Pulsed Short-Wave Diathermy Effects on Human Fibroblast Proliferation

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ABSTRACT. Hill J, Lewis M, Mills P, Kielty C. Pulsed short-wave diathermy effects on human fibroblast proliferation. Arch Phys Med Rehabil 2002;83:832-6.

Objectives: To investigate the influence of pulsed short-wave diathermy (PSWD) on fibroblast and chondrocyte cell proliferation rates and to establish the influences of different dosages applied.

Design: Four single-blind trials.

Setting: Laboratory, in vitro study.

Specimens: Human adult dermal fibroblast and chondrocyte cells were plated at known concentrations and incubated for 5 days.

Intervention: Exposure to PSWD, twice daily, on days 2, 3, and 4.

Main Outcome Measure: After crystal violet staining (day 5), optical density (cell number) was determined spectrophotometrically.

Results: PSWD, given at mean power of 48W for 10 minutes, increased fibroblast proliferation compared with control groups ($P<.001$). There was a relationship between cell proliferation and the amount of energy given ($P<0.001$). The optimal mean power for proliferation was estimated to be 13.8W. While keeping mean power constant at 6W, altering pulse duration and pulse repetition rate dosage parameters did not have a significant effect on proliferation ($P=.519$). Chondrocyte proliferation also increased with PSWD exposure of 6W at 10 minutes duration ($P=.015$). In addition, treatment time was significantly associated with chondrocyte proliferation ($P<.001$).

Conclusion: PSWD is associated with increased rates of fibroblast and chondrocyte proliferation in vitro, which is dose dependent. These results contribute to an understanding of the physiologic mechanisms underlying the therapeutic effects of PSWD.

Key Words: Chondrocytes; Diathermy; Electric stimulation therapy; Fibroblasts; Physical therapy; Rehabilitation.

PULSED SHORT-WAVE diathermy (PSWD) is an electrotherapy treatment modality that uses a specific radio-frequency band of the electromagnetic spectrum at 27.12MHz and wavelength of 11.6m.[1] Clinically, therapists have shown that PSWD has a therapeutic effect for a variety of musculoskeletal complaints.[2-4] Furthermore, evidence indicates that PSWD is extremely popular. In 1995, a UK national survey found that PSWD was being used almost ubiquitously in physiotherapy departments.[5] It also revealed that 98% of departments owned a PSWD machine and that 70% of these machines were being used at least 3 times a day, making PSWD the second most popular electrotherapy modality, after ultrasound. Surveys in Australia and North America reveal a similar picture.[6,7] However, despite both its widespread popularity and therapeutic usage, current understanding of both the physiologic benefits of PSWD and the optimum treatment settings to deliver it clinically have yet to be researched.[5,7,8]

Physiologic effects attributable to PSWD have been shown in the laboratory.[9-12] These effects include the in vivo acceleration of wound healing and nerve regeneration. In all of the cited studies, the investigators note that healing time is shorter in subjects exposed to PSWD than it is in untreated controls. In addition, a similar theme emerges in the different reports of these investigators as they provide possible explanations for the cellular mechanisms that may be accelerating the rate of healing. The common suggestion is that PSWD influences fibroblast cell proliferation rates. These cells are crucial to the healing process because they are involved in the synthesis of collagen, which repairs scar tissue. However, although this proposed cellular mechanism for PSWD has been widely acclaimed in the subsequent literature, in vitro studies to confirm its direct effects on fibroblasts or other closely related cells, such as chondrocytes, could not be identified.[13]

The relation between PSWD dosage and the optimum physiologic effect has also not been investigated. Clinically this has importance because the treatment effect may depend on the pattern and intensity of exposure. At present, a comprehensive selection of dosage variables is available, such as the mean power, pulse duration, pulse repetition rate, total time treated, and frequency of treatments. However, despite the wide choice of energy patterns, clinicians have scant empirical evidence on which to base their treatment selection because the physiologic influence of the various dosage parameters is still in question.[5]

This study was designed to investigate the hypothesis that PSWD directly accelerates fibroblast cell proliferation rates in vitro and to establish the influences of different applied dosage variables. We also studied the influence of PSWD on chondrocytes to assess whether this physiologic effect was cell specific.

