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Topical effectiveness of different concentrations of nanosilver solution on *Leishmania major* lesions in Balb/c mice

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ABSTRACT

Background & objectives: Cutaneous leishmaniasis is an infection caused by protozoan genus *Leishmania*. Although glucantime is commonly used for the treatment of leishmaniasis, it has some side effects including increased liver enzymes and electrocardiogram changes. In addition, the drug is expensive, the injection is painful, and research shows that resistance of parasite to glucantime is growing in different parts of the world. Therefore, scientists are paying more attention to develop new drugs such as nanosilver solution. The present study is an attempt to evaluate the *in vivo* topical effects of different concentrations of nanosilver solution in the treatment of leishmaniasis lesions.

Methods: In all, 90 female Balb/c mice aged 6–8 wk were infected with 2×10^6 viable stationary-phase promastigotes in the base of tail. Different concentrations (60, 80, 120, 130 and 2000 ppm) nanosilver solution were used in the present study to test the efficacy in the treatment of lesions. Clinical control of the infection trends was conducted weekly for 5 wk by measuring lesion diameter with standard Kulis-Vernieh. Data were analyzed by paired *t*-test, analysis of variance (ANOVA), and Tukey test.

Results: Mean lesion diameter pre- and post-treatment did not significantly differ between different treatment groups ($p > 0.05$). Likewise, a significant difference in splenic parasite load was also not observed between different treatment groups.

Interpretation & conclusion: Based on our results, different concentrations of nanosilver are ineffective in reducing mean sizes of lesions.

Key words Balb/c mice; *Leishmania major;* nanosilver; treatment of lesions

INTRODUCTION

Cutaneous leishmaniasis is an infection caused by *Leishmania* parasites. According to findings ~15,000 people are affected annually in Iran. The real rate of incidence is 4 to 5 times higher than the reported prevalence[1, 2]. Current therapies for treatment of *Leishmania* are compounds of pentavalent antimony (pentostam and glucantime) and amphotericin B, metronidazole, diamydin, pentamidine, allopurinol, ketoconazole, astrokonazol, dapson, monomycin, paramomycin, etc[3–6]. These drugs have side effects; for example glucantime elevate liver enzymes, change electrocardiogram, erupt cutaneous, painful injection, resistance of parasites to glucantime, etc.[3, 4, 7]. Disadvantage of glucantime and other drugs have more side effects that caused researchers attempt to find new drugs with more efficacy and less side effects.

Nanosilver solution is a new drug with anti-bacterial, antifungal and antiviral properties. Advantages of this drug compared to other drugs are efficacy at low concentration and long life duration[8–10]. Studies showed that nanosilver cement has high antibacterial activity and high effectiveness against multi resistant bacteria without cytotoxicity *in vitro*[11]. Recently, findings have demonstrated that nanosilver has anti-inflammatory effects and increases wound healing and dressings of wounds[12]. If these results are confirmed *in vivo*, nanosilver may be appropriate for ulcer treatment. In this study, we evaluated the effect of topical application of different concentrations of nanosilver solution on the leishmaniasis ulcer in Balb/c mice.

MATERIAL & METHODS

Preparation of nanosilver solution

Nanosilver powder was used with average particle size of 100 nm. We used colloid nanosilver particles (4000 ppm) that were manufactured by Nanoalvand Co., Iran. Dilutions of 60, 80, 120, 130 and 2000 ppm of nanosilver

4000 were prepared in double deionized water immediately before use.

Parasite culture

Iranian *Leishmania* strains (MRHO/IR/75/ER) were cultured in RPMI-1640 medium supplemented with 10% inactivated fetal calf serum (FCS), 100 mg/ml streptomycin and 100 IU/ml penicillin G at 25°C[13]. The promastigotes from stationary-growth phase of culture were used to infect inbred Balb/c mice.

In vivo study

Female Balb/c mice, 6 to 8 wk old with approximately 60–80 g were procured from the Animal Breeding Stock Facility of Pasteur Institute of Tehran. Exactly, 0.1 ml of the solution containing 2×10^6 *L. major* promastigotes (MRHO/IR/75/ER) was injected into the base of mice tail subcutaneously. After injection, the mice were kept in shelves in specific and appropriate conditions. After 5 wk, wound was formed at the base of the tail. The parasites were confirmed under a microscope after sampling the wound and colored it.

