Identification of gold nanoparticle in lymphocytes: a confirmation of direct intracellular penetration effect

Lenfositte altın nanopartikülünün tespit edilmesi: direkt intraselüler penetrasyon etkisinin doğrulanması

Viroj Wiwanitkit, Amornpun Sereemaspun, Rojrit Rojanathanes
Chulalongkorn University Faculty of Medicine, Bangkok, Thailand

Abstract

Objective: Nanoparticles differ from the same material at larger scale in chemical and physical properties. The effect of nanoparticle on the blood cell still needs scientific verification.
Material and Methods: The direct effect of gold nanoparticles on lymphocyte was direct assessed by in vitro assay.
Results: In this work, the author reported additional results from specific observation on lymphocyte exposure to gold nanoparticle.
Conclusion: This result confirms for direct intracellular penetration effect. *(Turk J Hematol 2009; 26: 29-30)*
Key words: Gold nanoparticle, lymphocyte

Received: October 9, 2007 Accepted: December 24, 2008

Özet

Amaç: Nanopartiküller, kimyasal ve fiziksel özellikleri açısından aynı maddenin daha büyük ölçekli olanından farklıdırlar. Nanopartikülün kan hücresi üzerindeki etkisi halen bilimsel doğrulama gerektirmektedir.
Yöntem ve Gereçler: Altın nanopartikülünün lenfosit üzerine direkt etkisinin in vitro yöntemlerle incelenmesi.
Bulgular: Bu çalışmada yazar, lenfositin altın nanopartikülüne maruziyeti üzerindeki gözleminden çıkardığı ek sonuçları bildirmektedir.
Sonuç: Bu bulgular direkt intraselüler penetrasyon etkisini doğrulamaktadır. *(Turk J Hematol 2009; 26: 29-30)*
Anahtar kelimeler: Altın nanopartikülü, lenfosit

Geliş tarihi: 09 Ekim 2007 Kabul tarihi: 24 Aralık 2008

Introduction

Nanoparticles differ from the same material in larger scale with respect to their specific chemical and physical properties [1]. The rapidly developing field of nanotechnology is likely to become a possible source through inhalation, ingestion, skin uptake, and injection of engineered nanomaterials [2]. In medicine, there is limited knowledge on the hematological effect of nanoparticles. The effect of the nanoparticle on the blood cell still needs scientific verification. In this work, the authors report the results from lymphocyte exposure to gold nanoparticle and confirm the direct intracellular penetration effect.

Address for Correspondence: Prof. Dr. Viroj Wiwanitkit, Wiwanitkit House, Bangkhae, Bangkok Thailand
Phone: 6624132436 E-mail: wviroj@yahoo.com

Materials and Methods

Ten EDTA blood samples (average absolute white count =5870.5±125.6/mm^3, lymphocyte count =25.4±16.2%) were used for further laboratory analysis. The sample was collected and transferred according to standard procedure of the medical laboratory. Gold nanoparticle solution in this work was performed according to standard classical Turkevich citrate reduction method [3]. 9 nanometer-sized gold nanoparticles were established and gold nanoparticle concentration was prepared at 44 ppm. Based on this preparation technique, the gold nanoparticles can be kept in a dark container at 4°C for over a month. Protocol for observation of the effect of gold nanoparticle on lymphocytes was set. First, a mixture of equal amount (500 microliter) of gold nanoparticle solution and EDTA blood sample was prepared. The mixture was left for incubation at room temperature (37°C). After the mixture was waited for 15 minutes, blood smear preparation was performed using Wright staining technique. The morphological changes in lymphocytes were then studied by clinical microscopy technique (conventional light microscopy) under high power field. One hundred lymphocytes were examined for each blood smear. Confirmation of the finding was also performed by phase contrast microscopy technique. All of the laboratory analysis was performed in a reference-accredited laboratory of the Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University.

Results

According to this work, intracellular gold nanoparticles could be observed in about 90.4±8.5% of lymphocytes (Figure1). No significant change was observed in the contour (shape) of normal and gold nanoparticle-penetrated lymphocytes. Concerning the gold nanoparticle-penetrated lymphocytes, the nanoparticle could be seen discretely in the cytoplasm.

