Immunization of albino mice against secondary infection with Hydatidosis by using protoscoleces treated with direct electrical current

Asmaa A.Ali , Saddam S.Yasseen
Department of Biology, Collage of Education, University of Mosul, Mosul Iraq
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Abstract
Immunization of BALB/c mice against secondary infection with Hydatid disease, by using protoscoleces (PSC) treated with direct electrical current for 2 min., was investigated.
Injection with about 2000PSC treated with direct current 5.77mA/cm^2, followed by injection with challenge dose, reduced cyst numbers (95.69%), Whereas, injection with PSC treated with direct current 11.82mA/cm^2 reduced cyst numbers (93.47%) in mice 3 months post infection.
Treatment with direct currents led to reduction of liver index and increase of spleen index within immunized groups.
It may well be concluded that direct electrical current potential 5.77 mA/cm^2 was most effective than 11.82 mA/cm^2.

Keywords: Direct electrical current , hydatid disease, immunization.

Introduction
Cystic echinococcosis, caused by *Echinococcus granulosus*, is an important zoonosis disease. Natural intermediate hosts, particulary cattle and sheep, become infected after ingestion of eggs released in feces of infected dogs. Humans are accidental hosts of this parasite, usually becoming infected through contact with infected dogs [1,2]. *E. granulosus* has worldwide geographical distribution, and in areas where the infection is endemic it constitutes a major public health problem and is a cause of important economical losses [1,3].
Immunity is thought to be most effective during the establishment phase of the infection. Once the hydatid cyst is established. The chronically infected host develops in most cases, cellular and humoral specific responses[4,5,6,7].These immune responses are efficiently evaded by *E. granulosus* and do not protect the host against the established hydatid cyst [8,9,10,11]. There is evidence that low voltage direct current (DC) (less than 10v) [12] is bactericidal and parasitocidal *in vitro* [13,14,15,16] and *in vivo* [14,17,18].Electric current may destroy cell physiological action by altering the passage of molecules through cell membrane [19]. There is also evidence showing that the electric current can modify the growth of bacteria [13,18]. Sharquie *et al*. [17] treated dermal leishmaniasis using direct current with the various current intensities. The killing effect of different DC electric potentials against *Leishmania major in vitro* and *in vivo* has been further investigated [14]. The complete destruction of human hydatid cyst protoscoleces by electrolysis device has also been reported [15,16]. In the present study , we attempted to optimize the voltage of induction for attenuation of hydatid cyst PSC using low voltage direct current, and immunization of white mice against secondary infection with Hydatidosis by the attenuated PSC.

Materials and methods
Parasite: sheep livers infected with *E. granulosus* hydatid cysts were collected from a slaughter house. They were immediately transferred to laboratory. The cysts were opened under the sterile condition.

Electrolysis device
An electrolysis device was designed in a rectangular shape having dimension of 2cm (length) x0.26cm (width) x7cm (height). Two flat carbon electrodes 2xcm were installed parallel to each other on the opposite side of the device, a distance of 1.72 cm between electrodes was selected as a potential [11].

Groups
The intervention groups
There are 2 groups of electrolysis solutions (phosphate buffer saline, pH 7.2), each containing about 2000/1ml live, freshly harvested PSC, the first group was subjected to direct electric current 2v = 5.77mA/cm^2 for 2min, the second group was subjected to 3v = 11.82 mA/cm^2 for 2min too (table 1).

The control group
There is one group consisting of (phosphate buffer solution ph 7.2) with about 2000PSC, which no current was applied.

Table (1): Injection protocol

Day	Group A	Group B	Control
٠	2000 PSC 5.77mA/cm^2 for 2min.	2000 PSC 11.82 mA/cm^2 for 2min.	None
٢٠	2000 PSC	2000 PSC	2000 PSC
٩٠	Dissection		

Procedure
The electrolysis cell was filled with the solution (PBS,Ph7.2),containing fresh, active protoscoleces. After setting the appropriate voltage and current intensity, the current was turned on, at the end of the exposure, the electric supply was turned off. The treated PSC were injected intraperitoneally in BALA/c mice.After 20 days all groups were injected with 2000PSC, for each, as a challenge dose. All groups were dissected 3 months post infection.
Numbers, diameters and the percentage of cysts reduction* [20,21],in addition to the weights of liver and spleen, and their organ indices, were evaluated.

