

Therapeutic ultrasound and essential oil of melaleuca alternifolia interfere with muscle regeneration?

Ultrassom terapêutico e óleo essencial de melaleuca alternifolia interferem na regeneração muscular?

¿El ultrasonido terapéutico y el aceite esencial de melaleuca alternifolia interfieren en la regeneración muscular?

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Abstract

Currently the Essentials oil of Tea Tree is used in healing, for possessing various medicinal properties. Phonophoresis is a method used in physiotherapy, where pharmacological substances associated with a coupling agent, are induced by ultrasonic waves in the underlying tissues. Our objectives are aimed at monitoring and qualitative analysis of the lesion treated with the compound and the method accuracy phonophoresis. We used twenty (20) rats, which are divided into two (2) different groups: Group I: Rats harmed without intervention and Group II: Rats victims treated with the compound by Phonophoresis. Histological analysis showed the material collected in samples from animals in group I, an extensive and diffuse inflammation, unconnected with large amounts of inflammatory cells, as in group 2 inflammation comes in mild areas with limited, there is also muscle fiber regeneration. The results seem to demonstrate the efficacy of Melaleuca alternifolia essential oil by phonophoresis in the muscle fiber in the lesion region.

Keywords: Phonophoresis; Tea tree oil; Musculoskeletal system; Drug effect.

Resumo

Atualmente o óleo essencial de Tea Tree é utilizado na cura, por possuir diversas propriedades medicinais. A fonoforese é um método utilizado na fisioterapia, onde substâncias farmacológicas associadas a um agente de acoplamento, são induzidas por ondas ultrassônicas nos tecidos subjacentes. Nossos objetivos visam o monitoramento e análise qualitativa da lesão tratada com o composto e a precisão do método fonoforese. Foram utilizados trinta (30) ratos, que são divididos em três (3) diferentes grupos: Grupo I: Ratos prejudicados sem intervenção. Grupo II: Ratos vítimas tratados com o composto por Fonoforese: Grupo III: Ratos feridos tratados com o composto. An análise histológica mostrou no material coletado em amostras de animais do grupo I, uma inflamação extensa e difusa, desvinculada de grande quantidade de células inflamatórias, já que no grupo 2 a inflamação vem em áreas leves com limitação, há também regeneração das fibras musculares. Os resultados parecem demonstrar eficácia do óleo essencial de Melaleuca alternifolia por fonoforese na fibra muscular na região da lesão.

Palavras-chave: Fonoforese; Óleo de melaleuca; Sistema musculo esquelético; Efeito de fármacos.

Resumen

Actualmente, el aceite esencial de Tea Tree se utiliza en la curación, ya que tiene varias propiedades medicinales. La fonoforesis es un método utilizado en fisioterapia, donde las sustancias farmacológicas asociadas con un agente de acoplamiento son inducidas por ondas ultrasónicas en los tejidos subyacentes. Nuestros objetivos son el seguimiento y análisis cualitativo de la lesión tratada con el compuesto y la precisión del método de fonoforesis. Se utilizaron treinta (30) ratas, las cuales se dividen en tres (3) grupos diferentes: Grupo I: Ratas dañadas sin intervención. Grupo II: Ratas víctimas tratadas con el compuesto por Fonoforesis: Grupo III: Ratas lesionadas tratadas con el compuesto. Un análisis histológico mostró en el material recolectado en muestras de animales del grupo I, una inflamación extensa y difusa, desvinculada de gran cantidad de células inflamatorias, ya que en el grupo 2 la inflamación se presenta en áreas claras con limitación, además hay regeneración de músculo fibras. Los resultados parecen demostrar la eficacia del aceite esencial de *Melaleuca alternifolia* por fonoforesis en la fibra musculoesquelética.

Palabras clave: Fonoforesis; Aceite de árbol de té; Sistema musculoesquelético; Efecto de la droga.

1. Introduction

The regenerative potential of skeletal striated muscles is a target of investigations, since there are phases of this process have not yet completely established (Cameron et al., 1992). This ability to repair injured tissue is essential to ensure homeostasis of the body and mind of the individual. With the reduction of the time of this tissue repair process generate great beneficial effects, such as the reduction of time spent in the hospital bed, avoiding complications in hospital that the individual is exposed, and thus allow more rapid return to their everyday activities and its general well-being. The muscle between the soft tissues, is the most plastic and changeable and can therefore respond to stimuli in normal and pathological. The occurrence of this plasticity due to the relationship between the muscle fibers and other components of the unit basic muscle how neuromuscular junction, extracellular matrix and satellite cells (Cunha et al., 2001).

