The different biological activity between the colloidal silver nanoparticle and the plasma silver nanoparticle in the human periodontal ligament cells

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ABSTRACT

The biological activity of the silver nanoparticles was tested in the primary cultured human periodontal ligament cells. Two types of silver nanoparticles (colloidal silver and plasma silver) were included in this study and MTT assay, cytotoxicity assay, and apoptosis assay were done. In results, the colloidal silver showed higher inhibition on the cellular proliferation than the plasma silver ($p<0.05$). The cytotoxicity was also higher in the colloidal silver ($p<0.05$). The colloidal silver showed higher apoptotic inducer than the plasma silver. In conclusion, the colloidal silver nanoparticle showed higher toxicity and inhibition of cellular proliferation than the plasma silver nanoparticle. Therefore, the production method might influence on the biological effect of the silver nanoparticle.

Keywords: plasma silver nanoparticle, colloidal silver nanoparticle, MTT assay, cytotoxicity assay, apoptosis

1 INTRODUCTION

The silver nanoparticle has been widely used in the field of cosmetics and medicine. It has been approved to use for topical application on skin as an ingredient of cosmetic products. In the field of medicine, it has been used as an additive of the burn-dressing. However, its biologic activity is not fully understood. According to published articles, it has anti-microbial effect and anti-inflammatory effect [1, 2].

In terms of producing methods, several different kinds of techniques have been introduced. The silver nanoparticle can be produced from ionic form of the silver [3, 4]. It can be also produced from burning out the silver metal at high temperature [5]. The physical and chemical properties of the silver nanoparticle may be different according to the producing method. However, the influence of the producing method on its biological properties has not been shown.

The objective of this study was to exam the biologic activity of the silver nanoparticle to the periodontal ligament (PDL) cells in terms of producing method.

2 MATERIALS AND METHOD

2.1 Cell cultures and MTT assay

The primary cultured human PDL cells were grown to 80% confluency in Dulbecco's modified Eagle's medium - low glucose (Gibco, BRL, Gaithersburg, MD) containing 1% penicillin/streptomycin, hFGF (100 μg/ml), and 10% fetal bovine serum (FBS) in 110mm culture dish under 37℃, 5% CO_2 condition. Twenty-four hours prior to the application of the silver nanoparticles, the cultures were washed 3 times with PBS and 10ml of serum-free medium was added to each plate. We added two different types of the silver nanoparticle to each grown dish. One was the colloidal silver (Nanomix®-SDW series, Nanopoly, Seoul, Korea) and the other was the plasma silver (NPC Inc., Iksan, Korea). The final concentrations were 2 μg/ml, 4 μg/ml, 10 μg/ml, and 20 μg/ml. The control was the PDL cells cultured without the silver nanoparticles. The MTT assay in the control and silver nanoparticle-treated cultures was assessed as previously described [6]: they were incubated in 6-well multiplates with yellow tetrazolium salt 3-(4, 5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (Cell proliferation kit I; Roche Molecular Biochemicals) for 4 hours at room temperature. Formazan crystals were solubilized overnight, and the product was quantified spectrophotometrically by measuring absorbance at 590 nm using a Victor Multilabel counter (Perkin-Elmer-Wallac, Freiburg, Germany).

2.2 Cytotoxicity assay and apoptosis assay

Cytotoxicity assay was done with commercial kit (CytoTox96®, Promega, WI). After 24 hours incubation with silver nanoparticles, the supernatant was collected for the analysis. The control was the supernatant of the cell culture without silver nanoparticle for 24 hours.

Apoptosis assay was done with the annexin V-FITC apoptosis detection kit I (BD biosciences, San Jose, CA). After 24 hours incubation with silver nanoparticles, the cells were fixed by 70% ethanol. They were washed twice with cold PBS and then 5 μ l of annexin V-FITC and 00 μ l of propidium iodide were applied for 15 minutes. After

washing twice with cold PBS, the slide was observed under the fluorescent microscopy.

2.3 Statistical analysis

The value from MTT assay and cytotoxicity assay were compared to the control by the independent samples t-test. The significance level was set as $p<0.05$.

3 RESULTS

The cells were observed under the phase contrast microscopy at 24 hours after the application of the silver nanoparticle (Fig. 1). The cellular morphology was changed and the cellular density was also different among groups. The cells in the colloidal silver group (Fig. 1b) were shown round shape and the cellular density was decreased compared to the others (Fig. 1a & c).

