Pulsed Versus Continuous Phenytoin Phonophoresis in Accelerating the Burn Wound Healing in Rats

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Abstract: Objective*:* this study was meant to help physical therapists determine whether phenytoin phonophoresis has better effect than topical application alone on the healing process of the burn wound. Moreover, it is meant to determine whether pulsed or continuous phonophoresis is the best to achieve optimum burn wound care. **Procedure**: forty five adult male Sprague-Dawley rats were used in this study ranging between 10-12 weeks of age, and weighing 200-250g at the start of the experiment. One deep full thickness (third degree) burn wound was induced on the trimmed back of the neck by contact for 10s with 127^{o}C heated metal stamp, 500mm^2 in area. The rats were randomly divided into three equal groups; each group included fifteen rats. The pulsed phonophoresis group (**PPG**) was treated with pulsed ultrasound (I=0.8W/cm^2 and F=3MHz for 5minutes) and phenytoin, the continuous phonophoresis group (**CPG**) was treated with continuous ultrasound (I=0.8W/cm^2 and F=3MHz for 5minutes) and phenytoin, and the control group (**CG**) received topical phenytoin application with placebo ultrasound. ***RESULTS:*** The findings showed significant difference in favor of the continuous phenytoin phonophoresis group (**CPG**) over the other two groups, i.e. the pulsed phenytoin phonophoresis group (**PPG**) and the control group (**CG**). Thus, these results support the suggestion that the application of continuous phenytoin phonophoresis over the burn wound is very effective in accelerating the burn wound healing than topical application of phenytoin or pulsed phenytoin phonophoresis over the burn wound.

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1. Introduction

Chronic wounds are always a major source of morbidity, leading to extensive disability, and are thereforelinkedto increased mortality; Petrie *et al.* **(2003**) stated that chronic wounds have a significant impact on public health and the outflow of healthcare resources.One of the oldest challenges for medical practice is thepursuitof better wound healing agents. One such agent that has been tried in wound healing is phenytoin. It was observed that phenytoin had a stimulatory effect - when orally administered - on gingival tissue hypertrophy, and this observation prompted its assessment and use topically for different kinds of wounds and ulcers such as war wounds, sores caused by venous stasis, atrophic ulcers and burns, and positive effects have been reported (**Modaghegh *et al.,* 1989**). The ability of phenytoin to accelerate ulcer healing was described more than 40 years ago (**Hollisaz *et al.,* 2004**). And, in 1958, a clinical study demonstrated that phenytoin sodium accelerates gingival wound healing compared with controls (**Sinha and Amarasena, 2008**). Studies have presented topical phenytoin asstimulating healing for burns, traumatic wounds, and diverse ulcers such as decubitus ulcers, venous stasis ulcers, diabetic ulcers, and leprosy trophic ulcers (**Bhatia and Prakash, 2004**).

Phonophoresis iswell-defined as the process of increasing skin absorption and penetration of the topical medications to the deep tissues using ultrasound. (**Saime *et al.,* 2010**).

On the other hand, ultrasound is a very wide spread modality among physical therapists, used in a wide variety of conditions, mostly for enhanced healing of soft tissues and drug administration through the skin. And thus, this study is meant to help physical therapists determine whether phenytoin phonophoresis has better effect than topical application alone on the healing process of the burn wound. Moreover, it is meant to determine whether pulsed or continuous phonophoresis is the best to achieve optimum burn wound care.

2. Method

Forty five adult male Sprague-Dawley rats were used in the study, ranging between 10-12 weeks of age, and weighing 220g-250g at the start of the experiment. Rats were weighed then anesthetized by intra-peritoneal injection of Thiopental sodium (80mg/kg). The hair in the back of the neck was trimmed, then one deep full thickness (third degree)

burn wound was induced on the back of the neck by contact for 10s with the 127°C heated metal stamp, 500mm^2 in area. The stamp was mounted on a special electro-mechanical device, built especially for the experiment. The temperature of the stamp and the contact duration were controlled electronically in the device to minimize the error that may result from the process of inducing the burn wound.The rats were then randomly divided into three equal groups; each group included fifteen rats.

a. **The pulsed phonophoresis group** (**PPG**): which received pulsed phenytoin phonophoresis using pulsed ultrasound.

b. **The continuous phonophoresis group** (**CPG**): which received continuous phenytoin phonophoresis using continuous ultrasound.

c. **The control group (CG):** which received topical phenytoin application. This group received placebo ultrasound, i.e., application of the ultrasound head while the device was being off.

All treatments were carried out on a daily basis for thirty consecutive days using 10% phenytoin preparation and the ultrasound device.

