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Cultured macrophages cause dissolucytosis of metallic silver

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Summary. The present study proves that cultured macrophages can liberate silver ions from metallic silver surfaces by a process called dissolucytosis. Macrophages (J774) were grown on a silver plate for different periods of time and after fixation in glutaraldehyde, they were subjected to autometallograhy in order to amplify possible cellular silver-sulphur nanocrystals. Light and electron microscopic analysis of the cells revealed that silver ions released from the plate had been taken up by the macrophages and accumulated in lysosome- like structures.

We found that the liberation of silver ions takes place extracellularly and is caused by chemical activity in a dissolution membrane, most likely secreted and organized by the macrophages. The liberation and the subsequent uptake of silver ions in the macrophages is a relatively fast process and the resulting silver-sulphur nanocrystals can be observed in macrophages that have been in contact with metallic silver for only a few minutes. Our findings indicate that the speed of dissolucytosis is highly influenced by the chemical nature of the object exposed to the dissolucytotic process which is likely to occur whenever macrophages encounter a non-phagocytosable foreign object.

Key words: Autometallography (AMG), Silver, Macrophages, Dissolucytosis, Lysosome

Introduction

Silver has been used as a medical remedy since the earliest times (Petering, 1976). In ancient Italy and Greece, it was recognized that a silver coin dropped into a bottle of water caused the water to stay fresh for extended periods.

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It is well known that a multitude of bacteria are killed or oppressed by silver ions (Fox et al., 1969; Modak and Fox, 1973; Hartmann et al., 1999; Brett, 2006) and those antibacterial properties of silver ions are still used in the clinic, in the form of antimicrobial dressings or foils to fight chronic ulcers and burn wounds (Gamelli et al., 1993; Leaper, 2006). Some catheters and tubes are silver coated to prevent infection in relation to installation of central venous catheters (Maki et al., 1988; Sampath et al., 1995; Bach et al., 1996) or intubation of patients to be artificially ventilated at intensive care units (Hartmann et al., 1999; Ramstedt et al., 2007).

It is generally believed that the toxic effect of silver on mammals is minimal because silver ions are bound to sulphide in the human body (Petering, 1976; Danscher, 1981). On the other hand silver ions have been found to be able to penetrate the blood-brain barrier in rats, where they accumulate in glia cells and neurons, primarily in large motorneurons in the brainstem, spinal cord and neurons in the cerebellar nuclei (Rungby and Danscher, 1983a). Silver ions have also been proved to affect the pyramidal neurons of the developing hippocampus (Rungby et al., 1987b). These data support the notion that silver ions may be toxic to neurons and that silver should therefore be handled cautiously, as it possibly increases the risk of toxic effects such as brain dysfunction and neuropathy. A decrease in blood leucocytes and nucleated bone marrow cells was found within 24 hours after application of silver sulfadiazine to mouse skin (Gamelli et al., 1993) and silver lactate has been shown to cause acute coagulation necrosis of mouse peritoneal macrophages in vitro (Ellermann-Eriksen et al., 1987; Rungby et al., 1987a) .

Despite these rather frightening data a majority of silver ions end up in the form of silver-sulphur nanocrystals related to collagen fibers and fibrils and also located in fibroblast lysosomes, neurons, gliacells and so on (Danscher, 1981; Rungby and Danscher, 1983b). It seems as if these silver-sulphur nanocrystals

are not very toxic (Kristiansen et al., 2008), but over time, a constant exposure to silver will result in argyria, i.e. a bluish discoloration of skin and mucosal membranes due to such accumulations of silver-sulphur nanocrystals (Aaseth et al., 1981; Wadhera and Fung, 2005).

To our knowledge *in vitro* dissolucytotic liberation of silver ions from metallic silver by macrophages has never been reported. In the present study, J774 macrophages were grown directly on a metallic silver surface in order to evaluate 1) whether dissolucytosis takes place *in vitro* and, if so, to map the time scale of the process; 2) whether the liberated silver ions are present as silver-sulphur nanocrystals (in the dissolution membrane) allowing them to be amplified with autometallography (AMG); 3) whether dissolucytotic liberated silver ions accumulate in lysosome like structures of the macrophages.