Results and discussion

The following results were obtained after injection white mice with PSC treated with direct current electricity.

The numbers, weights, diameters of cysts and their percentage of reduction are shown in Figure (1) and figure (2) , respectively .

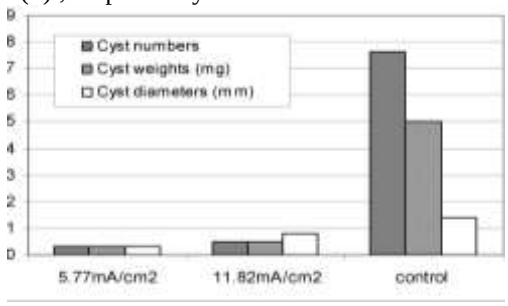

Figure (1): Effect of PSC treated with direct electric current on numbers, weights and diameters of secondary hydatid cysts in mice.

A greater proportion of hydatid cysts were reduced (95.69%) in group A (which injected with PSC treated with the current density of 5.77 mA/cm^2 for 2min.). Applying current density of 11.22mA/cm^2 for 2min. (group B) reduced cyst numbers (93.47%), in comparison with the control group.

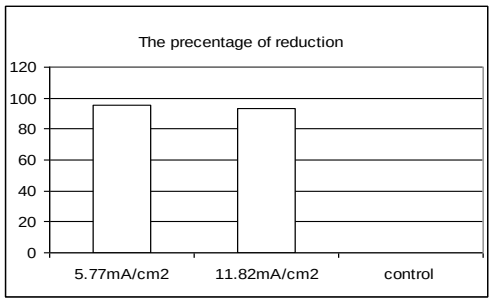

Figure (2): The percentage of reduction of cyst numbers of injected groups in comparison with the control group.

$$\text{Percentage of reduction} = 100 - \frac{\text{No.of cysts in treated mice}}{\text{No.of cysts in the control group}} * 100$$

The results of the present study indicate that injection of attenuated PSC with direct current potential of at lest 5.77mA/cm^2 can reduce the hydatid cyst numbers in BALB/c mice. It probably cause defect in PSC which lead to change in their characteristics including their active antigens. Direct electrical current may disrupt ion transport of parasite membrane to a greater extend resulting in destruction of its membrane and leading to loss of the viability of the parasites. In fact, biological membranes transport groups of ions and polar molecules that can maintain potentials and electrostatic charges, and the integrity of the cell membrane in related to these effects [19,22]. External electrical currents may also draw vital compounds from the cell cytoplasm and so inhibit the cells normal physiological activity [19]. Anatomical action of electric current appears to have two components, one direct and one (the generation of antimicrobial factors) indirect [23].

In the *in vivo* study, the potential 2v at a current 5.11 mA/cm^2 caused (95.69%) damage when used for 2min. and 3v at a current 11.22 mA/cm^2 caused (93.47%) damage when used for at the same time. Berg *et al.* [24] reported that the exposure to a current of 75mA for 4h. had no adverse effects on mammalian cells. Electropathy may not have a direct parasitical effect on the lesions of cutaneous leishmaniasis [19,23]. But enhance the healing capacity of the host tissues and/or their ability to inhibit the multiplication and dissemination of the parasites [25]. Electrical stimulation, for example, may induce migration of epidermal cells [26]; attract neutrophils and macrophages, stimulate fibroblast proliferation, alter blood flow [27], increase DNA synthesis [28]and increase the expression of receptors for transforming growth factor β [29]. It is known that growth factors participate in the healing of wounds and that transforming growth factor-β plays a fundamental role in collagen synthesis [29,30] and Kloth [18] have reported how electrical stimulation can reduce the numbers of the mast cells that have been associated with a variety of wound-healing complications.

The weights of liver, spleen and their organ indices after induction of direct current for different voltage, current densities and period of time are shown in Figure (3) and figure (4),respectively,the lesser value of liver organ index was (51.99), when applying a current density of 5.77mA/cm^2 after 2min. in comparison with the control group (68.67), where as the spleen index was (4.60) in comparison with the control group (3.06).