METHOD

In a controlled laboratory setting, 4 single-blind trials were completed to investigate the influences of PSWD irradiation on fibroblast and chondrocyte cell division rates. The purpose of the first experiment was to establish whether PSWD affects fibroblast proliferation, with the null hypothesis that exposure to PSWD does not change the fibroblast cell division rate. The second experiment examined the relation between the amount

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of energy given and fibroblast proliferation. The third experiment investigated whether the pattern of energy given was influential. Finally, the fourth experiment was performed to determine if chondrocyte proliferation was influenced by exposure to PSWD and, if so, whether varying the length of treatment time was a determinant of the rate of cell proliferation.

Outcome Measure

The outcome measure for each experiment was optical density (a proxy measure of cell number). At the start of each experiment, a known number of cells were plated into microtiter plates, which were given different treatments (ie, PSWD, no PSWD). Cells were then allowed to culture for 5 days. This time period was selected based on pilot study findings that 5 days gave the cells time to proliferate without reaching confluence. The outcome measure of optical density was then assessed on day 5. Cells were harvested and stained using a crystal violet assay.[14] Optical density was ascertained spectrophotometrically in a plate reader at a known wavelength of 600nm.

Cell Culture

Three human adult dermal fibroblast cell lines from different individuals (at low, <5 passages) and a single cell line of chondrocytes were routinely cultured in standard conditions, which were Dulbecco's minimal essential medium with 10% fetal calf serum at 37°C in a humidified atmosphere with 5% CO_2. The cells were dispersed from nearly confluent cultures by .025% trypsin in .02% ethylene diaminetetraacetic acid solution. Cells were then spun in a centrifuge at 1000rpm for 4 minutes, after adding equal quantities of Dulbecco's minimal essential medium. A measured volume of growth medium was used to resuspend the cell pellets. Cell counts were performed by using a Sysmex CC-108 hemocytometer,[a] and an average of 16 counts were taken. Cells were then plated out in triplicate at known concentrations (1000 cells) into the 96-well, flat-bottomed microtiter plates[b] and incubated at 37°C for 5 days to let them proliferate. For all experiments, control and treatment groups were taken to a temperature-controlled room at 32°C (verified daily). However, control groups were placed away from the PSWD field at a distance greater than 3m, as recommended.[15]

Treatment

A calibrated Megapulse II unit[c] produced the PSWD output. The PSWD drum electrode was placed 2cm from the microtiter plate, which was placed on a wooden table. The peak pulse power of 150W was kept constant throughout all experiments because Megapulse II PSWD units come with this variable preset in the machine. The same inductive drum electrode was used throughout the study. Exposure to PSWD treatment was performed on days 2, 3, and 4 at twice daily regular intervals (morning, afternoon).

The mean power (MP) was calculated as follows:

$$MP = \frac{PPP \times PD \times PRR}{1{,}000{,}000}$$

where PPP is peak pulse power (amplitude/intensity of the pulse, in W); PD is pulse duration (length of each pulse or "on" period, in μs), and PRR is pulse repetition rate (number of pulses delivered in 1s, in Hz).

Analysis

The investigator was blind to treatment and control groups for the crystal violet assay and the recording of optical density. Statistical significance was set at P less than .05 (2 tailed) and analysis was performed by using SPSS, version 9.0,[d] for Windows 95.

Experiment 1: Effect of PSWD on Fibroblast Proliferation

To assess PSWD effect on fibroblast proliferation, we compared the optical density of a control group not exposed to PSWD, with an exposed treatment group (n=18 wells per group). For the treatment group, the PSWD dosage was 48W (800Hz$\times$400μs) mean power, for 10 minutes' duration (as recommended by the manufacturer). The high mean power was selected because it was thought that if an effect were to be found, it would most likely be seen with the maximum amount of mean power given. For statistical analysis, optical density was compared between groups with the Mann-Whitney U test.

Experiment 2: Relation Between Mean Power Dosage and Fibroblast Proliferation

The relation between mean power dosage and fibroblast proliferation was determined by comparing the optical density of 7 separate treatment groups with different exposure dosages as follows: 1, 3, 4, 8, 12, 24, 48W (n=16 wells per group). These dosages reflect the range of power available for clinical application of PSWD, which this unit could reproduce. Treatment time was 10 minutes. A difference in optical density between treatment groups was tested by using the Kruskal-Wallis test. Also, a combination of mathematical methods, statistical regression, and differential calculus was used to derive the PSWD dosage that corresponded to optimal optical density under the conditions of this experiment. For this analysis, optical density and dosage values were transformed to their natural logarithms so that quadratic regression could be performed as well as satisfy the assumptions of parametric statistical procedures.

