Effects Of Various Doses Of Ultraviolet Radiation and pH On Viability Ofechinococcus Granulosus Protoscolices

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Citation

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Abstract

Three radiation doses of ultraviolet radiation 9000, 27000, 54000 erg/mm2/sec, three degree of pH: 4,7,9 and were used in the present study.
We found that the HCF was the better preservative solution than N.S. and gives protoscolices a relative protection against u.v. rays (protoscolices live more than 15 days in contrast with 10-12 days to N.S).
pH 4 has the greatest effect on protoscolices viability followed by pH 9, while pH:7 was a optional degree of pH.
The optimal conditions of preservative solution extracted from results of this study was HCF pH=7 in 30□C and 2000 Erg/mm2/sec has the lowest effects on viability in comparison with other u.v. radiation doses.

INTRODUCTION

Cystic echinococcosis (hydatidosis and/or hydatid disease) is an important public health and economic problem in Iraq. Its caused by larval stage (hydatid cyst or protoscolices) of Echinococcus granulosus..(Satoh , etal.,2005)

The life cycle of E.granulosus involves a large number of definitive and intermediate hosts distributed in different geographical and climatic zones. The distribution of hydatid diseases is world-wide, but the main endemic areas are where the sheep-dog-man cycle can be maintained (Kamhawi, 1995 and Mathis , etal., 2005)).

From an economic point of view, losses attributed to hydatid disease are mainly due to : (Anderson 1995 and Zhang ,etal.,2003)

1. The medical services offered for the patients with hydatid disease.
2. Losses caused by condemnation of organs affected by hydatid cysts livers and lungs from slaughtered animals.
3. Loss of weight in infected livestock.

With regard to vaccination, as a preventive measure, little progress has been made on the development of a vaccine against the adult as well as the metalestode of E. granulosus. (Ohnishi, 1986 and Walker etal., 2004).

It has been recognized that hydatid protoscolices remain viable for long periods in different media at different temperature. In the absence of nutritive factors, the protoscolices of E. granulosus do not survive in vitro for more than 4 day at 37 C. This survival time depend on temperature, pH and ionic concentration.

Radiation therapy has been attempted on the larval stage of Echinococcus granulosus, while the influence of z-ray radiation on eggs or protoscolices of Echinococcus granulosus, has been observed by a few investigators. (Webbe, 1995 and Stettler , etal., 2003)).

The bactericidal properties of ultraviolet (U.V.) radiation are well known and have included practical application in the sterilization of air and of water supplies.

Relatively little is known about the effects of such radiation upon metazoa of importance in public health. Some workers have been done on the effects of U.V. irradiation upon the eggs of helminthes (Eckert Deplazes,2004).

The aims of this study are to determine the effect of various un lethal doses of u.v. radiation on the protoscolices of E.granulosus and to determine the effect PH of the medium on the susceptibility of protoscolices to u.v. light.

MATERIALS AND METHODS

SOURCE OF ULTRAVIOLET RADIATION:

A “ famed-1 “ quartz lamp, used often in infant's nurseries,

kindergartens, hospitals and laboratory, was as a source of radiation.

The lamp as 700 (Philips,Holland) emitted u.v. rays in the region between 2537A and 3650A , and visible light of the wave lengths between 4050A and 5460A , (1nm=10A) so the radiation dose is equal (15 Erg/mm2/sec) when the distance between sample and source of u.v. light is 15cm (Stankiewicz, et al., 1970. Al-Saimary & Ali, 1997).

HYDATID CYST

Protoscolices of E.granulosus were collected from hepatid hydatid cysts (HCF) of sheep and irradiated immediately after aseptically removed from HHCF and enumerated by dilution count, and using aqueous eosin solution as available stain for detected the viability of protoscolices.

The percentages of protoscolices viability were examined in the following serial period times (0, 0.30, 1, 2, 3, 24, 48, 72, 96, 120) and contineous daily until death all of protoscolices.

EXPERIMENTAL DESIGN

The protoscolices isolated from hydatid cyst fluid preserved in two solution hydatid cyst fluid (HCF) and normal saline. (0.85% NaCl) (N.S.) in three degree of pH: 4,7,9.

The protoscolices divided into four groups irradiated as the following depend on (Al-Saimary & Ali, 1997).

1. I minute irradiation (900 Erg/mm2/sec).
2. 30 minute irradiation (27000 Erg/mm2/sec).
3. I hour minute irradiation (54000 Erg/mm2/sec).
4. Controlling group without irradiation.

Each above group divided into three major sub group in pH:4, 7, 9 and each these major divided in two minor sub group preserved in HCF or N.S.

After irradiation, we enumerate the percentages of viability of protoscolices continuous time period. The preservation temperature is 30 C.

RESULTS

(HCF) hydatid cyst fluid keep the protoscolices viable for 15 days, while (N.S) normal saline for 10 days in pH=7.

In pH4 & 9 the viability percentage decreased from 98 to zero of in (6 & 10) days, and (6&7) days after preserved in HCF & N.S respectively in control groups without any irradiation. Fig-1.

In the first radiation dose / minute (900 Erg/mm2/sec.) the protoscolices preservative in HCF still a live in 4, 7 and 7 day after irradiation at pH 4, 7, 9 respectively while in N.S still a live in 4, 7, 6 days after irradiated and preserved in the same pH degree respectively. Fig-2.

