Nanogold for the treatment of zoonotic cutaneous leishmaniasis caused by *Leishmania major* (MRHO/IR/75/ER): An animal trial with methanol extract of *Eucalyptus camaldulensis*

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**ABSTRACT**

This experimental study was conducted to assess of effectiveness of two nanogold concentrations against cutaneous leishmaniasis (CL) caused by Iranian strain of Leishmania major in BALB/c mice model. Cutaneous lesions were experimentally induced by inoculation with Iranian Leishmania major promastigotes (MRHO/IR/75/ER) in 61 BALB/c mice. The infected mice were enrolled to four groups: interventional groups which were treated with two different concentrations of nanogold and two control groups. Nanogold solutions applied topically twice daily for 28 days (N=30) and the results compared with control groups (N=31). The results showed that amastigote number into the lesions, were significantly decreased in interventional groups compared with control groups (*p=0.001*). Nanogold solutions were also decreased mortality rate in the mice (*p=0.003*). No statistically difference was found between 40 µg/ml and 0.4µg/ml of nanogold concentrations in amastigote reduction (*p=0.648*).

**Key words:** Leishmania major, Cutaneous leishmaniasis, Nanogold, BALB/c mice

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1. Introduction

Cutaneous leishmaniasis is prevalent in many parts of Iran in which Leishmania major and L.tropica are the primary causative agents of cutaneous leishmaniasis (Mohebali et al., 2004). Surveillance data indicate that the global number of CL cases has increased during the past decade. Such increases can be explained in part by improved diagnosis and case detection, but are also a result of inadequate vector or reservoir control and the emergence of antileishmanial drug resistance (Reithinger et al., 2007). Most of available drugs for treating leishmaniasis are toxic, need multiple injections, have limited efficacy and emerge drug resistance in some cases (Croft and Coombs, 2003; Hadighi et al., 2006). The first line drugs for treating all forms of leishmaniasis in Iran are antimonial compounds, particularly meglumine antimoniate (Glucantime®). Based on a few studies that have been carried out in recent years, about 10 to 15 percent of CL had not desirable response to meglumine antimoniate; consequently, it is necessary to find and evaluate a safe, local and suitable new drug (Mohebali et al., 2007).

The development of metal complexes as potent chemotherapeutic agents against leishmaniasis remains a priority since the drugs currently available are not totally safe or active (Navarro, 2009). The parasites of genus Leishmania are considered to have a similar metabolism to tumoral cells. Some research groups have tested metallic antitumoral compounds against kinetoplastid parasites, with interesting results (Brown, 1987). Antitumoral compounds interact with DNA, and have led some authors to propose that each DNA interacting compound could have been active against protozoa (Brown, 1987). One of the metallic compounds used, was gold. Complexes of gold with different organic drugs such as chloroquine, KETOconoazole, and pentamidine as ligands against malaria, trypanosomiasis, and leishmaniasis have been tested (Navarro et al., 2007).

On the other hand, recent interest in using gold nanoparticles (Au NPs) in medicine has altered the methods of diagnosis and treatment (Chen et al., 2008). For example, employing Au NPs in PCR has optimized the specificity of this diagnostic method (Li et al., 2005). Also some researchers have took advantage of Au NPs in transferring drugs into the biological cells, which provided a good basic for nuclear targeted delivery (Gu et al., 2009). This is a novel idea that gold nanoparticles can have effects in treatment of leishmaniasis. Therefore this study aimed to determine the cure rate of two doses of nanogold solutions against zoonotic cutaneous leishmaniasis in BALB/c model.

2. Methodology

2.1. Preparation of nanogold concentrations

The gold nanoparticles were produced by the methanol extract of Eucalyptus camaldulensis through the reduction of aqueous AuCl4- in the size range of 1.25-17.5 nm with average size of 5.5 nm (Ramezani et al., 2008). Gold nanoparticles in 40µg/ml concentrations (maximum doses) were achieved from stock in 400µg/ml then nanogold in 0.4µg/ml (minimum doses) was prepared from 40µg/ml diluted in sterile water.

As described in some studies, nanosystems are often accumulated at higher concentration than normal drugs, thereby enhancing bioavailability at the targeted site. Inhibitory concentration in biological activity of Gold, Cooper and Iridium complexes against Leishmania.spp (promastigotes & amastigotes) were 3.4, 4.1 & 3.1µg/ml, respectively. So we chose 0.4µg/ml concentration for minimum dose. (Navarro et al., 2007; Navarro et al., 2003; Mbongo et al., 1998). Choloroauric Acid HAuCl4 used in 40µg/ml concentration as the control group 1 to compare with gold in nano size.

2.2. Mice

Inbred BALB/c mice (6-8 weeks old) were purchased from the Laboratory Animal unit Pasteur Institute of Iran.