The attempt to understand the regenerative process in its entirety is justified by its importance in the context of sports, medicine, physiotherapy and other professions who study or work with the preservation, and health of human movement. This quest for preservation finds echoes in research in the scientific world to find shortcuts to get to the healing process faster and more efficiently. One of the healing topical components that have been gaining attention from researchers with encouraging results is the essential oil of *Melaleuca* popularly known as Tea Tree, (Almeida-Junior et al., 2022). Tea tree oil has great antioxidant, antifungal, antibacterial, anti-inflammatory and antihyperproliferative potential, (Zhong et al., 2021). To maintain the quality and integrity of *Melaleuca* oil, its extraction is carried out by steam, (Johns et al., 1992; Sevik et al., 2021). The penetration of drugs in subcutaneous tissue can be facilitated through the ultra sound treatment (UST) by phonoforesis method. The phonoforesis consists of topical application of pharmacological substances associated with some skin through the coupler agent by UST for the underlying tissues (Gaban et al., 2007; Machado, 2008; Morsoleto et al., 2015).

The monitoring and evaluation of lesion treated with aqueous composite based essential oil of Tea Tree (*Melaleuca Alternifolia*), the performance of the gelatin industrialized as experimental way coupler agent and the observation of the accuracy of the method of phonoforesis in applicability as a promoter of cutaneous permeation of compound used.

2. Methodology

This experimental project was carried out in the histology and microscopy laboratories and in the operating rooms of the animal experimentation facility at Fundação Hermínio Ometto (FHO) in Araras, SP. The procedures and handling of the animals followed the rigorous principles of CONEP for the use of animals in research. Being approved by the Research Ethics Committee and Scientific Merit of the FHO under registration number 054/2009. Fifteen male *Rattus nonerficus wistar* rats with an average weight of 200 g and 60 days old were used for this experiment, divided into 3 groups of 5 animals, which were maintained with balanced chow and water ad libitum, during light/dark periods for 12 hours, heated to an average ambient temperature of 25°C. essential oil of *Melaleuca alternifolia*, Bio Essence® incorporated into gelatin at a concentration of 10%.

The animals were anesthetized with an intraperitoneal injection of 0.3 ml of Ketamine and 0.1 ml of Xylazine for each 200g of body weight. The experimental lesion with 2 cm in length and 1.5 cm in depth on the right hind paw according to the protocol of Morsoleto et al., 2015. It was added to the animals after intervention, 10 drops of Dipyron added to each liter of water in their drinkers. This gel associated with 10% Melaleuca oil was applied on the surgical lesion in gentle circular movements with a BIOSET Sonacel Plus 1 MHz ultrasound device (Indústria de Tecnologia Eletrônica Ltda.), with an area of 0.5 cm² and the diameter of the 0.8 cm transducer tip, (Bazin & Kitchen, 1998).

