Cold atmospheric plasma jet against Leishmania major in vitro study

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Abstract

Cutaneous leishmaniasis is endemic in tropical and subtropical regions, due to the varied clinical protests, including a lump of skin and mucosal tissue to destroy it as a treat disease. In this study, the effect of cold atmospheric plasma on Cutaneous Leishmaniasis was investigated. Plasma device that used in this study was created from the electrical discharge ICP (Inductive Coupled Plasma), we use standard strain of L. major and after treatment by cold atmospheric plasma cells was counted. The result shows a significant reduction in cells which treated by cold atmospheric plasma.

Keywords: leishmaniasis is, Cold atmospheric plasma, Treatment.

INTRODUCTION

Today over 12 million people in the world are affected by leishmaniasis and approximately 350 million people are exposed to this disease (World Health Organization, 2009). More than 1.5 million individuals are reported to show the symptoms of Cutaneous Leishmaniasis (CL)(Herwaldt, 1999), annually. Leishmaniasis is a protozoan disease that occurs in humans in various forms, there are about 22 Leishmania species that cause various clinical manifestations. Leishmaniasis can result in visceral, cutaneous, or mucocutaneous infections according to the species the disease treatment has always been of great importance (John et al., 2006).

The main treatment choice for leishmaniasis is chemotherapy, mainly based on pentavalent antimonials. (Tanyuksel et al., 2003). However, these are extremely toxic and cause serious side effects, and there is a worldwide increasing frequency of chemo resistance to antimonials (Singh, 2006).

Cryotherapy is another treatment, in which liquid nitrogen and carbon dioxide are used in cases where the patient does not respond to conventional therapies. Hypopigmentation after treatment was noted in 68%, but repigmentation occurred within 2–3 months (Panagiotopoulos et al., 2005). In addition, electro-therapy by direct electricity can be used (effective) in order to decrease the size of the lesions (Sharquie et al., 1998).

UV radiation treatments of CL have been applied but the reported results indicate slow inactivation rates and high dose requirements which in turn may cause tissue damage (Fridman et al., 2006).

Many studies have shown that cold atmospheric plasma (CAP) inhibit microbial growth by inducing various microbial metabolic reactions in bacteria, fungi, and parasites through increased generation of reactive oxygen species or free radicals. Plasma is an ionized gas
that refers to the forth state of matter.

In vitro experiments that have been conducted so far, prove the therapeutic effects of CAP such as eradication of pathogens (Fridman et al., 2006), blood coagulation and tissue sterilization (Fridman et al., 2008), treatment of skin ulcers and tooth decay (Lee et al., 2009; Park et al., 2011), uptake and elimination of clogging arteries and skin rejuvenation and removing the scars (Lloyd et al., 2010; Laroussi, 2005).

In this research we studied the effects of cold atmospheric plasma on L. major that is a very important epidemic disease in tropical and sub tropical areas.

METHODS AND MATERIALS

Producing parasite

L. major (MHOM/64/IR/ER75) promastigotes were provided by the Department of Parasitology, Pasteur Institute of Iran and cultured at 24-26 °C in RPMI-1640 medium containing 10% fetal calf serum (FCS). At regular intervals, the parasites were harvested in the log phase of growth. The number of promastigotes was checked using a Neubauer slide. In sterile condition, 100 ml of the culture was transferred to a 24-well culture plate.

Plasma source specification

The plasma jet device consists of a Pyrex tube of 2 mm inner diameter and 1.4 mm external diameter, surrounded by copper electrodes in the width of 6 mm and connected to the power supply. The applied voltage to the electrode ionized the inlet gas fed into the tube and the generated plasma emitted out from end of the tube and propagates about a few centimeters into the ambient air. The power supply produces 10 KV high voltage pulses with a frequency of 6 kHz and 30 microsecond pulse, using helium 95% as the operating gas with 5% oxygen as additional gas to main feeding gas. Helium-oxygen gas flow rate was adjusted 2 liters per minute.

Cell preparation for plasma treatment

After preparing and transferring the cells to the wells, the cells were treated by cold plasma for 30, 60, 120 and 240 seconds in 3 repeats in similar conditions.

Cell counting

The cells were counted before and after treatment by Neubauer chamber and were counted under the microscope. The results of counting in both dilutions were compared. Consequently, the cells were examined by Invert microscope and Neubauer slide was used for cell direct counting.

In order to analyze the obtained data, we used T-test (P < 0.05).

RESULTS

We investigated the effect of cold atmospheric plasma on L. major promastigotes.

The microscopic results after treatment by cold atmospheric plasma

After 24 h of plasma treatment, no significant differences (P> 0.05) in the motility and viability of the promastigotes were detected. A significant difference was observed in the decreasing of the cell viability after 48 and 72 h. Moreover the motility of plasma treated promastigotes at 48 and 72 h became more circular.

In microscopic observation on treated promastigotes in 30 seconds, there was no difference in the frequency of activity than the control group and accumulation of promastigote (Figure 1.a). In treatment of 60 seconds, parasite activity was low, but higher density and aggregation was observed (Figure 1.b). In 120 seconds treatment, greater mass of cells was observed, while the activity of the parasite had decreased (Figure 1.c). Density of the cells was more in the margins of the well in comparison with the center of the well. In the 240 second plasma treatment, density of the promastigote decreased gradually from the margins to the center of the well (Figure 1.d). The activity of the cells dramatically decreased and large number of inactive promastigotes was clearly observed.