Testing and treatment groups

After 5 wk of inoculation, when lesions appeared at the base of tail, treatment was initiated. Animals were divided into 9 groups with at least 10 animals in each group. Clinical control of the infection trends was conducted weekly for 5 wk by measuring lesion diameter with standard Kulis-Vernieh. The animals were treated as follows:

(1) Control group 1: Without inoculation of parasites; (2) Control group 2: Parasites were injected without any treatment; (3) Control group 3: Treated with only distilled water; (4) Control group 4: Positive control group treated with peritoneal injection of amphotericin B (6 mg/kg body weight) daily for two weeks; and (5) Treatment group 1: Treated with intralesional injection of nanosilver 60 ppm solution; (6) Treatment group 2: Treated with intralesional injection of nanosilver 80 ppm solution; (7) Treatment group 3: Treated with topical spray of the nanosilver 120 ppm; (8) Treatment group 4: Treated with topical spray of the nanosilver 130 ppm; and (9) Treatment group 5: Treated with topical spray of the nanosilver 2000 ppm.

In treatment groups, mice with one nodule were selected. Nanosilver solutions was used in different concentrations (60, 80,120, 130 and 2000 ppm) and injected five times intralesionally every 4th day up to 16 days. Mice were followed for 5 wk and efficacy of nanosilver solutions was investigated in groups. Light microscopy was used with high magnification (×1000) for observation of amastigote forms in the smears prepared from lesions.

Determination of spleen parasite load

Positive effect of nanosilver solution on parasite load was investigated based on the method of cultivation (Limiting dilution method) and buffer changing. Spleen parasite load was determined in the second week after wound appearance and 2 months post-treatment. The final concentration was diluted with the same medium. Total weight of isolated organ (in this case is the spleen) was determined. Approximately, 20 mg of tissue in between two sterile glass slides in 1 ml medium (Graces insect medium containing 15% bovine serum inactivated by heat), was homogenized and the final concentration was diluted with the same medium. Various dilutions were prepared in the 96 well planted plates and were maintained for 3 week at 26°C. Wells were tested for 3 consecutive days for investigation of live promastigotes. Maximum dilution that positive parasite had been demonstrated was reported as concentration of parasite in milligram. Spleen parasite load was determined in the second week after wound appearance and 2 months post-treatment[14].

Statistical analysis

Statistical significance between groups was analyzed by Paired *t*-test and two-way analysis of variance (ANOVA) using SPSS version 17.5. Values of $p < 0.05$ were considered statistically significant.

RESULTS

In vivo studies (Nanosilver effect on lesion size)

In all, five nanosilver treated groups were compared with control groups, the mean lesion sizes did not decrease significantly after 5 wk treatment ($p > 0.05$) (Table 1). Statistically significant differences were not found between treatment groups and control groups pre- and 5 wk post-treatment (Table 2). In addition, significant differences were not observed in spleen parasite load pre- and post-treatment ($p > 0.05$) (Fig. 1).

DISCUSSION

We didn't find any significant difference in the ulcer size, mean lesion diameter and spleen parasite load after 5 wk treatment with nanosilver solution. These findings demonstrated that nanosilver treatment when compared with control group reduced mastigote numbers and pro-

Table 1. Therapeutic effects of different concentrations of nanosilver solution on the lesion sizes (mm) of cutaneous leishmaniasis induced by *L. major* in Balb/c mice compared to control groups

Groups	Time of treatment						
	Start (Mean ± SD)	Week 1 (Mean ± SD)	Week 2 (Mean ± SD)	Week 3 (Mean ± SD)	Week 4 (Mean ± SD)	Week 5 (Mean ± SD)	*P*-value*
Control	6.87 ± 2.84	7.71 ± 2.31	9.38 ± 3.89	9.01 ± 2.13	10.46 ± 1.50	12.72 ± 2.73	0.108
Distilled water	5.47 ± 1.63	9.61 ± 2.27	10.36 ± 2.94	12.95 ± 2.68	12.67 ± 0.035	13.15 ± 1.97	0.307
Amphotericin B (+) control	4.91 ± 0.601	6.08 ± 2.49	8.48 ± 2.96	7.58 ± 1.48	6.85 ± 1.12	6.75 ± 0.74	0.131
Topical inj. 60 ppm	7.44 ± 2.74	10.41 ± 3.41	10.21 ± 1.54	10.63 ± 3.56	10.08 ± 2.92	13.26 ± 1.31	0.127
Topical inj. 80 ppm	7.20 ± 2.64	8.03 ± 1.14	10.56 ± 2.82	9.69 ± 4.21	9.91 ± 2.51	10.81 ± 2.44	0.448
Topical spray 120 ppm	7.24 ± 1.88	10.41 ± 3.87	12.9 ± 5.08	14.3 ± 9.01	11.30 ± 2.17	9.20 ± 3.53	0.281
Topical spray 130 ppm	7.40 ± 1.91	10.30 ± 2.11	10.17 ± 2.20	9.70 ± 3.34	12.16 ± 5.42	12.52 ± 7.03	0.677
Topical spray 2000 ppm	6.54 ± 1.44	9.29 ± 1.81	10.96 ± 1.25	11.66 ± 2.46	15.05 ± 3.46	13.66 ± 2.86	0.534

*Statistical test ANOVA.

