



Is Shortwave Diathermy Effective on Mortality of Protoscolices?

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Abstract

AIM: A previous study confirmed the effect of microwaves on protoscolices *in vitro*. In the current study, the effect of shortwave diathermy (SWD) on the protoscolices of hydatid cysts was investigated.

MATERIALS AND METHODS: The hydatid cyst was collected from the slaughterhouse and its contents were drained sterilized. SWD therapy was done in continuous mode. The experiment was conducted based on electrode area, power of device, and exposure time. GraphPad and SPSS software was used for data analysis.

RESULTS: The study results showed that the two main factors of time exposure and power of the machine could significantly effective in the mortality rate of protoscolices compared to control ($p < 0.0001$). Scolicidal activity of SWD in 450 w was 4.5%, 8.3%, and 10%, respectively, after 2, 5, and 10 min with $\Delta T = 0.5^\circ\text{C}$, 14°C , and 16°C . The results of the study showed that the simultaneous effect of two factors of power and time exposure increased significantly death rate of protoscolices compared to control ($p < 0.05$).

CONCLUSION: Since the application of SWD has limited risks, ease of use, and relative low cost and it can induce hyperthermia in depth of tissues, continuous SWD can be used as a tool for preoperative in surgery. However, it is likely that scolicidal activity can be observed by changing the device mode in pulse short wave at a lower temperature and also the possible side effects need further investigation *in vivo*.

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Introduction

Hydatidosis or cystic echinococcosis (CE) is a chronic disease caused by the larval stage of *Echinococcus* spp. tapeworm [1].

The World Health Organization (WHO) considers the CE to be a neglected tropical disease because it not only causes a dangerous disease in humans but also lead to high medical and economic costs [2], [3]. The gold standard therapeutic methods for CE are surgical treatment. This is an invasive procedure and the risks; complications and costs are high [4]. In recent years, research has been conducted to find non-invasive treatment for the disease. Many researchers are trying to find effective and low-risk therapeutic procedures for the disease such as the use of radiofrequency electromagnetic waves, *in vitro*, and *in vivo* [5], [6].

Shortwave radio frequencies are a type of electromagnetic radiation which often referred to forms of high-frequency electromagnetic radiation, which are classified as non-ionizing radiation ($0.1-10^{13}$ Hz) with a wavelength of between 10 and 100 m [7].

A considerable period of time physiotherapists has utilized shortwave diathermy (SWD) in continuous and pulsed mode to cause thermal and non-thermal

effects on tissues. The most appropriate term for the waves used in this type of therapy would be 11 m. The frequency of oscillations of these waves corresponds to 27.12×10^6 Hz [8]. Continuous SWD was reported as an effective treatment for acute and chronic soft tissue. The machine for producing these waves induces oscillating electrical fields which produce magnetic fields. SWD machine is a signal generator that uses either inductive or capacitive electrodes to deliver energy to the body which led to considerable physiological effects in tissue [9]. Hence, SWD can change physiological processing consist of increasing blood flow, relieving joint stiffness, pain and inflammation, decreasing hematoma and edema, reduction in muscle spasm, increasing soft tissue elasticity, and acceleration in speeding the recovery of wounds [10], [11].

This kind of treatment is non-invasive and its application has limited risks, ease of use, and relative low-cost. It can be applied locally to the target tissue and can induce a significant deep-heating response in human soft tissues through and, most importantly, it proved efficacy in many clinical areas [10]. These features have caused; it has become very popular with clinical therapists. It is likely the clinical application of shortwave (radio-frequency) diathermy to become increasingly widespread in the next years. However, research of its effects and clinical efficacy are scarcity conspicuous.

To use this particular modality, it seems to require more practical scientific evidence for its efficacy. In the current *in vitro* study, the effect of SWD on the protoscolices of hydatid cysts based on two variables of the power and time exposure of radiation was investigated.

Materials and Methods

This experimental study was done on protoscolices obtained from the hydatid cyst.

Preparation of protoscolices and viability test

Sheep hydatid cysts were collected from abattoir of Arak city in Markazi Province and transferred to Parasitology Laboratory in Arak University of Medical Sciences. The content of cysts was completely depleted by a sterile syringe and washed 3 times with normal saline. The concentration of protoscolices was set by normal saline and the suspension containing 9000–10,000 protoscolices/milliliter was provided. The viability of protoscolices was determined by the eosin stain method. The suspension of live protoscolices was transferred to a dark container and stored at 4°C for future use [12]. This suspension was aliquot into identical tubes and in equal volume.