The results of the counting of living cells by Neubauer chamber

Following the treatment with CAP, each well was mixed and homogenized again by sampler; the media were diluted to 0.01 and 0.001 concentrations, and then a volume of 10 µL was transferred to Neubauer slide and was counted through the invert microscope. The data obtained from counting the present cells in two media with different concentrations were compared.

The average number of the cells in the control group was 1.5×10^7, while the average number of the cells in the CAP treated wells were 1.49×10^7, 1.41×10^7, 1.26×10^7, 9.9×10^6 in 30, 60, 120 and 240 seconds treatments, respectively. The average cell number in the 240 seconds treatment with CAP that showed a
significant decrease in the number of the promastigotes. The results show that treated promastigotes in 240 seconds, the amount of cells reduced significantly, which indicating that cold atmospheric plasma have lowering effect at this time on living cells.

**DISCUSSION**

In the present study, the effect of cold atmospheric plasma on L. major was assessed. The significant treatment was exposure of the cells to...
the cold atmospheric plasma for 240 seconds.

Plasma has been often observed to inactivate prokaryotic (bacteria) and eukaryotic (yeast and fungi) microbes with different degrees of efficacy (Muranyi et al., 2007).

Fridman treated CL promastigotes at various FE-DBD plasma doses and separately human Macrophage cultures have been treated to assess the difference in the rate of inactivation between different cell lines besides the dose required to inactivate the parasite. In their experiment about 20-30% of macrophages were inactivated in 2 minutes of plasma treatment, whereas 100% of promastigotes were inactivated in 20 seconds treatment) (Fridman et al., 2008). It is worthwhile to mention that the device applied in current study was CAP that affects the cells indirectly, while the device used by Fridman et al. was FE-DBD plasma which is in direct contact with the treated cells, leading to increase in the efficacy of the device.

R.S. Tipa effect of plasma on gram positive and gram negative bacteria they use argon compressed air and helium and the argon have better effect on inactivation of cells(Tipa et al.).

Ozgur KORU et al. studied the effects of hyperbaric oxygen (HBO) on Leishmania tropica promastigote forms (Koru et al., 2012).

The best treatment is induction of programmed cell death caused through the imbalance in reactive Oxygen species (ROS) production. In plasma treatment, uv-induced cell damage has two mechanisms: 1) effects directly on macromolecules in cells. 2) alteration in DNA, Protein and lipid. Plasma can modify DNA and conformat protein rearrangement and aggregation. In this procedure Leishmania spp. is constantly in contact with ROS, generated by its own physiological processes or by those of the host environment including host immune response and drug metabolism (Dolai et al., 2009), but Leishmanialacks the catalase and peroxidase antioxidant systems that contain enzymes with a key role in neutralizing the toxic effects of ROS in the cells of eukaryotic organisms.

Presence of an effective amount of ROS stimulates programmed cell death or apoptosis; however, it was recently shown that caspase-dependent programmed death in Leishmania does not exist. A caspase-like activity was detected which correlated with the loss of mitochondrial membrane potential. Furthermore, the occurrence of apoptotic characteristics including PS externalization, cell shrinkage and DNA fragmentation, destabilization of mitochondria, ROS dysregulation implies the evolution of alternative pathways of apoptosis in L. major (Alzate et al., 2006). Raja Moman et al. reported effective antibacterial effect of CAP on various species of bacteria (Moman et al., 2010). On the other hand, Fridman et al. evaluated the effect of direct and indirect plasma on inactivation of bacteria. Their results indicate that the direct treatment is more effective and destroyed the cells in 15 seconds, whereas 5 minutes treatment was required for cells death in the indirect plasma exposure method (Fridman et al., 2007). In another study, Routh et al. indicated that atmospheric plasma treatment for 25 minutes in room temperature effectively reduces the number of 2 different species, Escherichia coli and Staphylococcus aureus (Roth et al., 2000). Villager et al. examined the bactericide effect of cold atmospheric plasma produced by gas mixture of N2-O2 on E. coli in agar culture and reported that almost 100% of the bacteria were killed after 25 minutes exposure (villager et al. 2003). Tippa et al. treated the types of Gram positive (S. aureus) and Gram negative (P. aeroginos) with Argon and Helium plasma. They revealed that Argon plasma is more effective (Tippa et al).

In this study, cold atmospheric plasma effect on L. major was evaluated, the gas used was Helium which was 95% and we added 5% of Oxygen and the most plasma overall effect was in 240 seconds. According to Young et al.’s study in 2013, evaluating the effect of cold atmospheric plasma on inactivation of Fusariumoxysporum, Neurosporacrassa and Fusariumgraminearum fungi spores in 10, 60 and 180
seconds by Argon, results of treatment showed that in 180 seconds the fungi were inactivated (Young et al., 2013). As known Argon atoms had more electron levels in compare to Helium used in the study, therefore electrons separate with lower energy levels from the last electron level than Helium resulting in higher energy levels of active species, so Argon has a more overall effect than Helium.

According to several treatment choices used for Cutaneous Leishmaniasis control which the most common choice is the use of pentavalent Antimonials with reported side effects. Cold atmospheric plasma can be used as a physical choice for a proper alternate in Leishmaniasis treatment or destroying the disease factor. In this study Helium plasma was used which regarding the results, other gases like Argon, having more electron levels in its atom has a more and faster overall effect can be used. In addition the cold atmospheric plasma effect can be evaluated on Leishmaniasis Amastigoteform that because of unevolved structure is predicted to have a more effect than Promastigote form. Also if there exists, the plasma effect can be evaluated on the scar left from Leishmaniasis on the animal’s body.

REFERENCES


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