigid, deformable), but many other variables can be manipulated by researchers in order to create the desirable injury. Many disadvantages are linked to the dropping weights model such as the low speed performed by the object in free fall⁴. However, this model presents simple and easy reproductive physical principles, which allows the performance of several manipulations considered satisfactory for producing a great variety of encephalic injuries^{5,6}.

The characterization of a model and the description of the behavior of its main physiologic, pathologic and clinical variables is an essential phase for a posterior use of the same research in experimental surgeries. This study aims to investigate an animal experimental traumatic brain injury model for researching on experimental surgery using the rat.

Methods

After the approval by the ethics committee in research of the Health, Human and Technology Sciences School of Piauí (NOVAFAPI), 25 adult Wistar rats (*Rattus norvegicus albinus*), originally from the Animal Colony of the Piauí State University (UESPI), were clinically examined, selected and packed in standard cages, under 25°C ambient temperature and 12 hours cycle of light per day. These animals weighed between 230g and 300g and had about 100 to 120 days of age, all male. They were divided in equal groups of five animals.

Each animal underwent an anesthetics protocol watched by a veterinarian and based on the administration of pre-anaesthetic drugs (Xilazina 2%, 5mg/Kg, SC) and fentanila (fentanila citrate in a dose of 0,032mg/Kg intraperitoneally). The anesthesia was promoted by using Propofol in the dose of 64,63mg/Kg intraperitoneally. The clinical data (rectal temperature, cardiac and respiratory rate, capilar glycemia, response to algic stimulus and palpebral and pupillary reflexes) were evaluated and registered before the trauma (after analgesia and anesthesia) and in identical sequence of time intervals after the trauma for all the groups of animals: at the moment of impact and 5, 10, 30, 50 and 80 minutes after the impact.

To produce the brain injury, a controlled closed head impact by weight fall device was set up exclusively for this study according to descriptions of it in the literature^{2,5-8} and under discreet adaptations (Figure 1).



FIGURE 1 - Controlled falling weight closed head injury platform. **A.** Metal rod to support the driving weight duct. **B.** Driving weight stiff plastic duct (perfect metal sphere of known weight) measuring 1m of height. **C.** Platform basis for positioning the experimental animal with a head solid support.

The fall of a perfect metallic sphere(diameter of 20mm) launched from 1 metre of high by the interior of the duct (diameter of 22mm) produce trauma direct onto the animal skull hitting on its median portion, approximately in the center of the cranium cap with minimum range among each species(Figure 1). The friction produced by the sphere passage inside the duct was considered negligible and its trajectory was straight and perpendicular to the surface of the animal skull. Each group of animal underwent a different quantity of energy controlled by the use of identical spheres, but with different weights always launched from the same height: Group 1 = 0,234J; Group 2 = 0,5J , Group 3 = 0,762J e Group 4 = 1J of energy, being the group 5 a Sham in which the animals underwent the same anesthetic protocol, but not to the trauma. These values can be obtained multiplying the mass of the spheres (Kg) by the gravity force (10m/s^2) and the height from where it is launched (1 m). In all groups the head of the animal remained still and partially fixed on a solid base (Figure 1). Each animal received exclusively an only impact corresponding the energy destined to its group.

Several clinical parameters, besides the ones already mentioned, were evaluated at the moment of impact and in the intervals mentioned previously, such as: abnormal postures (decortication and decerebration), convulsions, apnea and death.

After completing all the evaluations in all intervals each animal was killed by deepening the anesthetics plan with Tiopental sodic. Each animal had, therefore, their encephalon removed by craniectomy produced with support of an electrical drill (Figure 2). Many macroscopic parameters were evaluated in this moment: skin laceration, hematomas in the scalp and extradural and subdural hematoma, subarachnoid hemorrhage and intracerebral hemorrhage.



FIGURE 2 - Details of removal of the skullcap for the extirpation of the brain.

Results

Concerning the anesthetics protocol adopted, excellent degrees of analgesia and anesthesia were observed, even when the animals underwent strong impacts (1J). Propofol allowed thorough anesthesia for about 25 to 30 minutes with the beginning of the action in up to 7 minutes and the analgesia was satisfactory for a period of 30-40 minutes with beginning of the action in up to 5 minutes. The opioid used had its dose repeated as the effect waned down until the clinical observation was completed after the impact. In this time interval a minimum interference of the use of this drug in the cardiac activity was observed, although the respiratory rate was sensitively modified by the administration of this opioid (Table 1), but according to what was observed in publications with the use of these drugs for experimental surgeries in rats⁹.