Table 1: Animal Weight Data for the Rats in the Three Various Group

Item	*Animal Weight (gr)*		
	CPG	*PPG*	*CG*
Mean	*250.80*	*252.40*	*253.13*
±SD	*18.38*	*20.55*	*19.28*
Maximum	*294*	*285*	*298*
Range	*77*	*66*	*77*
Minimum	*217*	*219*	*221*
F	*2305.846*		
p-value	*$8.93*10^{-15}$*		
Level of significance	*Non-Significant*		

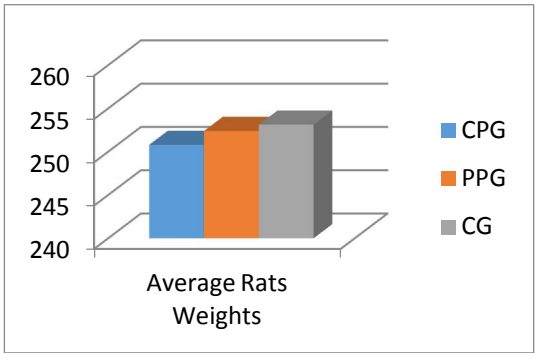

Figure 1: Mean values of weight among three various groups of the study

Wounds were photographed using the ***Olympus S310***high resolution digital camera. The photos were then transferred into a laptop to calculate the burn wound area. Computer aided design software (**AutoCAD**) was used to compute and compare the burn wound surface areas. Animals were inspected twice a week and the healing was assessed based on physical parameters especially wound contraction and epithelialization. The *mean* and the *standard deviation*were calculated for all groups after the end of the experiment. The mean, the standard deviation and range were used as a primary source of connecting facts about each parameter to measure central tendency. The *percentage of the absolute changes*was calculated within each group and between groups to detect level of significance for improvement.

Analysis of variance (**ANOVA**) was used to test significant differences between groups and $p<0.05$ was considered significant. Wound contraction was measured according to the Walker formula (**Meena *et al.*, 2011**):

$$\%WoundArea = \frac{WoundAreaondayX}{WoundAreaonFirstDay} * 100$$

$$\%Wound\ Contraction = 100 - \%Wound\ Area$$

Or the adjusted Walker formula

$$\%WoundArea = \frac{WoundareaondayX}{Woundareaonpreviousday} * 100$$

$$\%Wound\ Contraction = 100 - \%Wound\ Area$$

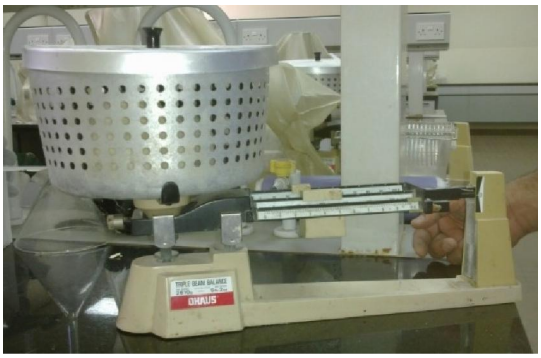

Figure 2: Weighing the rats at the start of the experiment

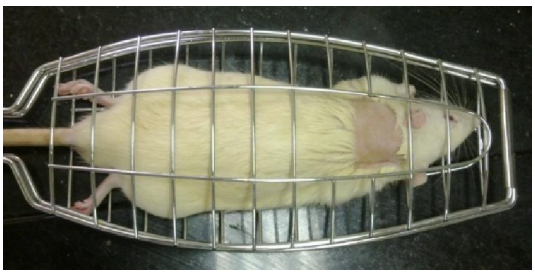

Figure 3: The rat was prepared by trimming the back of the neck, and then it was held in the special holder before inducing the third degree burn

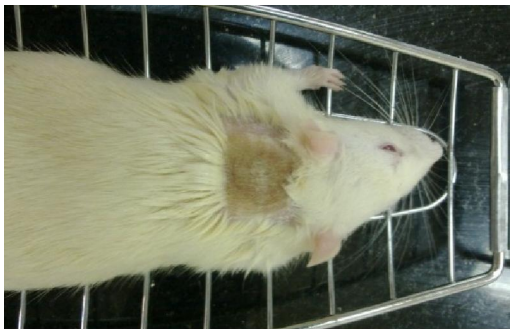

Figure 4: The rat after inducing the burn

3. Results

The average percentage wound contractions relative to the first day for the continuous phenytoin phonophoresis (**CPG**) ranged between 0.36% and -0.03% with an average 0.11±0.11%. As for the pulsed phenytoin phonophoresis group (**PPG**), the average percentage wound contractions ranged between 0.25% and -0.10% with an average -0.03±0.11%. Finally, the average percentage wound contractions for the control group (**CG**) ranged between 0.19% and -0.14% with the average -0.05±0.11%.