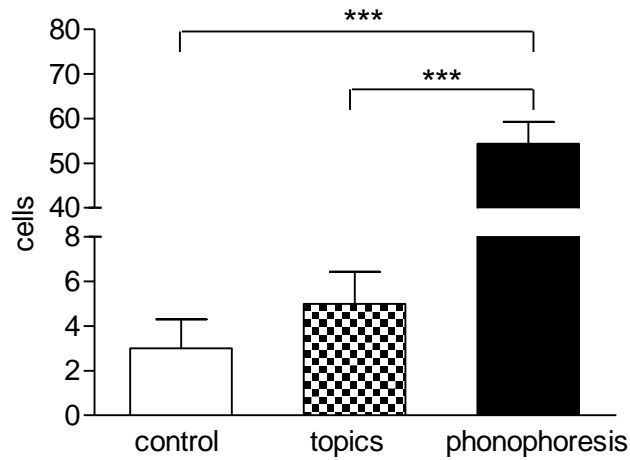
The transducer device was calibrated in a UPM DT-10 Ultrasound Power Meter radiometric scale with OHMIC precision with an accuracy of more or less 20mW. Rats were divided into three (3) groups. Group I (control, injured without treatment); group II (injured and treated with 10% Melaleuca gel); group III (injured patients, treated with the gel associated with 10% Melaleuca oil through ultrasound, Phonophoresis). Shortly before the surgical procedure to locate the incision, shaving was performed and the surgical procedure was performed with a skin incision in the right hind paw of the biting rats, removing the integumentary tissue and muscle tissue. in experimental laboratory. Adding 50 ml of Tea Tree essential oil to every 500 ml of gel. In each topical application or via phonophoresis, five ml were used per section in each animal. The treatment via phonophoresis (Cameron, 1992; Morsoleto et al., 2015; Mororó et al., 2020; Moraes et al., 2022) used an intensity of 0.3Wcm², frequency of 1 MHz continuously for one minute, with circular movements. In group II (topical treatment) the procedure was performed with the device turned off. After the ten predetermined sessions, the animals were euthanized on the 11th day, samples were collected to be prepared histologically and stained in Hematoxylin-Eosin. Histological slides were analyzed qualitatively and quantitatively and photo documented, protocol of Meijer et al., (1997). 10 fields were examined on each slide. Quantitative analysis was performed through field counting of inflammatory infiltrates, neoangiogenesis and the presence of muscle regeneration. Statistical analysis was performed via Origin 8.0 using statistical methods and One Way Anova retest Turkey.

3. Results

In Figure 1, we have the quantitative representation of muscle regeneration of the following groups: group I (Co control) injured without treatment, group II (T topic) injured and treated with tea tree gel, without ultrasound and group III (F phonophoresis) treated with tea tree gel delivered through ultrasound.

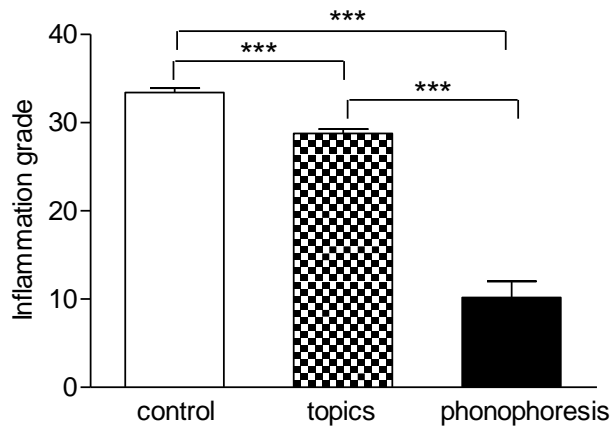
In statistical analysis of presence of muscular regeneration by field in the injury site, the Group III (F) presented greater number of cells per field parsed when compared to groups I. (*) shows the difference of Group I (Co) group III (F) and group II (T) (p 0.01).

Figure 1. Presence of muscle regeneration by observed field.



Source: Authors.

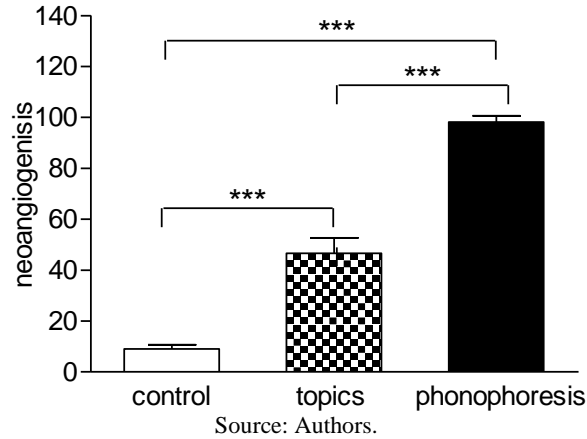
Figure 2. Degree of inflammatory infiltrates. Represents the inflammatory degree in the wound bed between the groups I (Co control), II (T topic) and III (F phonoforesis).



Source: Authors.