2.3. Parasite preparation, inoculation and distribution of BALB/c mice within groups

Sixty one BALB/c mice were injected with Iranian strain of Leishmania major promastigotes (MRHO/IR/75/ER) into the base tail of the mice subcutaneously by inoculums of 108 promastigotes /ml. After infected all the mice (~1 month), they divided into 4 groups: Group 1, treated with high dose of nanogold solution (40µg/ml), Group 2, treated with low dose of nanogold solution (0.4µg/ml), Control group 1, treated with HAuCl4 (Merck) and Control group 2, kept without any treatment. Mice were treated topically twice a day for continuous 28 days. Before treatment initiation, the size of lesions was measured by caliper and also their body weights of each mouse were recorded. Impression smears were prepared to counting numbers of amastigotes. Amastigotes were counted randomly in 10 fields of each slide with high magnification (x1000) (WHO, 1991). Lesion size and weight were measured every week. Lesion size was measured by a digital caliper (Guangio®) in millimeters in two diameters (a, b) then it calculated according to this formula: LS= (a + b)/ 2. Body weight was measured by balance. Their impression smears were prepared weeks 1,3 and 4 after the end of treatment courses. Survival rate was exhibited as the percentage of surviving mice every week.

Secondary infection was determined by lesion’s observation and microscopically survey of slides. Secondary infection was reported positive when more than 5 microorganisms observed (WHO, 1991).
2.4. Statistical analysis
Statistical significance between groups was analyzed by Student’s t test, analysis of variance (ANOVA) and Chi-Square analysis using SPSS version 13.5. Values of $P < 0.05$ were considered statistically significant.

3. Results
On weeks 5 and 8 of post treatment, amastigote counts into the lesions of nanogold groups were statistically decreased ($p=0.001$) whereas too many of amastigotes were still observed into the lesion of the control groups (Tab. 1).

Table 1. Amastigote counts before and after treatment by nanogold concentrations ($N=30$) and control groups ($N=31$).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before treatment Mean ± SE</th>
<th>After treatment Mean ± SE</th>
<th>Difference</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 5</td>
<td>Week 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nanogold (max) 40µg/ml</td>
<td>122.27±76</td>
<td>84.07±27.05</td>
<td>38.20±9.83</td>
<td>$p=0.05$</td>
</tr>
<tr>
<td>Nanogold 0.4µg/ml (min)</td>
<td>98.73±29.10</td>
<td>71.77±44.98</td>
<td>26.96±19.62</td>
<td>$p=0.05$</td>
</tr>
<tr>
<td>HSAuCl4 (control) 40µg/ml</td>
<td>241.67±27.59</td>
<td>270.20±35.12</td>
<td>28.53±41.42</td>
<td>$p=0.05$</td>
</tr>
<tr>
<td>No treatment (control2)</td>
<td>206.88±20.43</td>
<td>284.58±44.48</td>
<td>79.70±45.99</td>
<td>$p=0.05$</td>
</tr>
</tbody>
</table>

The increment number of amastigotes in week 5 may be pertinent to insufficient time for this concentration of nanogold.

Differences between nanogold groups compared to control groups at week 8 post treatment were significant by ANOVA ($p=0.001$).

No statistically difference was found between 40 µg/ml and 0.4µg/ml of nanogold concentrations in amastigote reduction by ANOVA ($p=0.648$).

The mean lesion sizes decreased 4 weeks after treatment with 40 µg/ml concentrations of anogold but this difference was not statistically significant in 8 weeks ($p=0.061$) (Fig. 1).

4. Discussion
Accumulation of nanosystems at the targeted site are often higher than normal drugs and usually leads to reduced systemic toxicity. One of several varieties of engineered nanosystems is gold nanoparticles (Au NPs). Chrysotherapy, the use of gold in medicine, has a long history. Ancient cultures in Egypt, India and China used gold to treat diseases such as smallpox, skin ulcers, syphilis and measles (Chen et al., 2008). In this study, we used two concentrations of nanogold with 40 nm sizes on L.major in vivo conditions. Our results showed the effectiveness of different concentrations of nanogold on proliferation of amastigote forms of L. major in macrophage. The mean of mouse skin lesion sizes were decreased at the end of treatment courses but the difference was not statistically significant. Interestingly mortality rates in nanogold-treated groups compared to all control groups, were decreased significantly at the end of treatment courses. Based on previous studies, One investigation showed that a complex of gold [Au (dppz)2]Cl3 induced a potent dose dependent antiproliiferation activity against promastigotes of Leishmania mexicana (Navarro et al., 2007). Another study showed that gold sodium thiomalate inhibited growth of amastigotes in vivo in hamsters and in vitro (Abruzzo and Horatius, 1969). Other researchers who investigated efficacy of this compound against Kala-azar noted that all 10 patients showed an excellent clinical response to
treatment with sodium aurothiomalate (Singh et al., 1989). Because of this finding and enhancing bioactivity of nanoparticles in contrast with micro materials, this study conducted to determine if nanogold had antileishmanial activity on BALB/c mice or not. The survey which studied antimicrobial sensitivity of Streptococcus mutans to nanogold confirmed our founding on secondary infection (Hernandez-Sierraeal., 2008). In all groups, secondary infection was increased after treatment and gold nanoparticles had not antimicrobial effect. Other gold compounds like gold (I) complexes had antimicrobial effect against Gram-positive bacteria and this behavior was related to phosphine and aminothiol band in the complexes (Nomiyaetal., 2000).

In conclusion, nanogold had therapeutic effect on cutaneous leishmaniasis and it seems to decrease progression of the disease in BALB/c animal model. The study needs to be repeated with larger groups of animals and more concentrations before obtaining any conclusion about the efficacy of Iranian nanogold.

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References