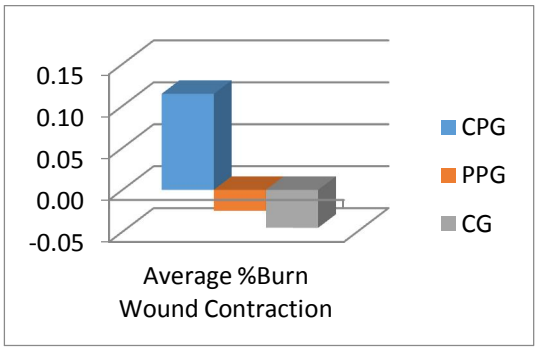

Figure 5: Average percentage burn wound contraction of three various groups of the study relative to the first day

The statistical analysis of the mean differences of the percentage burn wound contractions relative to first day for all groups after thirty days of therapy revealed the following results:

There was a highly statistical significant decrease (p=6.26x10^{-4}<0.05) in burn wound area in the continuous phenytoin phonophoresis group (**CPG**) compared to the other therapeutic group (**PPG**) and the control group (**CG**).

However, there was no significant difference (p=0.62>0.05) in burn wound contraction between the **PPG** and **CG**, i.e., no significant difference whether the rats received the pulsed phenytoin phonophoresis regime or just got the topical application of phenytoin on the burn wounds.

The percentage wound contraction relative to the previous day for the continuous phenytoin phonophoresis (**CPG**) ranged between 19.32% and 29.41% with an average 23.73±3.91%. As for the pulsed phenytoin phonophoresis group (**PPG**), the percentage wound contraction ranged between 14.39% and 18.99% with an average 16.86±1.44%. Finally, the percentage wound contraction for the control group (**CG**) ranged between 9.48% and 28.75% with the average 16.45±5.83%.

The modification of Walker formula was to accentuate the progress of the burn wound healing process during small time intervals, i.e., between a specific day and the previous day without always going back to the first day as it is the case using the Walker formula.

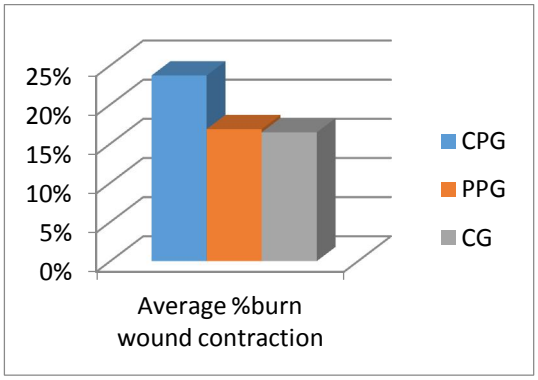

Figure 6: Average percentage burn wound contraction of three various groups of the study relative to the previous day according to the modified Walker Formula

Analysis of variance (**ANOVA**) was used to test the significant differences between the groups. The findings of this experiment calculated using the modified Walker formula were analyzed.

There was a highly statistical significant decrease (p= 2.73x10^{-5}<0.05) in burn wound area in the continuous phenytoin phonophoresis group (**CPG**) compared to the other therapeutic group (**PPG**) and the control group (**CG**).

However, there was no significant difference (p=0.80>0.05) in burn wound contraction between the **PPG** and **CG**, i.e., no significant difference whether the rats received the pulsed phenytoin phonophoresis regime or just got the topical application of phenytoin on the burn wounds.

4. Discussion

The burn wounds kept expanding at many instances during the first few days of the experiment. The expansion can be partly due to the inaccuracy in the visual assessment of burns; burns are dynamic wounds and they are in a state of change for up to 72

hours after injury and may be subjectiveto resuscitation conditions. Thermal trauma causes two different sorts of injury within the burn wound. An immediate and permanent injury, and a late and possibly reversible injury in the area closestto the center site, called the zone of stasis which is characterized by a recognized edema, which in turn is surrounded by an area of inflammation and active edema formation due to vasodilatation and increased micro-vascular permeability. Excessive local edema increaseis followed by a decrease in perfusion, leading to more local tissue ischemia. Local microcirculation is compromised to the worst extent between twelve to 24 hours post-burn. **Atiyeh *et al.* (2005)** claimed that the depth of superficial burns appears to remain stable with time, whereas that of deep dermal burns increases with time, and burn wounds continue to demarcate for several days.