TABLE 1 - Analysis of clinical and physiological parameters of the anesthetic protocol based in use of xylazine, fentanyl and propofol approximately 10 minutes after anesthetic induction

Parameters	Mean value	Reference value
Cardiac rate	280 bpm ^a	261-600 bpm ^d
Respiratory rate	52 rrpm ^b	70 – 150 irpm ^d
Rectal Temperature	35°C	36,5 – 38,5 °C ^d
Motricity	Abolished ^c	
Pupillary reflex	Abolished ^c	
Palpebral reflex	Reduced ^c	

^a Beats per minute. ^b Respiratory rate per minute. ^c Compared to the same animal before administration of analgesic and anesthetic. ^d Based on Marques and Caetano¹⁰

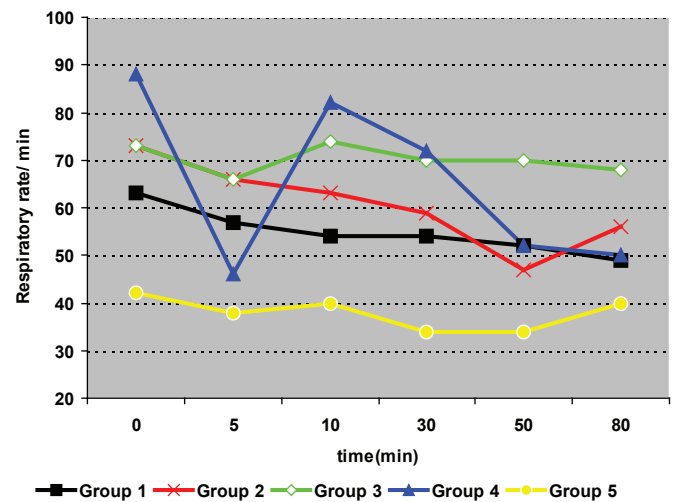
The clinical alterations observed in each experimental group were as bigger and more intense as greater was the employed energy for the impact keeping a direct relation between the manifestation and the energy dispensed on the cranium. No clinical variable suffered important variation while the impact of energy of 0,234 and 0,5 joules was used (Table 2). After the effect of the anesthetic, these animals presented normal movement and reflexes. Convulsions and abnormal postures began to manifest only in animals underwent to impacts of the order of 0.762J and were present in all animals in Group 4 (Table 2).

TABLE 2 – Number of animals with alterations in the clinical parameters after the impact and their respective groups

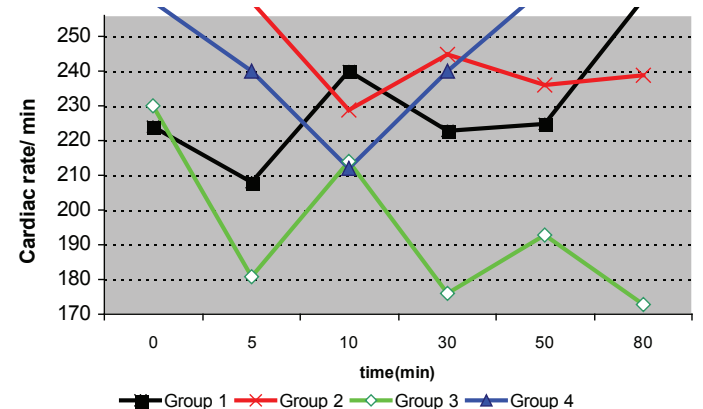
Group	Convulsion	Abnormal postures		Deaths
		Decortication	Decerebration	
1	0	0	0	0
2	0	0	1	0
3	2	0	1	1
4	5	1	0	4
5	0	0	0	0

All these events happened immediately after the impact. The majority of this group died in up to 10 minutes from the moment of the impact despite the reanimation attempts. The convulsions were focal and limited to the posterior limbs and tail lasting about 30 seconds. Bradypnea were more intense in the groups 3 and 4 and immediately after the impact. The depression of the cardiorespiratory function was sensibly greater in the animals of group 4 (Figure 3) and only an animal of this group survived

to the 80 minutes of observation. In these animals there was an important tachydyspnea in the moment of the impact followed by the sudden and progressive fall of the frequency in the following 5 minutes (Figure 3).