In the present study, a decrease in weight and organ index of liver in mice injected with treated PSC with direct electrical current was obtained, compared with the control group, in which an obvious increase was noticed, which is similar to that obtained by Ali [31], Gottstein [32],Sarciron et al.[33].Gottstein &Hemphill [34],Ali&Salih[35], Ali&Yaseen [36] on *E. granulosus* and *E. multilocularis*. This increase may be attributed, as suggested by the above mentioned authors, to the presence of granulomas or necrotic areas, which alternate the nature of these tissues, in addition to the numerous numbers of parasites attacking the liver. On the other hand, a decrease in the weight and organ index of liver, noticed in the present study may be attributed to the reduction of cyst numbers in mice injected with treated PSC by the direct current. This is similar to what has been reported by Dalmi *et al.,* [16]. In contrast, an increase in the spleen weight and organ index, was obtained in mice injected with treated PSC in comparison with the control group. This may by attributed to the mitogenic effect of the treated PSC by direct electrical current on T and B lymphocytes in spleen.

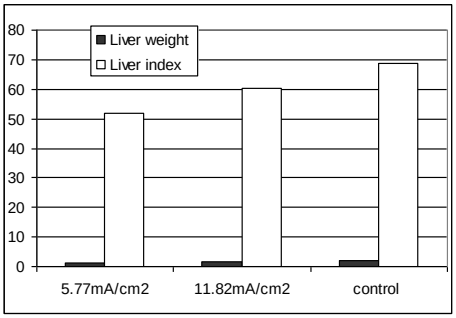

Figure (3): The effect of PSC treated with direct current on weight and index of liver.

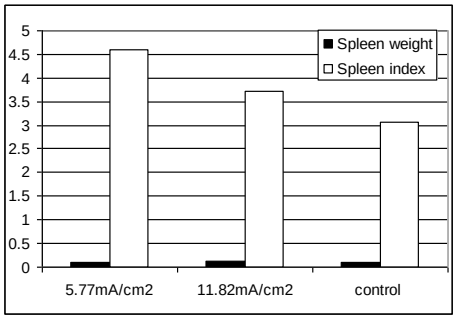

Figure (4): The effect of PSC treated with direct current on weight and index of spleen.

In this *in vivo* study, the results obtained in the present work indicated that, low voltage direct current can be regarded as an affective, fast and inexpensive method for the attenuation of PSC. This method could be used for immunizing of mice against secondary infection with hydatid disease. Hence, further research on safe and effective methods of applying direct current for attenuation other parasites is still required.

Acknowledgment

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تمنيع الفئران البيض ضد الاصابة الثانوية بداء الاكياس العدرية باستخدام الرؤيسات الأولية المعاملة بتيار كهربائي مستمر

أسماء عبد العزيز علي و صدام سالم ياسين

قسم علوم الحياة، كلية التربية، جامعة الموصل، الموصل ، العراق

(تاريخ الاستلام: / /٢٠٠٧ ، تاريخ القبول: / / ٢٠٠٧)

الملخص

تم التحري عن تمنيع الفئران البيض BALB/c ضد الاصابة بداء الاكياس العدرية الثانوي باستخدام الرؤيسات الاولية المعاملة بتيار كهربائي مستمر لمدة دقيقتين.

ادى حقن الفئران بنحو ٢٠٠٠ رؤيس اولي معامل بتيار مباشر ٥,٧٧ملي امبير/سم^{٢}، والمتبوع بحقن جرعة تحدي، الى اختزال اعداد الاكياس بنسبة (٩٥,٦٩%). بينما ادى حقن الرؤيسات الأولية المعاملة بتيار كهربائي مباشر ١١,٨٢ ملي امبير/سم^{٢} الى اختزال اعداد الاكياس بنسبة (٩٣,٤٧%) بعد ثلاثة اشهر من الاصابة.

كما ادى حقن الرؤيسات الأولية المعاملة بتيار كهربائي مستمر الى اختزال معامل الكبد و زيادة في معامل الطحال في المجاميع الممنعة. تبين من النتائج ان التيار الكهربائي المستمر ذو الشدة ٥,٧٧ ملي امبير/سم^{٢} كان اكثر تاثيرا من التيار ١١,٨٢ ملي امبير/سم^{٢}.

الكلمات الدالة: التيار الكهربائي المباشر، داء الاكياس العدرية، تمنيع.