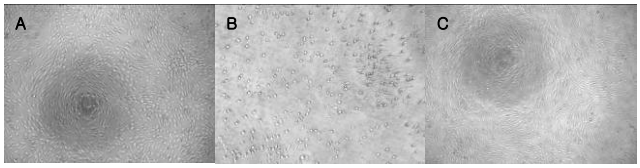

Figure 1. The findings of the phase contrast microscopy. A. control, B. 20μ g/ml colloidal silver, C. 20μ g/ml plasma silver

The results of MTT assay were shown in Figure 2. The values at 24 hours after the colloidal silver treatment were 0.069±0.001, 0.065±0.003, 0.071±0.004, and 0.072±0.008 in 2 μ g/ml, 4 μ g/ml, 10 μ g/ml, and 20 μ g/ml, respectively. When compared them to the control (1.212±0.220), the difference was statistically significant (P=0.001 in all groups). The values at 24 hours after the plasma silver treatment were 0.954±0.210, 0.944±0.632, 0.661±0.112, and 0.830±0.017 in 2 μ g/ml, 4 μ g/ml, 10 μ g/ml, and 20 μ g/ml, respectively. When compared them to the control (1.013 ± 0.275), the difference was not statistically significant (P>0.05 in all groups).

The values at 48 hours after the colloidal silver treatment were 0.076±0.002, 0.074±0.003, 0.085±0.003, and 0.091±0.004 in 2 μ g/ml, 4 μ g/ml, 10 μ g/ml, and 20 μ g/ml, respectively. When compared them to the control (1.246±0.283), the difference was statistically significant (P=0.002 in all groups). The values at 48 hours after the plasma silver treatment were 1.087±0.153, 0.956±0.113, 0.922±0.124, and 0.698±0.078 in 2 μ g/ml, 4 μ g/ml, 10 μ g/ml, and 20 μ g/ml, respectively. When compared them to the control (1.080 ± 0.392), the difference was not statistically significant (P>0.05 in all groups).

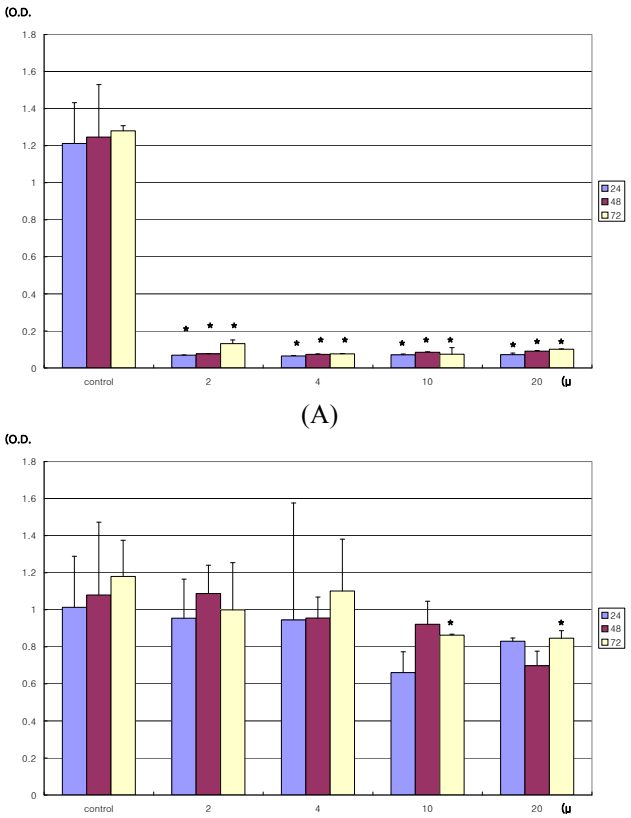

Figure 2. The results of MTT assay. The group of colloidal silver nanoparticle (A) showed lower value than the group of plasma silver nanoparticle (B).

The values at 72 hours after the colloidal silver treatment were 0.131±0.020, 0.076±0.003, 0.074±0.036, and 0.101±0.002 in 2 μ g/ml, 4 μ g/ml, 10 μ g/ml, and 20 μ g/ml, respectively. When compared them to the control (1.280±0.028), the difference was statistically significant (P<0.001 in all groups). The values at 72 hours after the plasma silver treatment were 0.999±0.255, 1.101±0.280, 0.863±0.006, and 0.847±0.040 in 2 μ g/ml, 4 μ g/ml, 10 μ g/ml, and 20 μ g/ml, respectively. When compared them to the control (1.180±0.195), the difference was statistically significant in 10 μ g/ml and 20 μ g/ml (P=0.002 and 0.003, respectively).