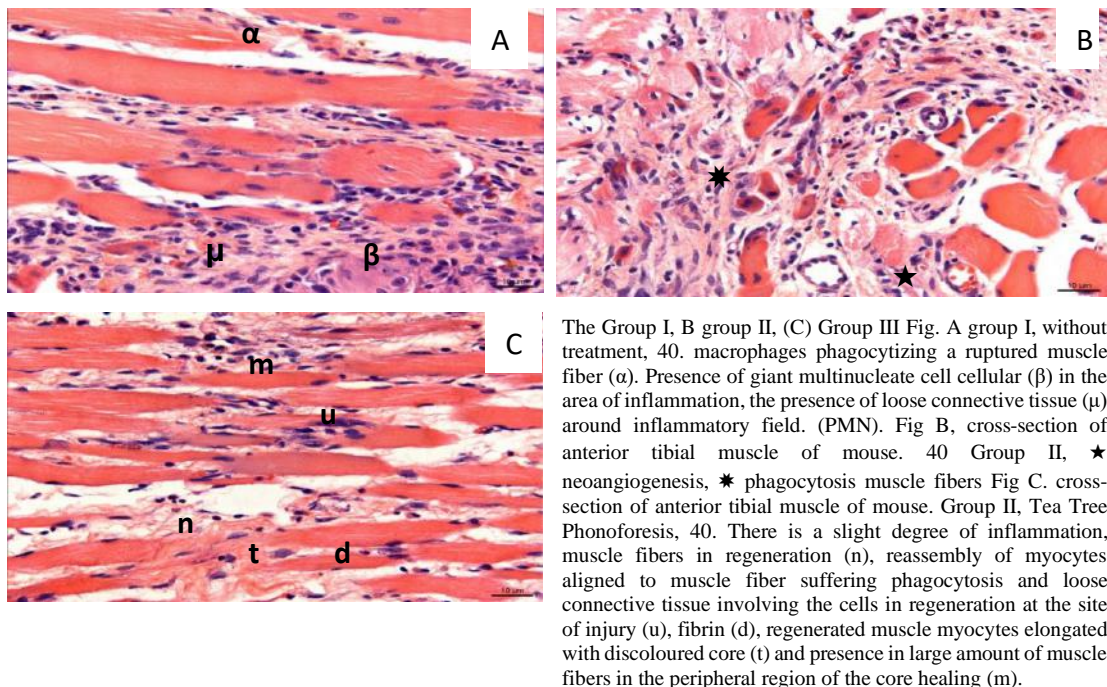
Statistical analysis of degree of inflammatory infiltrates per field present in the location of the injury site, group I (control) presented greater statistical score and the Groups II and III presented the lowest score. (*) evidence of difference between Groups (p 0.01).

Figure 3. Presence of neo-vascularization. Represents the neo-vascularization between the groups I (Co control), II (T topic) and III (F phonoforesis).



The Figure 3 represents the statistical analysis of presence of neo-vascularization by field in the location of the injury. The Group III presented greater number of vessels up when compared to the group I. (*) shows the difference of Group I (p 0.01).

Figure 4. Histological sections of groups A, B and C.



Source: Authors.

Figure 4A. Cross section of the rat tibialis anterior muscle. Group I, no treatment, Bar = 10 μ m, 40x magnification. A group of macrophages is observed phagocytosing a torn muscle fiber (α). Presence of multinucleated cellular giant cells (β) in

the area of inflammation, presence of loose connective tissue (μ) throughout the inflammatory field and large presence of giant cells called polymorphonuclear cells (PMN).

Figure 4B. Cross section of the rat tibialis anterior muscle. Group II, Tea Tree + Phonophoresis, Bar = 10 μ m, 40x magnification. Mild inflammation is observed, regenerating muscle fibers(n), myocytes aligned to reconstitute the phagocytosed muscle fiber and loose connective tissue involving regenerating cells at the injury site (u), regenerated muscle fibrin(d), myocytes elongated with an isochoric core(t) and the presence of a large number of muscle fibers with a peripheral core in the healing region (m). Figure C. cross-section of anterior tibial muscle of rat. Group II, Tea Tree Phonoforesis, 40. There is a slight degree of inflammation, muscle fibers in regeneration (n), reassembly of myocytes aligned to muscle fiber suffering phagocytosis and loose connective tissue involving the cells in regeneration at the site of injury (u), fibrin (d), regenerated muscle myocytes elongated with discoloured core (t) and presence in large amount of muscle fibers in the peripheral region of the core healing (m).