Materials and methods

Cell cultures

J774 macrophages were grown under standard conditions as previously described (Brunk et al., 1997; Li et al., 2000; Zhao et al., 2000). In brief, the cells were grown in 200 ml Nunc plastic flasks (Nunc A/S, Roskilde, Denmark) in a medium consisting of RPMI 1640 + GlutaMAX ™+ 25 mM HEPES (GIBCO®, supplied by Invitrogen, Taastrup, Denmark) supplemented with 10% fetal bovine serum (Biochrom AG, Berlin, Germany), 1% Penicillin-streptomycin (10 U/ml Penicilllin G sodium + 10 µg/ml streptomycinsulfate in 0.85% saline) and 1% non-essential amino acids. The plastic flasks containing the cells were incubated at 37.1°C and 5% CO_2 (Heraeus, Heracell 150, Axeb Danmark A/S). The cells were sub-cultivated at confluence two or three times a week. When sub-cultivating the old medium was replaced by 10 ml of fresh medium and the cells were mechanically loosened using a plastic scraper (Nunc A/S, Roskilde, Denmark). Afterwards, 5 ml of this cell suspension was transferred to each of two new glass flasks, adding an additional 12 ml of fresh medium to each flask.

Silver exposure

Macrophages were grown directly on a metallic silver plate (1.77 cm^2, 99.95% pure silver, Good-Fellow, Cambridge, UK), placed in a 24-well tray (Nunc A/S, Roskilde, Denmark). The cells were mechanically loosened from the bottom of the flask by scraping and 0.5 ml of the cell suspension was added to every well having a silver plate placed at the bottom, together with 0.5 ml of fresh medium (500.000 cells/ml). Then the tray was placed in the incubator. The macrophages were grown on the metallic silver plates for 5 min, 10 min, 15 min, 30 min, 60 min, 2 h, 3 h, 4 h, 5 h, 6 h, 12 h and 24 h, respectively, using five wells at each time point, repeating all experiments three times. After incubation on the metallic silver plate, 0.5 ml of the medium was removed and the cells were loosened mechanically and placed on a glass cover slip (1.77 cm^2). 0.5 ml of fresh medium was added and the cells were incubated for 24 hours. To show the process of dissolucytosis silver grids (3 mm, mesh 300) were placed in the wells for 24 hours instead of a silver plate.

Controls

Macrophages grown directly on a thin glass cover slip placed in a standard well were mechanically loosened and incubated for 24 hours. For every exposure time the number of control wells (n=5) is equal to the number of exposed wells.

Preparation for silver AMG staining

After 24 hours of incubation the medium was replaced with 3% glutaraldehyde for 20 minutes. The glutaraldehyde was removed and the fixed cells were rinsed several times in distilled water before being AMG developed.

Cells attached to silver grids were fixed in 3% glutaraldehyde and embedded in Epon. Semi-thin sections were cut and subjected to AMG development.

The AMG developer

The developer consisted of 1) 60 ml gum arabic solution, 2) 10 ml sodium citrate buffer (25.5 g of citric acid $1H_2O$+23.5 g sodium citrate 2 H_2O to 100 ml distilled water). Immediately before use 3) 15 ml reductor, 0.85 g of hydroquinone dissolved in 15 ml of distilled water, 40°C, and 4) 15 ml of a solution containing silver ions, 0.12 g silver lactate in 15 ml distilled water, 40°C, were added and the AMG developer was thoroughly stirred (Danscher, 1981; Danscher and Stoltenberg, 2006).

AMG development

The macrophage containing cover slips were placed in a jar filled with AMG developer and placed in a water bath at 26°C. The set-up was covered with a dark hood and developed for 60 min. The AMG developer was then replaced by a 5% sodium thiosulphate solution for 10 min in order to stop the process, and the cover slips were finally rinsed several times in distilled water while still in the jars. The cell cultures were counterstained with toluidine blue, dehydrated and embedded in DePeX (Bie & Berntsen A/S, Herlev, Denmark) and mounted upside down on a glass slide.

Electron microscopical analysis

Macrophages were grown on silver plates or silver grids in the same way as the cells destined to be

analyzed in the light microscope. At the end of the exposure, 0.5 ml medium from each well was removed and the cells were mechanically loosened. The cell suspensions from three wells were added to 1.5 ml of freshly made medium and placed on a glass slip at the bottom of a plastic bottle (Chamber Slide TM, Nunc A/S, Roskilde, Denmark). After 24 hours incubation the medium was replaced with 3% glutaraldehyde and the bottles were stored in a refrigerator at 5°C. A drop of Epon was placed on the glass slip and an Epon block was set on top. After 24 hours the Epon block was broken off and 3 µm semi-thin sections were cut and placed on glass slides.