Discussion

The effect of nanoparticle in hematology has been poorly clarified. There are some recent reports on the application of nanoparticles in lymphocytes for diagnostic purposes, such as immunophenotyping [4,5]. The entry of gold into the white blood cell has been demonstrated previously [4-7]. Pittet et al. [6] proposed that labeled cells could be detected by magnetic resonance imaging, fluorescence reflectance imaging, fluorescence-mediated tomography, confocal microscopy and flow cytometry, and could be purified based on their fluorescent or magnetic properties. Wiwanitkit et al. [7] previously noted accumulation of gold nanoparticle in the white blood cell. However, there are many points that need further study and verification. In that work, Wiwanitkit et al. used only a single sample system, which might not be a confirmation. They reported the observation of nanoparticles in the granulocytes [7], and this might be due to the phagocytic mechanism, which cannot confirm that it is the direct effect of gold nanoparticle on the white blood cell. However, there is no confirmation on the actual direct effect of gold nanoparticle on lymphocytes. The purpose of this report was to confirm the penetration effect of gold nanoparticle into the lymphocyte, a non-phagocytic cell.

Here, we were able to demonstrate that the gold can enter into the lymphocyte. Since the lymphocyte has no phagocytosis activity, the only mechanism is the direct penetration of gold nanoparticle into the cytoplasm of the lymphocyte. This can confirm the usefulness of nanoparticle as a novel drug delivery system to the lymphocyte [8]. Fahmy [8] reported that because of its versatility, the nanoparticle system, which combines specific receptor targeting with an imaging agent and drug delivery, was suited to both basic science and applications, from developing therapeutic strategies for autoimmune and alloimmune diseases, to noninvasive tracking of pathogenic lymphocyte migration.

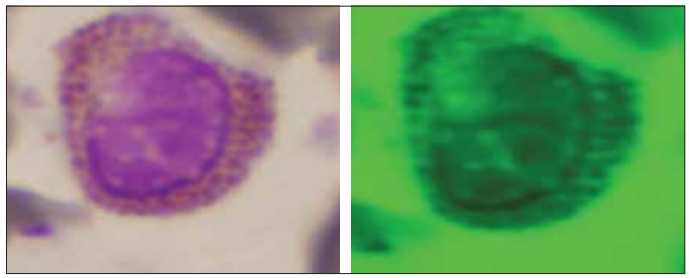

Figure 1. Accumulation of gold nanoparticle in white blood cell (appearing as black particle within the cytoplasm of the lymphocyte) **A.** Before phase contrast **B**. After phase contrast

However, the exact mechanism of direct penetration of the gold nanoparticle into the cytoplasm of lymphocytes requires further studies because the reported pore size in the lymphocyte membrane is about 4 nmx2.5 nm, which is smaller than the diameter of our gold nanoparticle. The authors believe that there might be some undiscovered mechanism that helps direct penetration of gold nanoparticles into the lymphocytes.

Comparing normal to gold nanoparticle-penetrated lymphocytes, no significant change was observed in the contour of the normal and gold nanoparticle-penetrated lymphocytes. Lymphocytes were still round. This can confirm the fact that nanoparticles are small and have no bulk effect on the cellular shape of lymphocytes. Of interest, the observed nanoparticles were in the cytoplasm but not the nucleus.

The nanoparticle could be seen discretely in the cytoplasm of the gold nanoparticle-penetrated lymphocytes. The question that remains to be determined in future research is why no nanoparticle was observed in the nucleus, even though it is smaller in size than the nuclear membrane pore.

In conclusion, a re-confirmation of the previous report that nanoparticles can be observed in the blood cell can be derived from this work, and it can be said that the direct penetration is a confirmed mechanism.

References

1. Seaton A. Nanotechnology and the occupational physician. Occup Med (Lond) 2006;56:312-6.
2. Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 2005;113:823-39.
3. Haruta M. When gold is not noble: catalysis by nanoparticles. Chem Rec 2003;3:75-87.
4. Siiman O, Gordon K, Burshteyn A, Maples JA, Whitesell JK. Immunophenotyping using gold or silver nanoparticle-polystyrene bead conjugates with multiple light scatter. Cytometry 2000;41:298-307.
5. Bene L, Szentesi G, Matyus L, Gaspar R, Damjanovich S. Nanoparticle energy transfer on the cell surface. J Mol Recognit 2005;18:236-53.
6. Pittet MJ, Swirski FK, Reynolds F, Josephson L, Weissleder R. Labeling of immune cells for in vivo imaging using magnetofluorescent nanoparticles. Nat Protoc 2006;1:73-9.
7. Wiwanitkit V, Sereemaspun A, Rojanathanes R. Effect of gold nanoparticle on the microscopic morphology of white blood cell. Cytopathology 2007 Dec 18 [Epub ahead of print].
8. Fahmy TM, Fong PM, Park J, Constable T, Saltzman WM. Nanosystems for simultaneous imaging and drug delivery to T cells. AAPS J 2007;9:E171-80.