The results of the cytotoxicity assay were shown in Fig. 3. The O.D. value of the control was 0.180±0.005. The O.D. values of the colloidal silver were 0.143±0.003, 0.115±0.001, 0.087±0.001, and 0.090±0.003 in 2 μ g/ml, 4 μ g/ml, 10 μ g/ml, and 20 μ g/ml, respectively. When compared to the control, they were significantly different (P<0.005). The O.D. values of the plasma silver were 0.188±0.005, 0.181±0.002, 0.192±0.002, and 0.154±0.001 in 2 μ g/ml, 4 μ g/ml, 10 μ g/ml, and 20 μ g/ml, respectively. When compared to the control, the values of 10 μ g/ml and

20 μ g/ml were significantly different (P<0.05 and P<0.005, respectively).

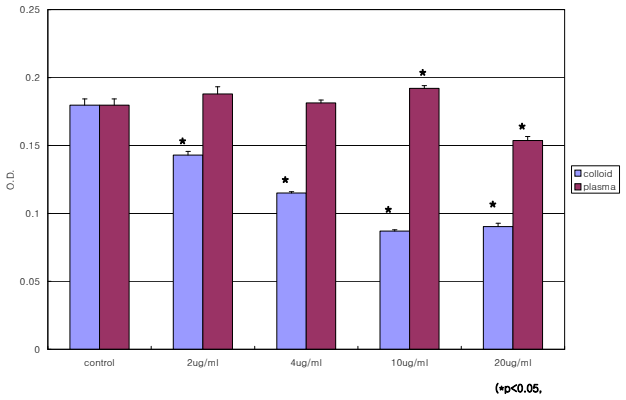

Figure 3. The results of the cytotoxicity assay

The results of the apoptosis assay were shown in Fig. 4. The expression of PI and annexin V was higher in the colloidal silver and the plasma silver compared to the control.

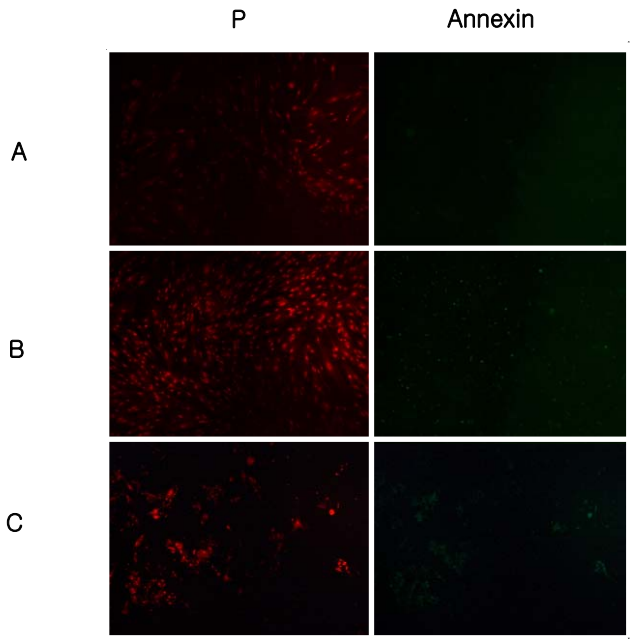

Figure 4. Fluorescent microscopic view (A: control, B: plasma silver, C: colloidal silver, P: propidium iodide)

4 DISCUSSION

There has been several different kind of method for producing the silver nanoparticles [3-5]. For these silver nanoparticles to be used in biomedical field, a key issue is their surface chemistry and functionalization [7]. In this article, we showed that the effect on cellular proliferation, cytotoxicity, and apoptosis were different between the colloidal silver and the plasma silver in the PDL cells.

The silver nanoparticle must be soluble in aqueous solution to be utilized in biological system. The water soluble nanoparticle can be produced by various method and the important factors are solvent selection, control of nucleation, and aggregation [8]. After purifying and drying, the nanoparticle produced from salts by chemical reaction is redissolved in water to form a clear red/ purple solution [9]. The size of chemically synthesized nanoparticle determines the color of solution, for example, 0.8 to 80 nm with the color range from yellow-orange to red-purple to blue-green. The color of the plasma silver solution was metallic gray (data not shown).