Experiment 3: Effect of Different Patterns of Energy on Fibroblast Proliferation

To investigate whether different patterns of PSWD energy influenced fibroblast proliferation rate, the pulse repetition rate and pulse duration were varied while a constant mean power was maintained. A dosage of 6W (mean power) was selected because this power was easily reproduced with 3 different pulse patterns using the Megapulse II PSWD that had been calibrated for this study. The patterns of energy used were as follows: group 1=100μs$\times$400Hz; group 2=200μs$\times$200Hz; and group 3=400μs$\times$100Hz (n=11 wells per group). Treatment time was 10 minutes. Optical density of the 3 groups was compared by using the Jonckheere-Terpstra statistical test.[16]

Experiment 4: PSWD Effect on Chondrocyte Proliferation and the Influence of Dosage Time

This final experiment first investigated whether chondrocyte cells were influenced in the same way as fibroblasts and, then, whether variations in PSWD dosage duration influenced chondrocyte proliferation rate when mean power was kept constant at 6W. This was determined by comparing the optical density of a control group (no PSWD) with 4 treatment groups (PSWD) that had different exposure times: 5, 10, 15, and 20 minutes, respectively (n=91 wells in total). For statistical analysis, the Mann-Whitney U test was used to compare optical density between the control group and each of the treatment groups separately. The Kruskal-Wallis test was used to assess formally whether optical density is influenced by dosage duration.

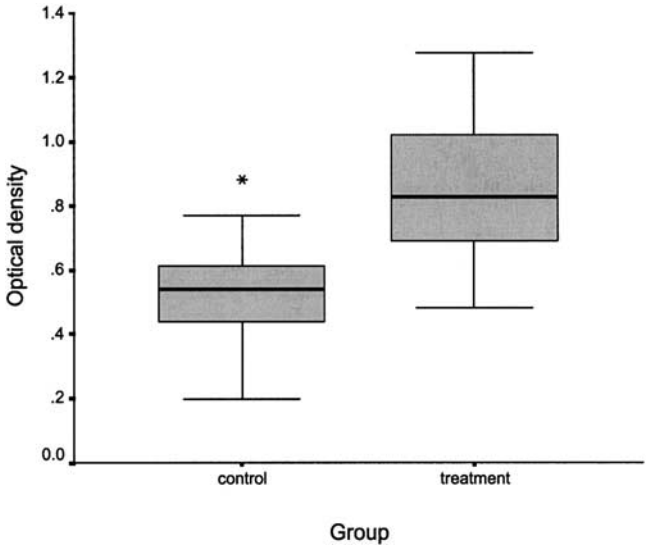

Fig 1. Boxplot showing the relation between the optical density of fibroblasts and PSWD treatment. NOTE. The shaded box represents the interquartile range (IQR) with the median as the center line; the whiskers represent the largest and smallest values excluding outlier observations. *Outlier observations.

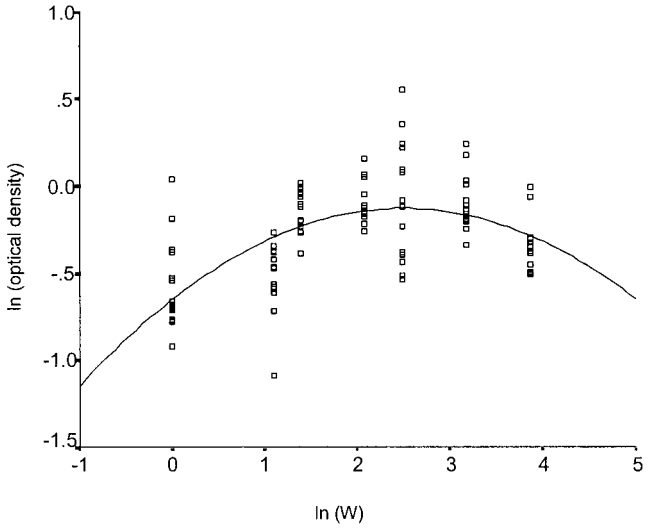

Fig 3. Regression modelling for curve estimation of relation between the optical density (OD) of fibroblasts and dosage of PSWD. Regression equation: $\ln(OD) = -.65 + .42 \times \ln(W) - .08 \times (\ln[W])^2$. Optimal dosage was 13.8W, derived by differential calculus where the gradient of the equation $[d(\ln[OD])/d(\ln[W]) = .42 - 2 \times .08 \times \ln(W)]$ was equal to zero.

RESULTS

Experiment 1 PSWD Effect on Fibroblast Proliferation

PSWD given at mean power dosage of 48W for 10 minutes, twice daily, was significantly associated with fibroblast cell division rate in vitro (U=42.0, P<.001) (fig 1). The median optical density was .54 in the control group and .83 in the treatment group.