The second radiation dose 30 minute (27000 Erg/mm2/sec.) showed a different patterns of effects, the percentages of protoscolices viability decrease in the lower level in (3,5,4) and (2,4,2) days after irradiated and preserved in HCF and N.S. at pH=4,7 and 9 respectively in each of solutions. Fig-3.

The greatest effects u.v. radiation on percentages of protoscolices viability were showed in the third radiation dose/hour (54000 Erg/mm2/sec.) , the protoscolices still alive in (12,72,48) and (12,48,24) hrs after irradiated and preserved in HCF and N.S. at pH=4,7 and 9 respectively. Fig-4.

Figure 1: Percentage of protoscolices viability after preserved in various pH preservative solutions control group.

Figure 2: Percentage of protoscolices viability after irradiation with 1minute (900 Erg/mm2/sec.) preserved in various pH preservative solutions.

Figure 3: Percentage of protoscolices viability after irradiation with 30 minute (27000 Erg/mm2/sec.) preserved in various pH preservative solutions.

Figure 4: Percentage of protoscolices viability after irradiation with 60 minute (54000 Erg/mm2/sec.) preserved in various pH preservative solutions.

DISCUSSION

In this investigation, the study of various factors –(such as pH and nutritional factor presence in hydatid cyst fluid)- that effected on irradiation with ultra violet light which found necessary, so, little information available about the combination factors.

In radiation view-there are two hypothesises about effects of u.v. rays on the protein and peptide compounds of living microorganisms:

1. The protein particles were absorbed and photons of uv-rays in the specific location of this protein, this

absorbance was qualified with the extinction coefficient and the numbers of specific locations of protein.

2. Chemical reactions were occurred in the absorbance location, however, the general structure of protein was changed or transformed into other structure (Mclaren & Shugar, 1964 , Eckert Deplazes,2004 and Satoh , etal.,2005).

Also the pyruvic acid and lactic acid were built from alonine irradiated with u.v. light by deamination, and the most important effect of u.v. rays was in DNA particle, the pyrimidia-dimers between two nitrogen bases in 254 nm of u.v. light, however the replication and transcription, also the ability of division and multiplication of irradiated living cells was lost and/or changed (Al-Dulaimy, 1987 and Stettler , et al., 2003).

In the present study we found that the PH:4 effect on the viability of protoscolices in the control group and these effect was increased with the high radiation dose, while the PH:9 showed a low effects than those of PH4. PH7 was a typical pH that not interacts with action of u.v. rays and keep the viability for a long period time.

In another view the hydatid cyst fluid was a more better and still the protoscolices a live longest than normal saline, and the HCF give a relative protection from action of u.v. rays because have a nutritional factors and a numbers of minerals, compound such as triglycerides, protein, fatty acid, carbohydrates and others (Andersen, 1995 and Zhang , etal.,2003), that we think it may be protect the protoscolices from a direct effect of u.v. light and help the protoscolices to live in along period time because its having above essential or main nutritional factors, rather than normal saline that is not give the protoscolices any u.v. light protein and its make a simple medium with degree of PH to elevate action of u.v. light on viability of protoscolices, that's showed in our result.

The results of our study were confirmed the results of the previous studies, of the previous studies; Zhang , etal.,2003 suggested that there are a number of factors may complicate assigning a limiting value to the effects of u.v. radiation, specimens may clump together or be situated in the containing vessel in such fashion that some are protected from the full effects of the radiation. Another factor may be natural range of resistance in the organism itself. Finally the wavelength of u.v. radiation is most important.

Morar & Felden , 2003 showed that free protoscolices could survive for 56 days in Krebs Ringer's solution. Stettler , et al., 2003 reported that scolices survived beyond 27 days at 8 C in 0.15m-NaCl. Mathis , et al., 2005 found that 8 days when they were kept in hydatid fluid at 10 and 20 C, while those in the intact cyst were still alive after 16 days storage at 1 and 10 C. Al-Masudi (1989) showed that the protoscolices still alive in N.S., KRS and KRS+HCF for 16,28 and 40 days respectively without irradiation, while in u.v. irradiation group of protoscolices still alive in KRS+HCF for 28, 20, 16, 12 and 12 days after irradiation with 4.5, 9, 18, 27, 54 Kerg/mm2.

Marchiondo, et al. (1994) used physiological saline solution containing 1,000 IU/ml of PencillinG and 100 mg/ml of streptomycin as a preservative solution. Finally Al-Saimary & Ali, 1997 found that NS keep the protoscolices viable for 432hrs and KRS+HCF for 696 hrs.

CONCLUSIONS AND RECOMMENDATIONS

1. Hydatid cyst fluid was the better preservative solution keep the protoscolices viable longer than 15 days in contract with normal saline (10-12 days).

2. pH:7 is optimal degree of pH for preservation of protoscolices, while pH 4 and 9 are decreased the viability of protoscolices.

3. We calculated the LD50 (60% lethal dose) of each of studied radiation doses 9000, 27000 and 54000 Erg/mm2/sec..

4. HCF is giving a relative protection against actions of u.v. rays

5. Continuous work about attenuation of protoscolices by using more of radiation doses will go on and study the attenuation of protoscolices in vitro by injected the laboratory animals and use the results of our study to practical application. In vitro and make optimal conditions for preservation of protoscolices.

6. We made a comparison to susceptibility to u.v. rays of protoscolices of hydatid cyst isolated from various intermediate hosts (Human, Cattle, Camel, Goat and others).

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