**FIGURE 3** – Ranges of mean value of the respiratory rate in different experimental groups and in Sham during the clinical evaluation intervals after impact at time 0 min.

The behavior of the cardio vascular function can be appreciated in Figure 4 and the Group shunt did not present inferior to 260 beatings in one minute frequencies considered by the researchers as an reference point for the bradycardia based on Marques and Caetano¹⁰.

**FIGURE 4** – Evolution of mean value of the cardiac rate in different groups during the time of clinical observation after impact.

The behavior of capilar glycemia in the different groups can be appreciated in Figure 5.

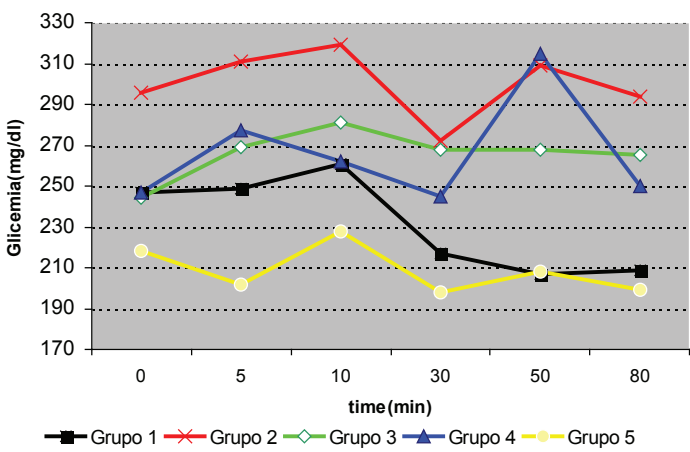


FIGURE 5 – Evolution of mean value of the capillary blood glucose levels from each experimental group during the time of clinical observation since the moment of impact (0 min) and in the group 5 (Sham).

More sudden variations were noticed in group 4 and generally there was an increase in the glycemic levels in the first minutes in all animals that underwent the trauma.

Figure 6 shows ranges in rectal temperature in each group. Only the group that underwent the greater energy of impact presented abnormalities in the behavior of the rectal temperature along of the clinical observation unconfirming with the standard observed for the rest of the groups of test and in relation to the control.

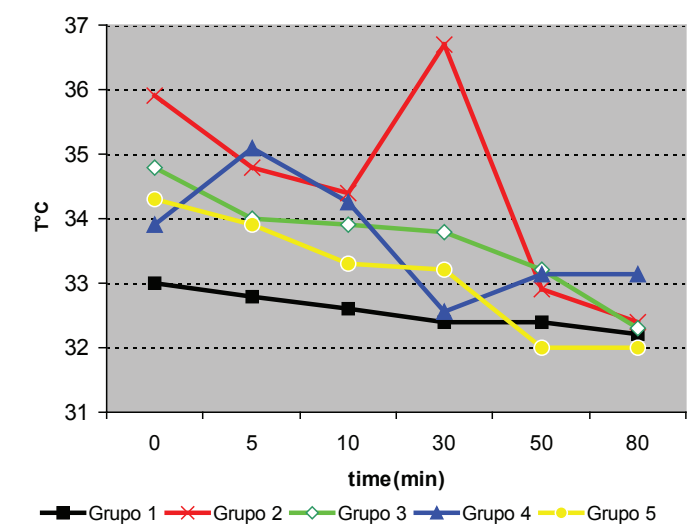


FIGURE 6 – Evolution of mean rectal temperature in different experimental groups and in the Sham group during the time of clinical evaluation after impact.

The macroscopic alterations noticed during the extirpation of the encephala were discreet even in the groups that underwent to great energy traumas and consisted in focal and diffuse lesions (Table 3).

TABLE 3 – Number of animals with focal and diffuse injuries during the macroscopic analyses of the cranium after impact.

Traumatic injury	Group				
	1	2	3	4	5
SCALP laceration	0	0	0	0	0
Hematoma/hemorrhage					
Galeal	0	0	1	1	0
Subgaleal	0	0	1	1	0
Extradural	0	0	1	1	0
Subdural	0	0	0	0	0
Subarachnoid	0	0	0	0	0
Intracerebral	0	0	0	2	0

Discussion

In the last years, many researchers have been dedicated to produce experimentally the biomechanics of the encephalic injury^{2,3}. Form the studies of Lighthall¹¹ a new model was created to produce controlled cortical traumas capable of generate focal lesions. Dixon *et al.*¹² adapted a model of Lighthall for the rat and, therefore, by the characteristics of these animals, researches with controlled cranium traumas gained popularity and greater reproductibility⁷.