Experiment 2: Relation Between Mean Power Dosage and Fibroblast Proliferation

The median optical density corresponding to each treatment dosage of PSWD is shown in figure 2. The lowest recorded median optical density was .51 given by 1W treatment, and the highest was .89 given by 12W treatment. Differences in optical density between treatment groups was statistically significant (χ^2=57.9, P<.001). The relation between increasing dosage of PSWD and optical density at 5 days could be modelled as a curve of best fit by using quadratic regression (R^2=.37, P<.001) (fig 3). The estimated optimal optical density was found at the PSWD dosage corresponding to 13.8W.

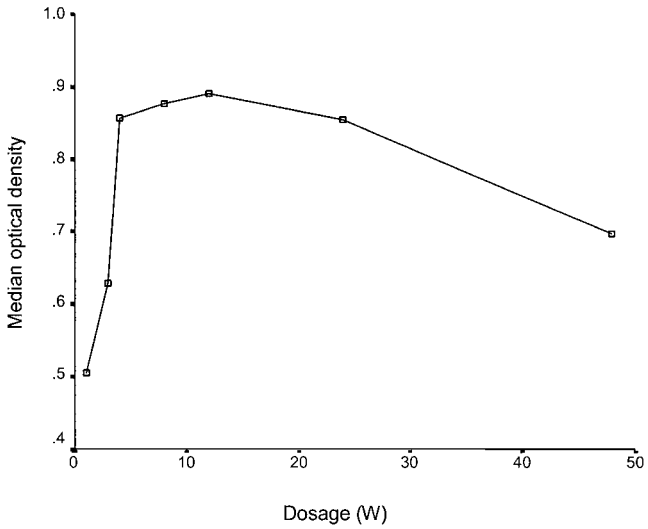

Fig 2. Association between the optical density of fibroblasts and dosage of PSWD.

Experiment 3: Effect of Different Patterns of Energy on Fibroblast Proliferation

Altering the pulse duration and pulse repetition rate while keeping the mean power constant at 6W did not affect cell proliferation rate (Jonckheere-Terpstra=182, P=.519). The median optical density was .12 at 400μs and 100Hz; .13 at 200μs and 200Hz; and .13 at 100μs and 400Hz (fig 4).

Experiment 4: PSWD Effect on Chondrocyte Proliferation and the Influence of Dosage Time

At constant dosages of 6W PSWD, chondrocyte proliferation varied significantly with treatment duration (χ^2=40.2, P<.001) (fig 5). Five minutes of treatment time resulted in the highest levels of optical density (median=.67) compared with 10-minute (median=.49), 15-minute (median=.58), and 20-minute (median=.55) treatment times. All treatment groups had significantly better optical densities than the control group ($U \geq 54$, $P \leq .015$ in each case).

DISCUSSION

In our study, we showed that PSWD significantly influenced fibroblast and chondrocyte proliferation in vitro. This finding may help in understanding the physiology underlying the therapeutic effects of PSWD in clinical practice, and it supports previous hypotheses regarding the cellular mechanisms by which PSWD influences fibroblast proliferation.[9-12] In addition, the dosage was important because there was a relation between in vitro cell proliferation and PSWD exposure. It was the

amount of energy given (mean power) and treatment duration that influenced cell proliferation, in contrast to the pattern of energy given (pulse duration and pulse repetition rate), which did not have a significant effect. There appears to be an optimum window of energy to influence cell proliferation in vitro; as in our study, we estimated a peak effect to be 13.8W (mean power). Furthermore, cell proliferation was highest with the 5-minute treatment duration compared with longer treatments.

Debate concerning the appropriate treatment parameters to use clinically has so far been dominated by anecdotal evidence. Based on a literature review, it has been proposed that relatively longer treatments with higher outputs are beneficial.[17] However, when PSWD is applied in the laboratory, our results suggest that it is possible to overexpose fibroblast cells to treatment and that a specific window of mean power and time duration is influential. Caution is needed when comparing our data with previous clinical recommendations because there are several problems with generalizing the results of laboratory experiments to the clinical setting. For example, greater amounts of energy may be required to overcome the impedance of human superficial tissue such as skin and fat, which are absent in an in vitro study. In addition, blood flow may help to dissipate thermal energy so that treatments longer than those in the laboratory are required. Research into the influence of different dosages on cell proliferation in vivo is therefore required to give clinicians information on the best treatment parameters to use. This need is made greater by the high popularity and usage of PSWD as an electrotherapy modality.

The molecular mechanism that may account for the effects of PSWD on cell proliferation is still unclear. It has been suggested that the molecular agitation caused by pulsed electromagnetic fields may initiate a series of trigger reactions (eg, the binding of hormones and neurotransmitters to their receptor sites).[18] Furthermore, it has also been suggested that these triggers may be the stimulation of gene transformation through heat shock protein and osmotic stress gradient mechanisms.[19] It is therefore plausible that PSWD may activate these specific molecular triggers.

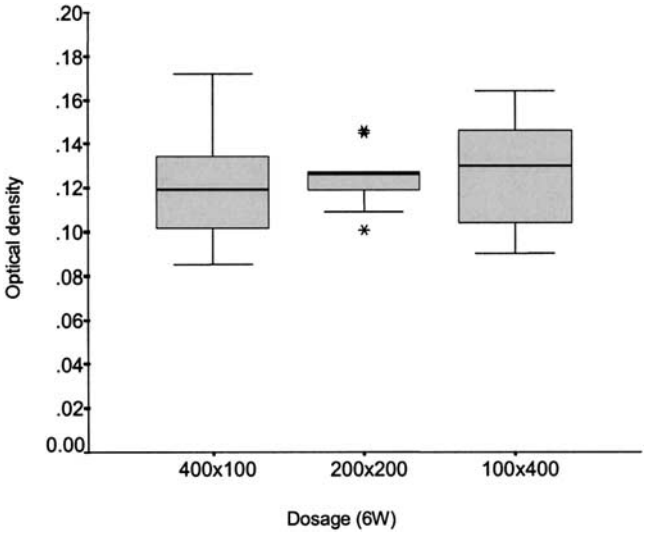

Fig 4. Boxplot showing the relation between the optical density of fibroblasts and PSWD dosage factors, pulse duration, and pulse repetition rate, given a constant power of 6W. NOTE: The shaded box represents the IQR with the median as the center line; the whiskers represent the largest and smallest values excluding outlier observations. * Outlier observations.

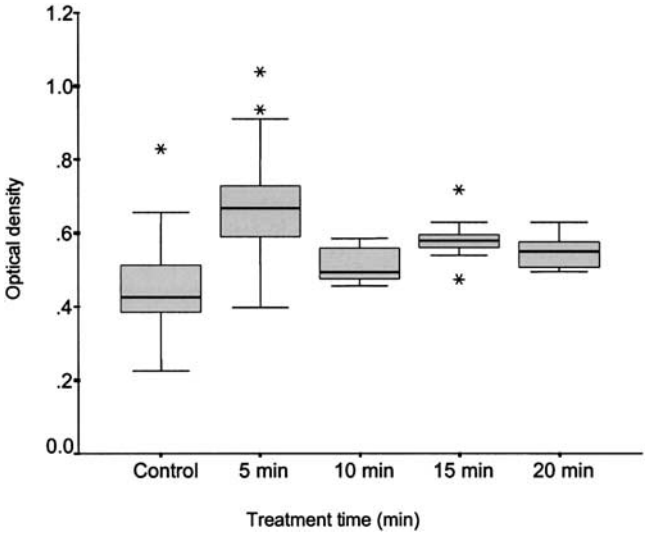

Fig 5. Boxplot showing the association between the optical density of chondrocytes and PSWD treatment time, with dosage set at 6W. NOTE: The shaded box represents the IQR with the median as the center line; the whiskers represent the largest and smallest values excluding outlier observations. *Outlier observations.

In recent months, public concern regarding the effects of radio-frequency devices has escalated, mainly because of fears surrounding the use of mobile phones. It is possible that these concerns will filter through to arouse suspicions about the clinical safety of PSWD equipment. Current UK safety guidelines for the use of PSWD already advise caution with respect to the treatment of rapidly dividing tissues. Pathologic conditions known to be sensitive to increased cell proliferation rates are specifically contraindicated.[1,20,21] However, once independent confirmation of this cellular mechanism for PSWD's effects has been established, clinical guidelines may need to be reviewed.

CONCLUSION

PSWD has a significant influence on fibroblast and chondrocyte proliferation in the laboratory setting. This effect is associated with treatment dosage and time. These in vitro results contribute to an understanding of the underlying cellular mechanism for the therapeutic effects of PSWD. Safety guidelines may need to be reviewed and further research is required to investigate the optimum treatment times and mean power dosages to use clinically.

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