In figures 4A, B and C, histological sections of groups I, II and III can be seen. Evidence of the inflammatory process quite accentuated in I, losing expressiveness in II and III. Regeneration of muscle fibers, phagocytosis and neovascularization. Following the events, the examination of the samples referring to group II (Figure 4B) showed that the inflammation is mild when compared to the control group, and it can also be observed in the muscle fibers due to regeneration, the action of the loose connective tissue present in all processes. involving the cells at the focus of the scar lesion and the presence of regenerated fibrin, elongated muscle myocytes with an isochoric nucleus and the presence of a large amount of muscle fibers in the peripheral region of the healing nucleus (Rubin et al., 2005; Rocha & Cavallieri, 2007).

4. Discussion

The tissue repair is a process that encompasses various blood cells, cell arrays and some processes, where the principal is inflammation. Inflammation is at the start of the repair of injured tissue, acts as a protective response causing a tissue reaction to an aggression-inducing vascularised tissue, pathogenic to the release of inflammatory mediators (Golab et al., 2007). The ability to intervene and accelerate this physiological process is essential to your optimization because the entire process of inflammation only ends when the offending agent is removed and all mediators of inflammatory response are destroyed (chart type 2) (Olsson et al., 2006; Morsoleto et al., 2015).

Obvious in cells, PMN group I increase the disabilities caused by injury, making aggregation work for muscle cells myocytes reconstruction, thereby filling the region with loose connective tissue and muscle not. The delay in regeneration favors more healthy tissue and therefore the muscle tissue turns into a loose collagen tissue that evolves into a fibrous tissue (dense collagen), thus making a thicker scar, hard and with little elasticity (Pereira, 2004). Concurring with the results found in Group I, (Abbas et al., 2012), claim that the evolution of tissue repair process encompasses several events that involve local and systemic factors in an attempt to reestablish the normal structure and function of the injured tissue. is at the beginning of this repair attempt, initially there is presence of neutrophils that possess immediate action destroying pests, bacteria and other foreign agents in the area, but can prolong the inflammation causing tissue injury so exacerbated

When this inflammatory process persists a longer time, week to months, either through prolonged exposure to toxic agents and aggressors, by certain microorganisms or even by autoimmunity, it is called chronic inflammation, which this will associate the presence of other cells and mononuclear leukocytes and B-lymphocytes, eosinophils, mast cells, T the plasma cells, macrophages, the actions of angiogenesis and tissue necrosis and fibrosis obvious characteristics, on the results of Group I. When this inflammatory process persists a longer time, week to months, either through prolonged exposure to toxic agents and aggressors, by certain microorganisms or even by autoimmunity, it is called chronic inflammation, which this will associate the presence of other cells and mononuclear leukocytes and B-lymphocytes, eosinophils, mast cells, T the plasma cells, macrophages,

the actions of angiogenesis and tissue necrosis and fibrosis obvious characteristics, on the results of the group I (Morsoleto et al, 2015; Silva et al., 2007), claim that the inflammation and scarring process begins almost at the same time, i.e. working together since the beginning.

The repair process is the reabsorption of cellular debris and the release of growth factors released by neighboring cells inducing cell multiplication with parenchymateous cells. Initially are released in the area injured chemical mediators that communicate with the preserved cells initiating the inflammatory processes. After the action of phagocyte, arise in place, fibroblasts and endothelial cells from proliferating capillaries within the limits of healthy cells, i.e., the higher the number of phagocytic higher occupancy by vascularised tissue, connective tissue, and spot neo formed from non-victim, found similar to results of this work.

The healing process then consists in replacement of injured tissue by a vascularised tissue, connective tissue (figures 1, 2, 3), where the beginning of the whole process is the installation of inflammation is responsible for the arrival of the Phagocytic then the proliferation of fibroblasts and endothelial cells that result in scar tissue. The reduction of inflammation, as well as the acceleration of healing with the aggregation of fibroblasts and large amount of vessels up shown in Group II (figures 1,2) can be justified by the anti-inflammatory action of the essential oil of Tea Tree (*Melaleuca Alternifolia*), because according to Silva (1998), Santos et al., 2022 this essential oil has many medicinal properties as antiseptic, bactericidal, germicidal, scarring, antifungal, anti-inflammatory, painkiller, among others. (Golab et al., 2007; Hart et al., 2000; Cox et al., 2001; Santos et al., 2022), still claim that this essential oil is a secure means of preventing the inflammation. In addition, possess immune stimulant properties interacts in the production of white blood cells and a direct action in microorganisms, so an alternative treatment for patients with low resistance or diseases that weaken your immune system and promote the emergence of unwelcome diseases in your body (Hammer et al., 2003).