SWD treatment

SWD irradiation was performed using SWD instrument (CURAplus 419; ENRAF-NONIUS).

To produce a uniform electric field, two same electrode surface area (8.5 cm²) was used. Furthermore, to produce the density of the electric field lines, two electrode with different sizes (8.5 cm², 2.5 cm²) was used. Samples were treated with SWD using “contra planar” technique. Hence, the electrodes were placed in parallel with samples (samples were placed in 1 cm from each electrode). All experiments were done in continuous mode. The experiment was conducted as follows:

- A. Comparison of the mortality rate of protoscolices based on electrode area in 350 w power. Samples were divided into two groups, including four subgroups according to time exposure (0, 0.5, 1, and 2 min). The first group was exposed to SWD by the same electrodes with an area of 8.5 cm² and the second group was treated with exposure by two non-identical electrodes (8.5 and 4.25 cm²).
- B. Comparison of the mortality rate of protoscolices based on power of the device with two non-identical electrodes (8.5 and 4.25 cm²) in 350 and 450 w power. Samples were divided into two groups, including three subgroups

according to time exposure (0, 5, and 10 min). The 1st and 2nd groups, respectively, were exposed to SWD in 350 and 450 W.

The protoscolices mortality rate was calculated as follows:

Mortality rate (%) = (number of dead protoscolices in each subgroup-number of dead protoscolices before irradiation/total protoscolices) × 100

The temperature change in the protoscolices suspensions was monitored before and after the irradiation with a thermocouple (Tp-01, Lutron Electronic Enterprise Co., Taiwan). The temperature of the suspension was measured with an accuracy of 0.1°C when the probe was inserted into the suspension. The temperature was shown as ΔT, representing the temperature difference in protoscolices suspension before and after the irradiation [5].

Statistical analysis

GraphPad Prism (version 6) and SPSS (version 16) software was used for data analysis. The mortality rate and ΔT were presented as mean values in three separate experiments and expressed as mean ± SD. Differences between the subgroups and the control were analyzed with a repeated measure test.

Results

The comparison of protoscolices mortality rate and ΔT when the radiation power was constant (350W), but the area of electrodes was changed is shown in Table 1.

Table 1: Comparison of the mortality rate of protoscolices in similar power (350 w) irradiation of SWD but different electrode area

Exposure time (min)	Electrodes size identical (8.5 cm ²)		Electrodes size different (8.5 and 4.25 cm ²)	
	Mortality (%)	ΔT (°C)	Mortality (%)	ΔT (°C)
0	0.33 ± 0	0	0.33 ± 0	0
0.5	2.5 ± 0.7	4.5 ± 0.7	3.25 ± 0.85	4.3 ± 0.71
1	3.5 ± 0.71	5.5 ± 0.7	3.85 ± 0.7	4.5 ± 0.7
2	4 ± 1.4	4.5 ± 0.7	4.15 ± 0.71	4 ± 1.41

SWD: Shortwave diathermy.

The comparison of protoscolices mortality rate and ΔT when the area of electrodes was constant but the radiation power was changed is shown in Table 2.

Table 2: Comparison of the mortality rate of protoscolices in similar electrode area (non-identical electrodes) but different power SWD

Exposure time (min)	350 w power		450 w power	
	Mortality (%)	ΔT (°C)	Mortality (%)	ΔT (°C)
0	0.33 ± 0	0	0.33 ± 0	0
5	5.5 ± 0.57	7.1 ± 0.3	8.3 ± 1.5	14 ± 2.6
10	7.1 ± 0.57	12.5 ± 0.7	10.5 ± 0.7	16 ± 2.1

SWD: Shortwave diathermy.

The comparison of repeated measure analysis for the mortality rate of protoscolices based on exposure radiation time with the power of SWD and electrodes surface area is shown in Figure 1.