The method producing nanoparticle based on the salts, the nanoparticles are protected by ligand and their characteristics are largely dependent on the kind of the ligand. The producing method for colloidal silver nanoparticle can be found in many published articles [3, 4]. However, the producing method for plasma silver nanoparticle can be found in the patent [5]. The inhibitory effect on PDL cells of colloidal silver was more prominent than that of plasma silver (Fig. 2). Until 48 hours, all tested concentration of plasma silver (2 μ g/ml to 20 μ g/ml) was failed to show the difference to the control (P>0.05; Fig. 2a). However, every tested concentration of colloidal silver (2 μ g/ml to 20 μ g/ml) showed the statistically significant difference to the control (P<0.005; Fig. 2b). At 72 hours after the application of plasma silver, 10 μ g/ml and 20 μ g/ml showed the statistically significant difference to the control (P=0.002 and 0.003, respectively; Fig. 2a). These differences might be explained as follows. First, the presence of ligand in the colloidal silver might influence on its biologic activity. There have been many reports that explain the effect of the ligand on the stability of nanoparticle. However, the reports provided by both producers said that there was no ligand in the water except for the silver nanoparticles. Therefore, the ligand effects could not explain the differences. Second, the producing method might influence on its biologic activity. After drying out, the nanoparticle can be observed by TEM. The colloidal silver showed the tendency of aggregation due to the diamagnetic property (Fig. 5A). However, the plasma silver did not show the tendency of aggregation (Fig. 5B) and it showed the paramagnetic property [5].

The therapeutic advantage of these characteristics should be determined by further study. Generally, silver nanoparticle has antimicrobial effect [2] and anti-inflammatory effect [10]. The therapeutic concentration for the antimicrobial effect has been different according to the product. There have been very few reports on its anti-inflammatory effect. The silver nanoparticles in an animal model show that rapid healing and improved cosmetic appearance occur in a dose-dependent manner and it is

mediated by the reduction in wound inflammation and the modulation of fibrogenic cytokines [1]. If the silver nanoparticle is used for the toothpaste, its effect on the PDL cells must be tested in terms of the silver type and the concentration. If both tested nanoparticle had similar antimicrobial effect and anti-inflammatory effect, the toxicity should be considered in next. As our best knowledge, there has been no report comparing the anti-inflammatory effect among the different types of nanoparticle. However, the antimicrobial effect has been shown similar value.

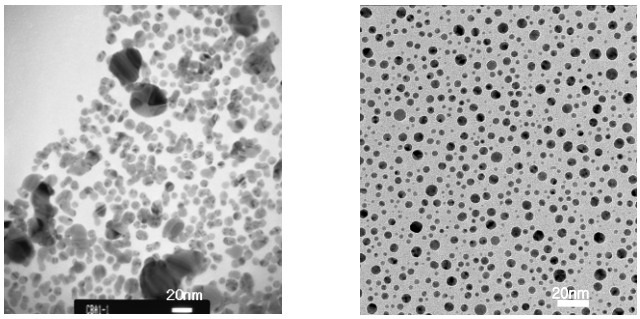

Figure 5. TEM images. The colloidal silver (A) showed more aggregation than the plasma silver (B).

The results of the cytotoxicity assay were shown that both silver nanoparticles were not toxic to the cell in the range of tested concentration. In case of the colloidal silver, the O.D. value was significantly lower than the control. Though the O.D. value of the plasma silver was generally higher than the control, it was not significant mostly. Only 10 μ g/ml of plasma silver was shown significantly high toxicity compared to the control ($P<0.05$). However, silver nanoparticle itself could increase the enzyme activity and it was dose-dependent (data not shown). Therefore, 6.7% increased LDH activity in 10 μ g/ml might be partly due to the silver nanoparticle in the culture media. The results of the cytotoxicity were shown that the inhibition of the cellular proliferation after the treatment of the silver nanoparticle was not due to the cellular lysis, but due to the apoptosis.

5 CONCLUSION

In conclusion, the colloidal silver was stronger apoptosis inducer to PDL cells than the plasma silver. The apoptosis was also dependent on time and concentration. To be utilized the silver nanoparticles in the area of the periodontology, the biologic property of each silver nanoparticle should be considered.

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