Another cause for the expansion in the burn wounds during the first two weeks is mechanical in nature; the rats were adding to the burn wound areas through aggressive contact and friction of the wound against the different objects in the cages, such as the water bottles, the metallic constructions in the cage…

According to this study, it was observed in all groups that there was an obvious drop in burn wound areas at the end of the treatment period. Regarding the decrease in burn wound areas for both the pulsed phenytoin phonophoresis group (**PPG**) and the control group (**CG**) that received topical application of phenytoin, no significant difference was found (p=0.62>0.05, F=0.25) [Walker formula] and (p=0.8>0.05, F=0.07) [modified Walker formula]. However, regarding the continuous phenytoin phonophoresis group (**CPG**), which underwent a dramatic decrease in burn wound areas, the statistical analysis demonstrated a significant difference (p=0.0025<0.05, F=11.05) [Walker formula] and (p=0.000001<0.05, F=38.0) [modified Walker formula] between the (**CPG**) and (**PPG**), and (p=0.00065<0.05, F=14.72) [Walker formula] and (p=0.00059<0.05, F=15.01) [modified Walker formula] between (**CPG**) and (**CG**).

Although in this study the histological evaluation of the burn wounds was not performed, but the calculation of burn wound contraction, i.e. the decrease in burn wound areas was significant.

Ultrasound promoted wound contraction of pressure ulcers (**Maeshige *et al*., 2010**), and since wounds that remain open heal mainly by contraction, these findings indicate that promotion of wound contraction contributes to healing of pressure ulcer in chronic wounds. Also, **Ramli *et al.* (2009)** confirmed that direct application of ultrasound resulted in enhanced angiogenesis.

On the other hand, Phenytoin (*Diphenylhydantoin, 5, 5-diphenylimidazolidine-2, 4-dione*) or diphenylhydantoin sodium which is an effective anti-epileptic medication whose capacity to accelerate ulcer healing was reported more than 40 years ago (**Hollisaz *et al.*, 2004**). In their study, **Carneiro *et al.* (2002)** concluded that phenytoin is effective in suppressing burn wound bacteria and relieving pain thereby promoting healing.

The study was to investigate the effectiveness of combining both, the ultrasound therapy and phenytoin, to accelerate the healing of burn wounds, through pulsed and continuous phonophoresis.

Therefore, according to this study, the effectiveness of combining continuous ultrasound therapy with phenytoin in accelerating the healing of third degree burn wounds was obvious. And thus, continuous phenytoin phonophoresis on burn wounds is recommended to accelerate the healing process.

Summary

The effects of pulsed versus continuous phenytoin phonophoresis in accelerating the burn wound healing in rats were investigated in this study.

After trimming the hair in the back of the neck, the rats were anesthetized, then, one deep full thickness (third degree) burn wound was induced by contact for 10s with the 127^{o}C heated metal stamp, 500mm^2 in area. The stamp was mounted on a special electro-mechanical device, built especially for the experiment.

The temperature of the stamp and the contact duration were controlled electronically in the device to minimize the errors that may result from the process of inducing the burn wound.

The rats were randomly divided into three equal groups; each group included fifteen rats.

a. The pulsed phonophoresis group (**PPG**): fifteen rats received pulsed phenytoin phonophoresis using pulsed ultrasound (I=0.8W/cm^2 and F=3MHz) and Phenytoin, for five minutes daily for thirty consecutive days.

b. The continuous phonophoresis group (**CPG**): fifteen rats received continuous phenytoin phonophoresis using continuous ultrasound (I=0.8W/cm^2 and F=3MHz) and Phenytoin, for five minutes daily for thirty consecutive days.

c. The control group (**CG**): fifteen rats received topical Phenytoin application daily for thirty consecutive days. This group received placebo ultrasound, i.e., application of the ultrasound head while the device was turned off.

According to the study, the percentage wound contraction for the continuous phenytoin phonophoresis (**CPG**) ranged between 19.32% and 29.41% with an average 23.73±3.91%. As for the pulsed phenytoin phonophoresis group (**PPG**), the

percentage wound contraction ranged between 14.39% and 18.99% with an average 16.86±1.44%. Finally, the percentage wound contraction for the control group (**CG**) ranged between 9.48% and 28.75% with the average 16.45±5.83%.

The findings showed significant difference in favor of the continuous phenytoin phonophoresis group (**CPG**) over the other two groups, i.e. the pulsed phenytoin phonophoresis group (**PPG**) and the control group (**CG**). Thus, these results support the suggestion that the application of continuous phenytoin phonophoresis over the burn wound is very effective in accelerating the burn wound healing than topical application of phenytoin or pulsed phenytoin phonophoresis over the burn wound.

Conclusion

Continuous phenytoin phonophoresis was effective in accelerating the healing process of burn wounds in rats having third degree full thickness dermal burns.

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