The silver grids were taken out of the medium, fixated in 3% glutaraldehyde, dehydrated and embedded in Epon. Semi-thin sections were cut and placed on glass slides. Then the sections were subjected to AMG development, rinsed carefully in distilled water, and finally counterstained with toluidin blue. After analysis in the light microscope selected sections were reembedded on top of a blank Epon block. This Epon block was trimmed and ultrathin sections were cut, placed on copper grids and stained with uranyl and lead (Danscher, 1981; Danscher and Stoltenberg, 2006).

Estimation of the number of silver-containing cells

From the series of cultures exposed to silver for different periods of time 3 samples of each given exposure time were randomly chosen. In these randomly chosen samples, 200 cells were systematically counted from the centre of the cover slip using the 40x objective on the microscope and a counting grid from the analySIS TM software pack. The presence and absence of AMG staining in each cell were noted. The proportion of the AMG stained macrophages at each time was calculated as an average of three samples. The results for each exposure time are plotted in a semi logarithmic scale together with error bars showing SD.

Results

Fase-contrast microscopy of J774 macrophage cell cultures reveals that the cells attach to the metallic silver plate as readily and effectively as they attach to the glass cover slips of the control wells. Light and electron microscopic analysis of the cultures showed an overwhelming presence of AMG enhanced silver-sulphur nanocrystals (AMG silver grains) in a multitude of macrophages while the controls were completely void of staining.

Through a period from 5 minutes to 24 hours after having attached to a metallic silver surface, the number of macrophages containing AMG silver grains in the cytoplasm increased exponentially. After 5 minutes the silver plate grown macrophages showed an uptake of silver ions in approximately 40% of the cells, while almost 100% of the cells contained AMG silver grains after 1 h (Fig. 1). There is also an increase in the intensity of AMG silver staining, and after 24 h of exposure the AMG staining was so intense that the cytoplasm of all the macrophages was completely covered by black AMG silver grains (Fig. 2a-d).

At the ultrastructural levels the amplified AMG silver grains were found to be rounded, but with distinct straight planes, indicating their crystalline nature. The silver amplified nanocrystals were constantly found in lysosome-like structures in the cytoplasm (Fig. 2f).

All the control cells were completely void of AMG staining (Fig. 2e).

Analysis of metallic silver grids placed in J774 macrophage cultures showed that the macrophages do not attach directly to the metallic silver surface, but to a bio-film, coined the dissolution membrane (Fig. 3a-c). Silver ions leaking from the dissolution membrane are taken up by the macrophages (the dissolucytes) and end up in lysosome-like structures where they again can be found accumulated in silver-sulphur nanocrystals (Fig. 3c). A few sparsely or unstained macrophages could be seen attaching to the silver grids after 24 hours of exposure (Fig. 3b). A phenomenon suggested to be the result of macrophages leaving the dissolution membrane after being loaded with silver thus making space for new macrophages.

Discussion

Recently it was proved that J774 macrophages are capable of liberating gold ions from metallic gold by controlling the chemical milieu in an ultra-thin biofilm layer intervened between the macrophage and the metal surface. This biofilm, coined the dissolution membrane, is most likely partly secreted and molecularly organized by the macrophages with the purpose of facilitating the

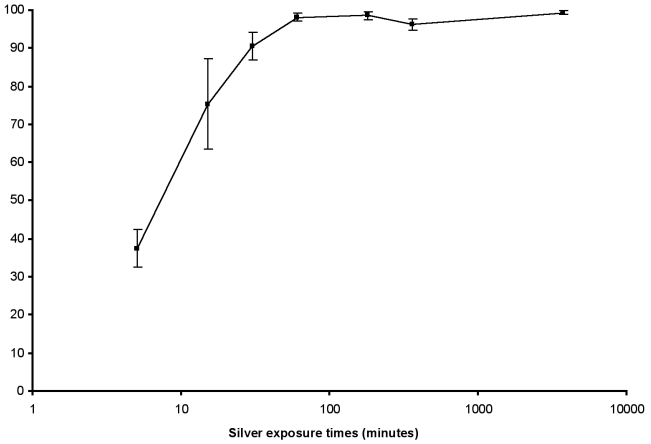

Fig. 1. The proportion of J774 macrophages (%) containing AMG silver grains evaluated by light microscopy. Value means are the average of n=3 cultures for each time point (5, 15, 30, 60, 180, 360, 1440 min). Each culture was exposed to a metallic silver plate. Notice that the x-axis is log-scale. Error bars are ± SD.