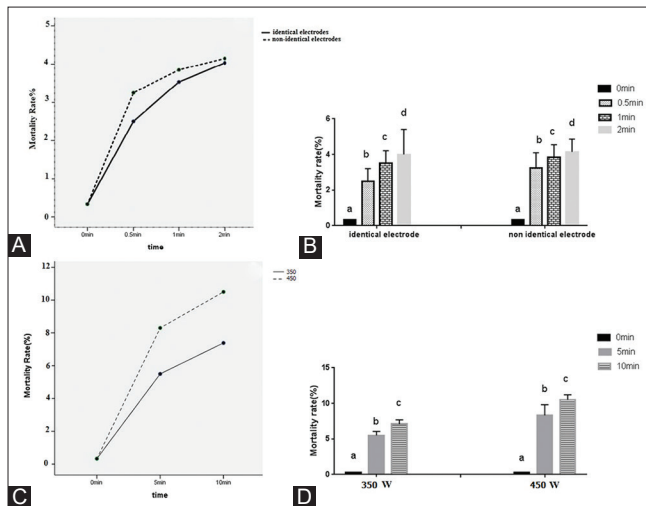


Figure 1: (A) Diagram of the repeated measure test based on exposure time and electrodes surface area; (B) diagram of the repeated measure test based on exposure time and power of SWD; (C,D) different letters "a, b, c, d" refer to significant differences according to repeated measure test ($p < 0.05$). "a" letter means, there was a significant difference between "a" group with "b" group and "c" group and "d" group, but there was not a significant difference between groups with the same letter

Discussion

Since the World Health Organization's working group on Hydatidosis proposed urgently needs to new treatment protocol which would be more effective with fewer complications, so often researchers are studying the subject [13].

The purpose of the evaluation of new therapeutic methods is to find applicable procedures with low complications that require little hospital facilities [14].

Radio frequency electromagnetic is known as non-invasive procedure has been enabled by the advance of various medical technologies [15]. The researchers showed that electromagnetic field (REF) effect on cell membrane leads to changes in transport of ions across the membrane [16].

Furthermore, the cell membrane can become more fluid [17], [18]. The permeability of the cell was changed, possibly allowing some potentially harmful molecules to enter and allow more movement of proteins and other molecules in and through the membrane [19]. Both integral and peripheral proteins in the membrane can also be damaged by high temperatures and, if extremely high, heat might cause these proteins to break down, or denature [16]. Vent Hoff's law states that a chemical reaction speed is temperature-dependent and for every 10° (100°C) rise in temperature, it enhances

two or three-fold. Thus, temperature increasing of tissue from the 37°C to $40\text{--}45^\circ\text{C}$ will accelerate the metabolism of the cell as the rate of consumption of oxygen and expenditure energy increase 1.5 times normal [8].

Some studies indicated that hyperthermia, locally or whole body, is a method for treatment some malignant tumors. One of the effective ways of inducing hyperthermia is applied of microwave. These waves rapidly raised temperature locally in areas with a low depth [20]. However, we need an alternative method in heating deep laying tissues and heating a relatively large volume of tissue. SDW can induce hyperthermia in length deep. It can produce heating depend on electrode surface area in a large volume [8], [10].

Therapeutically, a temperature increase of more than 1°C causes relieving in mild inflammation and an arising temperature between 2 and 3°C is led to decreasing in muscle spasm and pain, changes in tissue extensibility are happening with a rising temperature $3\text{--}4^\circ\text{C}$. If the rising temperature is lower than 1°C , the effect of the thermal of SWD on cell behavior will reversible [21].

From 0 up 2 min, although the change in electrode surface area has not been effective in increasing the mortality rate of protoscolices. However, the mortality rate was, respectively, 3.25%, 3.85, and 4.15% ($\Delta 4\text{--}4.3^\circ\text{C}$) at the end of 0.5, 1, and 2 min. Moazeni *et al.* indicated a mortality rate of protoscolices by warm water at temperature ($\Delta 20\text{--}24^\circ\text{C}$) was 0.4 and 2.9% at 1 and 2 min *in vitro*.

It is likely that instead of a radiation session (1 min), we use several 1-min sessions of radiation or using a pulse SWD use as alternative method, so the mortality rate would be reached to the optimum level or level which can be effective as a pre-surgery.

Our study showed that 0 up 10 min time has been an effective factor in increasing the mortality rate ($p < 0.0001$). However, another effective factor in enhancing the mortality rate of protoscolices was power of machine, and this was directly related to the temperature rise, so mortality rate was, respectively, 8.3% and 10% ($\Delta 14^\circ$ and 16°C) at the end of 5 and 10 min. Moazeni *et al.* indicated that the mortality rate of protoscolices by warm water at the lowest temperature ($\Delta 20\text{--}24^\circ\text{C}$) was 10.2 and 20.4%, respectively, at 5 and 10 min *in vitro*.

It is likely that according to research conducted, using PSWD, selecting the appropriate time session and electrode surface (for localized electric density) can provide a proper effect in relatively low temperatures.

Further studies are needed to provide deeper insight in such subtle power and thermal phenomena determine any side effects *in vivo* that possible effect on protoscolices.

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