It is suggested in this study that the action of the essential oil of Tea Tree was increased by phonoforesis. According Bazin & Kicthen, (1998) and Moraes et al., (2022) claim that the acoustic waves produced by this equipment increases the permeability of the membrane of platelets, which releases serotonin and other chemicals, moreover, the UST causes the mechanical vibration on cell membrane molecules, thus this permeability is increased, favoring the entry of drug percutaneous favoring that side effects caused by chemical mediators in an inflammation to be minimised Rocha et al., (2007). An important factor in the applicability of phonoforesis is choosing its parameters, calls for the continuous regime on grounds of time optimization of ultrasound interaction with drugs and the fabric, (Olsson et al., 2006; Machado, 2008; Mororó et al., 2020).

In continuous mode, the generation of ultrasonic wave remains constant throughout the implementation, pulsed wave mode already has varying intervals. However, it is more frequent in clinical routine ultrasound equipment act in pulsed regime frequently fixed 100 Hz pulse repetition, which allow the mechanical effect of therapy outweighs the thermal effect, favoring the process of repair of connective tissues (Rubin et al., 2005; Pereira, 2004). So, based on these concepts, researched and published was resolved using continuous wave emission mode ultra wave sonic in Group II. By its very nature, the ultrasound waves cannot travel through the air, i.e., in order to have the proper transmission of energy ultra-sonorous to the tissues depends on an agent between coupler and transducer metal skin. According to literature the ideal agent in acoustic terms is water, but needs to be maintained between the transducer and the fabric, so the water can only be used in its liquid form, if it is contained within a container or turned into aqueous gels having acoustic properties similar to water and with a high viscosity, thereby facilitating the application of the transducer to the skin sliding and, there are reports that authors also used in his studies with positive results other compounds like couplers creams and ointments.

These authors listed as key requirements for the coupling means the conditions of absence of gas bubbles, high viscosity, sterility, economically cheap (Abbas et al., 2012). Muscle regeneration course in Group III (figure 1) cannot be justified by Silva et al., (2007) and Rocha & Cavallieri (2007). because in their studies claim that the satellite cells remain undifferentiated adult

myoblasts or quiescent between the plasma membrane of the muscle fiber and the basal membrane when stimulated, these cells are enabled, proliferate and joins the muscle fiber. After a muscle trauma, basal lamina break, with the help of satellite cells macrophages migrate to the region of the injury producing growth factors, such as platelet-derived growth factor (PDGF) that has the function of chemo taxis, proliferation and differentiation of satellite cells and stimulate the proliferation of fibroblasts, but this process can be harmful, because the fibroblasts deposited more rapidly than the muscle satellite cell, taking a hard and fibrous tissue.

To join the muscle regeneration process, mediated by inflammatory cells in the event lesion, leukocytes are polymorphs found at places since the initial injury, macrophages can be seen on 6:0 and increasingly since the predominant cell type of lesion focus. Upon arrival at the site of injury also macrophages release chemo taxis factors to the satellite cells. Satellite cells to cross the barrier of the basal lâmina release enzymes (trypsin and prophase), which have the function of dissolving the membrane, to the satellite cells have free way to the center of the lesion and begin the process of muscle regeneration. The proliferation of these cells can still be stimulated by fibroblast growth factors, which enhance the postoperative period, plaster fiber and innervations.

5. Conclusion

As phonophoresis is the physical mechanism where mechanical sound vibrations above 20,000 Hz cross the epidermis, drugs can be delivered to these sound waves and enter through the tissues. Being an interesting alternative of drug administration. Because it is a non-invasive technique, it has been widely used, associated with several anti-inflammatory agents. In this work, the authors concluded that the presented findings seem to indicate that when the gel with association of Tea Tree essential oil (*Melaleuca Alternifolia*), used via phonophoresis, produced statistical improvements in the resolution of the lesion.

We suggest other researches that address various concentrations of drugs, using laboratory techniques that recover essential elements of the substance used in the treated tissues.

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