Table 2. Comparison of lesion size of cutaneous leishmaniasis in Balb/c mice treated with different concentrations of nanosilver solution in pre- and post-treatment

Groups	Size of lesion (mm)		*P*-value
	Pre-treatment Mean ± S.D.	Post-treatment Mean ± S.D.	
Control group (n=5)	6.87 ± 2.84	12.72 ± 2.73	0.108
Distilled water (n=5)	5.47 ± 1.63	13.15 ± 1.97	0.307
Amphotericin B (n=5)	4.91 ± 0.601	6.75 ± 0.74	0.131
Topical inj. 60 ppm (n=5)	7.44 ± 2.74	13.26 ± 1.31	0.127
Topical inj. 80 ppm (n=5)	7.20 ± 2.64	10.81 ± 2.44	0.448
Topical spray120 ppm (n=5)	7.24 ± 1.88	9.20 ± 3.53	0.281
Topical spray130 ppm (n=5)	7.40 ± 1.91	12.52 ± 7.03	0.677
Topical spray 2000 ppm (n=5)	6.54 ± 1.44	13.66 ± 2.86	0.534

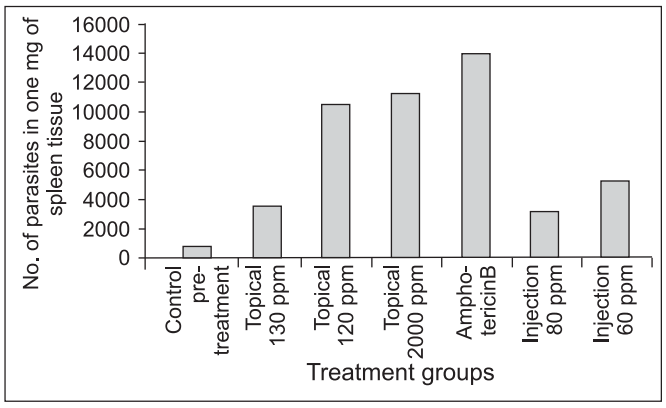

Fig. 1: Comparison of splenic parasite load in pre- and post-treatment of mice for the different groups.

liferation of them but this reduction was not significant. In addition, different concentrations of nanosilver didn't decrease the mean lesion size significantly. Secondary infection was significantly decreased in nanosilver-treated groups compared with control groups. According to a previous study, spleen parasite load decreased significantly after treatment with nanosilver solution[15]. One randomized clinical trial demonstrated that wound healing time in nanosilver treated groups compared with control groups decreased significantly and nanosilver increased bacterial clearance from infected wounds. Nanosilver use did not have any adverse effect for healing[16]. Another study showed that nanosilver decreased the wound healing time in superficial burn wounds but not deep burn wounds and demonstrated that it enhances re-epithelization and don't have any effect on other phase of wound healing such as new tissue formation, angiogenesis and proliferation[17].

Silver has been used to treat different infections for many years; silver nano-particle form usage has

opened new ways of treatment with development of nano-technology[14]. Nanosilver compounds show impact on a wide range of microorganisms including bacteria, viruses, fungi, and even protect against protozoa and influenza[15, 18, 19].

Recent studies suggested that nanosilver has a potent anti-inflammatory effect and enhance wound healing. Nanosilver has antibacterial effects against a large number of bacterial species. According to findings, bacterial resistance to elemental silver is extremely rare[12]. The exact mechanisms of antibacterial effect of nanosilver are not yet elucidated. Some possible mechanisms are effective on peptidoglycan cell wall, the plasma membrane, bacterial (cytoplasmic) DNA, bacterial proteins especially enzymes involved in vital cellular processes[12]. According to some findings, antibacterial effect of nanosilver is size and dose dependent. Smaller sizes of nanosilver have more antibacterial effects and exhibit more on various range of bacteria[20,21].

Few findings showed that nanosilver is more effective on gram-negative bacteria than gram-positive bacteria. Gram-negative bacteria are more sensitive to nanosilver than gram-positive bacteria[22, 23].

Despite the beneficial effects of nanosilver, toxicity of nanosilver is still unknown. Nanosilver damage DNA, denature proteins and enzymes and produce free radicals and some studies showed that nanosilver is cytotoxic to several different cell lines. The teratogenicity of nanosilver in humans is indistinctive and is not reported in the literature but *in vivo* (animal studies) and *in vitro* studies showed nanosilver can exhibit a significant level of toxicity. So prescription of nanosilver for healing wounds in human needs to be investigated[24, 25].

CONCLUSION

Based on our results, different concentrations of nanosilver are ineffective in reducing mean sizes of lesions. However, nanosilver can be used in treating secondary infections of cutaneous leishmaniasis.

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