Even this model of encephalic lesion has great number of variants which comprehend from devices with mechanisms of fall of weights to devices which operate by triggering a pneumatic piston, all producing closed head injury. An important aspect of these models is the facility to determine the energy involved in the trauma. The weight of the object as well as the height from where the weight is launched can be manipulated in order to provide the trauma different levels of potential gravitational energy in the future converted in cinetic energy to be dispersed over the cranium of the animal. While in models under pneumatic pressure it is able to reach high speed producing severe lesions as we elevate the impact pressure, in the models of fall of weight the speed of the object is around only 2m/s⁴. Nevertheless, the manipulation of the variables previously mentioned allowed this study to analyze and correlate increasing levels of energy with different clinical and pathological alterations.

The traumatic brain injury study allowed the production of several types of lesions, however, these were macroscopically discreet and not very frequent, almost always restrict to very elevated level of energy (0,762 and 1J) which caused the death of the experimental animals in few minutes (Table 3). The use of this quantity of energy probably did not allow margin for studies of therapeutic approaches unless mechanisms of mechanical ventilation and cardiac reanimation, considering that the majority of these animals developed important cardio respiratory depression and 100% of these animals present convulsions (Table 2 and Figures 3 and 4) referred by literature¹³ as an important cause of secondary cerebral lesion. The causes of secondary lesions, classified as of systemic genesis (hypotension, hypoxia, hypoglycemia and hyperthermia) and intracranial (cerebral edema, vasospasm, convulsions) are the aim of studies that propose to developing therapeutic approaches for the cranial trauma once the primary lesion can not be reversed¹⁴.

This model was limited in the production of primary lesions macroscopically meaningful once it is not capable of creating cranial fractures with levels of energy compatible with life after the impact. It presented discreet focal lesions in few animals and only at elevated levels of energy, but in accordance with what was described in similar studies^{4,11,12}. In this study this model was capable to reproduce the causes of secondary systemic cerebral lesions (represented by bradypnea and bradycardia illustrated in Figures 3 and 4) as well as intracranial (represented by convulsions and cerebral edema illustrated in Table 2) and these findings are in agreement with what was presented by similar studies^{2,4}. The cardiac and respiratory rate decrease is part of a classic triad of intracranial hypertension known as "Cushing response"¹⁵ and reflects the intracranial compartment high pressure establishment of the animals that underwent the trauma (Figures 3 and 4). The increased cardiac rate observed in the Sham along all the period of clinical observation is due to the inhibition of the vagal tone by fentanila⁹. It is observed, in Figure 4, that despite this inhibition the cardiac rate of the animals of groups 1, 2, 3 and 4 falls below 261 beatings per minute after the trauma.

The mortality in the group that underwent the impact of 0,5J was zero, which was not in agreement with what was observed by similar studies in which the same energy was related to 43% of mortality¹⁶. This disagreement was attributed to the weight and platform characteristics used in that study. In groups 1, 2 and 3 the reflex recovering and the motricity happened about 50 minutes after the anesthetic induction and although the mortality in group 4 had achieved 80% the only animal that survived the trauma had its recovery similar to those from the previous groups.

Concerning capillary blood glucose, some studies have demonstrated that the range in the plasmatic levels of glucose may not be a meaningful factor for the result of traumatic cerebral lesions in models of cerebral injury by liquid perfusion¹⁷. However, in this model, only the animals that underwent cranial trauma had elevations in their capillary blood glucose and this elevation was as greater as the used energy for the impact was, translating the neuroendocrine and metabolic response to the trauma. It was attributed greater importance to the behavior of the capillary blood glucose along the time in detriment to its absolute values. Mortality was greater in the groups where glycemia had great deflections or remained elevated in relation to the pre-traumatic levels agreeing with previous studies about the theme¹⁸. There are still few clarifications about the real influence of hyperglycemia in the progression of the cerebral damage related to the trauma, although it is admissible a reserved prognosis to hyperglycemic patients after cranial traumas¹⁸.

The decrease of the rectal temperature after the trauma and along clinical evaluation was attributed to the inhibitory action of the fentanila over the hypothalamic thermoregulator center described also in other publications⁹. Nevertheless, it is possible to observe greater ranges in temperature of the groups that underwent the impacts of greater energy with proportionately greater cerebral damage indicating an important contribution of encephalic trauma for limitation in the temperature control in these groups (Figure 6).