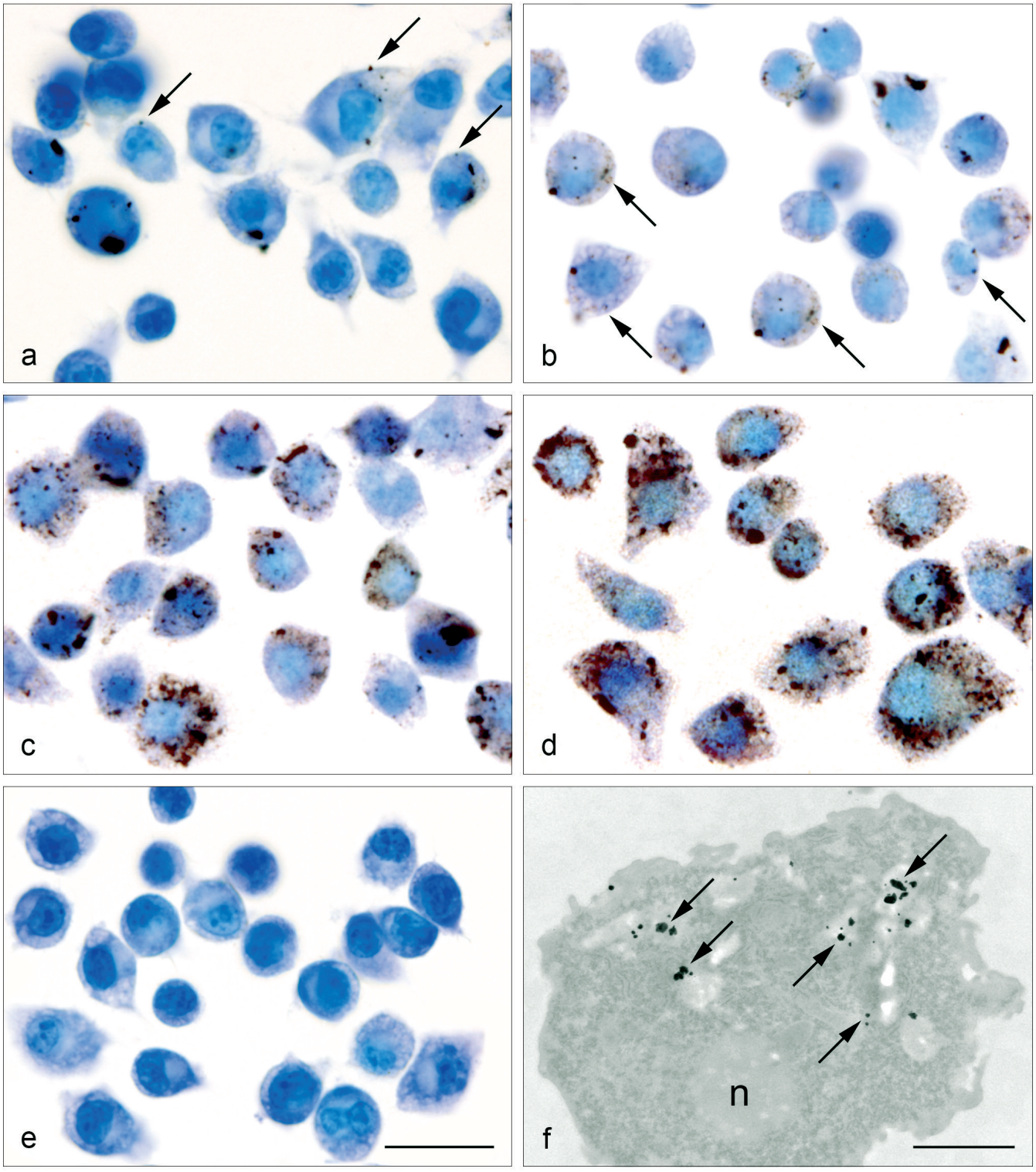

Fig. 2. AMG enhanced silver-sulphur nanocrystals (AMG silver grains) in the cytoplasm of J774 macrophages. Light micrograph of J774 macrophages exposed to metallic silver plates for 5 minutes **(a)**, 1 h **(b)**, 12 h **(c)** and 24 h **(d)**. Note the sparse staining i.e. few and small AMG silver grains (arrows a, b), increasing to huge staining i.e. several and larger AMG silver grains **(c, d)**. **e.** