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Effect of *Magnetospirillum gryphiswaldense* on serum iron levels in mice

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ABSTRACT

Background and Objectives: The Magnetotactic bacterium *Magnetospirillum gryphiswaldense* (MSR-1) mineralizes the magnetite (Fe_3O_4) crystals and organizes a highly ordered intracellular structure, called the magnetosome. Iron transport system supports the biogenesis of magnetite. Although iron is an essential trace element for many metabolic pathways of the body, increase or decrease in iron will cause many diseases. Mice were infected by MSR-1 to study survival of bacteria in mice when injected by different routes. The aim of this study was to investigate whether bacterial magnetite formation could take up Fe^{2+} ions from the blood an animal model.

Materials and Methods: In this study, MSR-1 at a dose lower than LD_{50} in 200 µl volume of PBS buffer was injected as intravascular (i.v), peritoneal (i.p) and subcutaneous (s.c) in mice. Number of viable bacterial was determined in organs such as liver, spleen and lymph node by measuring colony-forming unit (CFU). Moreover, serum iron level was evaluated by using commercial kits.

Results and Conclusion: According to CFU measurements, after 96 hours, mice can clear MSR-1 from their body with different routes of injection. We have also shown that MSR-1 bacteria can affect the blood iron level in mice. The serum iron level decreased from control level in the first 24 h after i.v injection ($P < 0.05$). Our research on optimizing the biological magnetic system is still continuing.

Keywords: *Magnetospirillum gryphiswaldense*, Serum Iron Level, Mice

INTRODUCTION

The magnetotactic α-proteobacterium, *Magnetospirillum gryphiswaldense* (MSR-1) is a gram-negative, motile, aquatic and heterotrophic bacteria (1). Bacteria require a large quantity of iron to synthesize intracellular magnetic particles that termed magnetosomes, biomineralizes up to 100 cubo-octahedral magnetite (Fe_3O_4) crystals per cell, which is accompanied by the intracellular accumulation of tremendous amounts of iron (up to 4% of the dry weight) (2). This amount indicates that MSR-1 use very efficient systems to uptake, transport, and precipitation of iron that, however, are still poorly understood (3). On the basis of spectroscopic and biochemical analyses, it was suggested that for bacterial magnetite formation, Fe^{3+} was taken up from the environment and subsequently reduced intracellular (4, 5). A biochemical pool of iron is formed in the cells, essentially composed of ferritin and Fe^{2+} (6). Magnetite biomineralization proceeds first by transport of Fe^{2+} ions and ferritin into invaginated magnetosome vesicles where Fe^{2+} and Fe^{3+} ions co-precipitate (7). Final magnetite growth then occurs in fully formed mature magnetosomes (6, 7).

Although iron is the mineral element that is essential for microbial growth and is also essential trace element for many metabolic pathways in body, increase or decrease of iron will cause many disorders. These complications fall into two main groups. In primary haemochromatosis the iron overload is a consequence of a breakdown of a «switch» in the gut which controls the uptake of iron. In secondary

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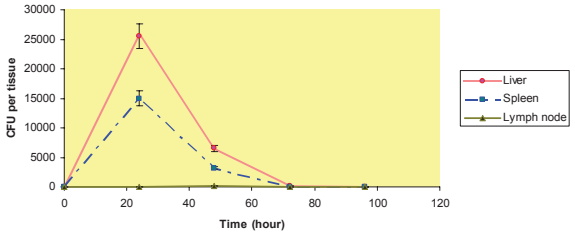

Fig.1. CFU per organ after i.v injection- 0, 24, 48, 72 & 96 hour after i.v injection, the number of colony forming units (CFU) in liver, spleen & lymph nodes.

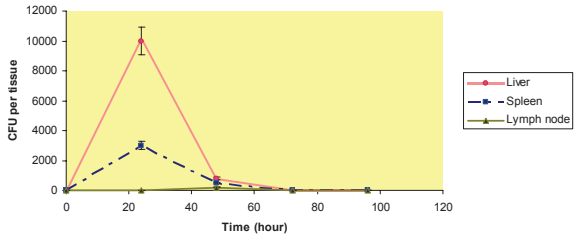

Fig. 2. CFU per organ after i.p injection- 0, 24, 48 & 72 hour after i.p injection, the number of colony forming units (CFU) in liver, spleen & lymph nodes.

haemochromatosis the excess iron results from multiple blood transfusions administered because of a genetic blood disease (8, 9).

As a matter of fact, the unique crystalline and magnetic property of magnetosomes in *Magnetospirillum gryphiswaldense* has brought this entity into the focus of multidisciplinary interest as they are used in biomedical applications (10). The aim of this study was to survey of effect of bacterial magnetite formation that takes up Fe^{2+} ions from the blood in animal model.

MATERIALS AND METHODS

Bacteria strain and culture condition. *Magnetospirillum gryphiswaldense MSR-1* (DSM 6361) was purchased from Deutsche Sammlung von Mikro organism und Zellkulturen. The bacteria were grown at 28°C with modified *Magnetospirillum* growth medium (11, 12) that containing 500 µM ferric citrate as previously described (12).

Preparing mice. Female BALB/c mice obtained from the Animal Department of Pasteur Institute of Iran, Tehran, Iran, weighing 18-24 g divided into 3 different groups for injection as intravascular (i.v), peritoneal (i.p) and subcutaneous (s.c). Eeach group had at least 4 different times of incubation (24 h, 48 h, 72 h & 96 h) (n = 36) and control group (n = 9). Mice were placed in polypropylene cages with stainless steel lids at an ambient temperature of 25 ± 2ºC with a 12 h light/dark period. The animals had free access to standard pellet chow and drinking water.

Determination of Lethal dose (LD_{50}). The bacterial pellets were washed in PBS buffer and additional dilutions were made in water to obtain different cell densities used to precisely calculate the LD_{50} dose. Then, 1×10^7 to 1×10^{12} CFU of bacteria were injected in mice and monitored for survival for 10 days after infection.

MSR-1 injection & Bacterial Clearance. MSR-1 (1×10^9 CFU) were injected with 200 µl volume of PBS as intravascular, peritoneal and subcutaneous to mice; the animal were sacrificed in 24, 48, 72 & 96 hours after injection, and the spleen, liver and lymph nodes were aseptically removed from each animal separately. The samples were rinsed with 5 ml sterile PBS, weighed and homogenized, then centrifuged at 1,000 rpm for 5 min (13). To evaluate the bacteria burden, the tissues separately homogenized in 5 ml PBS. Serial dilutions of earth tissue extraet were spread on *Magnetospirillum* growth medium plates and the number of colonies was counted after incubated for 10 days at 37ºC.

Blood samples Collection. At indicated time point (24, 48 and 72 h) after i.v, i.p & s.c injection of MSR-1, the blood samples were collected by cardiac puncture into centrifuge tube (15). Collection of samples were done between 8-10 am since serum iron levels is affected by the time of day among other parameters the serum iron level of each sample was determined by using commercial kit (Pars Azmoon, Tehran, Iran) with the sensitivity of 5 µg/dl (16).

RESULTS

Bacteria growth. After *Magnetospirillum* growth medium (supplemented with ferric citrate) had been prepared, bacteria colonies were visible about 1 mm in size after 5-7 days on medium. The colonies had a white-to-creamy appearance.

LD_{50} determination of MSR-1. Survival estimates of 10 mice per group until 10 days with five doses of MSR-1 were as fallows: 1×10^6, 10^7, 10^8, 10^9, 10^{10}, $10^{11}$to 10^{12} CFU per ml. The LD_{50} was determined

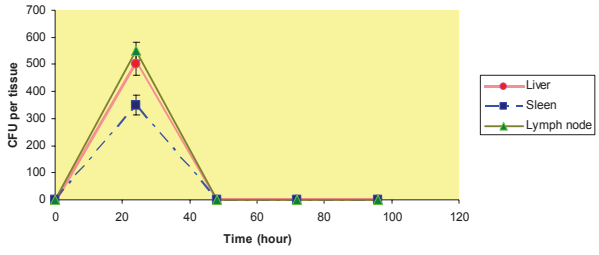

Fig.3. CFU per organ after s.c injection- 0, 24, 48 & 72 hour after s.c injection, the number of colony forming units(CFU) in liver, spleen & lymph nodes.

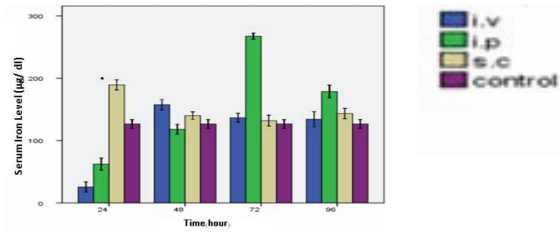

Fig. 4. Serum iron level. Serum iron level was determined by using commercial kit after 24, 48, 72 & 96 hour; i.v, i.p & s.c injection.

as1 × 10^9 CFU per ml. T-test was utilized for statistical analysis between groups. The LD_{50} determinations were repeat-ed twice with 10 mice per dose of the bacteria.

Clearance of bacteria. We had done a separate experiment with viable counts to investigate the distribution of MSR-1 for at last 96 hours. After 24 h period of i.v. injection, the numbers of CFUs recovered were higher in liver and spleen compared with lymph nodes. This trend reversed by hour 48, and 72, no viable bacteria were found in lymph nodes; The bacteria were found in liver and spleen in 48 and 72 h, and by hour 96 no viable bacteria were found in liver and spleen (Fig. 1); by hour 48 this trend changed and less viable bacteria were found in liver, spleen and lymph nodes after i.p and s.c injection compared with time point of 24 h (Fig. 2, 3). No viable bacteria were found in liver, spleen and lymph nodes after 72 h (Fig. 1, 2 & 3).

Serum Iron level. The serum iron level has been decreased to 20% of control level in the first 24 h after i.v injection ($P < 0.05$). In contrast, after 48 h it has been increased over 100% of control level ($P < 0.05$) and after 2 h came back to normal level, there were not a significant different in serum iron level ($P > 0.05$). In i.p injection after the first 24 h there was a significant change in serum iron level ($P < 0.05$), after 48 h it has been decreased a little compare with control level ($P < 0.05$) and after 72 h it has been increased compare with control level ($P < 0.05$), and s.c injection there were significant changes ($P < 0.05$) (Fig. 4).

Statistical analysis. In this study, t-tests was done for analysis serum iron level to compare differences between experimental and control mice. Statistical significance was determined by $P < 0.05$. The experiments results were repeated twice with a minimum of 3 mice per dose, group & day of the bacteria. However, the standard deviations (SD) was consistently < 10% of the mean.

DISCUSSION

Many previous studies have shown that MSR-1 could uptake and transport iron from the environment (17). In this study we have reported effect of MSR-1 on serum iron level in mice. On the other hand, we propose to identify basic survey of phenomenon after MSR-1 injection to animals models.

The bacteria were injected into mice for LD_{50} determination (LD_{50} determination was needed for bacteria injection). After LD_{50} determination, bacteria were injected i.v, i.p and s.c to identify the best effects of iron level changes and bacteria clearance in mice.

We have shown the role of bacteria on serum iron level by different routes of injection (i.v, i.p & s.c) in indicated time points (24, 48, 72, 96 h) and also the CFUs measurements have shown that after 48 hours from i.p & s.c injection and 72 h after i.v injection, mice could clear MSR-1from body. As it is shown in our data, MSR-1 bacteria could be used to decrease iron levels in mice.

In summary, we suggest a new application for using magnetite characterization of MSR-1 in biomedicine. Our research on optimizing the biological magnetic system is still continuing.

Based on our data, we can suggest the survey of MSR-1 effect on iron overloaded diseases in animal models and also survey of mechanism of clearance and immunity system response to MSR-1.

In conclusion, we suggest that *Magnetospirillum gryphiswaldense* can uptake, transport, and precipitate iron in mouse and 72 hours after intake of *MSR-1,* it will be cleared.

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