Conclusion

The use of the animal model for the traumatic brain injury by fall of weight represents an alternative of low cost and easy reproduction that allows great variation in the levels of energy trauma and produces lesions and clinical parameters compatible with the objectives of the studies in experimental surgery for the cranium encephalic trauma in rats.

References

1. Simard JM, Kahle KT, Gerzanich V. Molecular mechanisms of microvascular failure in central nervous system injury - synergistic roles of NKCC1 and SUR1/TRPM4. *J Neurosurg.* 2010;113:622-9.
2. Cernak I. Animal models of head trauma. *NeuroRx.* 2005;2(3):410-22.
3. Park HK, Fernandez I, Dujovny M, Diaz FG. Experimental animal models of traumatic brain injury: medical and biomechanical mechanism. *Crit Rev Neurosurg.* 1999;9:44-52.
4. Dvilevicius AE, Prandini MN, Dobrowolski S, Barbosa AC. Estudo de traumatismo cranioencefálico experimental em ratos com aparelho de impacto cortical controlado. *Arq Bras Neurocir.*

- 2008;27(2):42-6.
5. Marmarou A, Foda MA, van den Brink W, Campbell J, Kita H, Demetriadou K. A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. *J Neurosurg.* 1994;80(2):291-300.
 6. Foda MA, Marmarou A. A new model of diffuse brain injury in rats. Part II: Pathophysiology and biomechanics. *J Neurosurg.* 1994;80(2):301-13.
 7. Onyszchuk G, Al-Hafeza B, He Y, Bilgen M, Berman NEJ, Brooks WM. A Mouse model of sensorimotor controlled cortical impact: characterization using longitudinal magnetic resonance imaging, behavioral assessments and histology. *J Neurosci Methods.* 2007;160(2):187-96.
 8. Adelson PD, Robichaud P, Hamilton RL, Kochanek PM. A model of traumatic brain injury in the immature rat. *J Neurosurg.* 1996;85:877-84.
 9. Schossler JE, Schossler DR. Avaliação clínica da anestesia geral pela tiletamina-zolazepan associada ao fentanil em ratos (*Rattus norvegicus albinus*). *Acta Cir Bras.* 1993;8(1):32-4.
 10. Marques RG, Caetano CER. Parâmetros fisiológicos em animais de pequeno e médio porte. In: Marques RG. *Técnica Operatória e Cirurgia Experimental*. Rio de Janeiro: Guanabara Koogan; 2005.
 11. Lighthall JW. Controlled cortical impact: a new experimental brain injury model. *J Neurotrauma.* 1988;5:1-15.
 12. Dixon CE, Clifton GL, Lighthall JW, Yaghmai AA, Hayes RL. A controlled cortical impact model of traumatic brain injury in the rat. *J Neurosci Methods.* 1991;39:253-62.
 13. Carvalho LFA, Affonseca CA, Guerra SD, Ferreira AR, Goulart EMA. Traumatismo cranioencefálico grave em crianças e adolescentes. *Rev Bras Ter Intensiva.* [serial on the Internet] 2007 Mar; 19(1). Available from URL: <http://www.scielo.br/rbti>
 14. Seppelt I. Intracranial hypertension after traumatic brain injury. *Indian J Crit Care Med.* 2004;8:120-6.
 15. Shalit MN, Cotev S. Interrelationship between blood pressure and regional cerebral blood flow in experimental intracranial hypertension. *J Neurosurg.* 1974;40(5):594-602.
 16. Shapira Y, Shohami E, Sidi A, Soffer D, Freeman S, Cotev S. Experimental closed head injury in rats: mechanical, pathophysiologic, and neurologic properties. *Crit Care Med.* 1988;16:258-65.
 17. Vink R, Golding EM, Williams JP, McIntosh TK. Blood glucose concentration does not affect outcome in brain trauma: A 31P MRS study. *J Cereb Blood Flow Metab.* 1997;17(1):50-3.
 18. Melo JRT, Reis RC, Lemos-Júnior LP, Coelho HMS, Almeida CER, Oliveira-Filho J. Hyperglycemia in pediatric head trauma patients: A cross-sectional study. *Arq Neuropsiquiatr.* 2009;67(3-B):804-6.

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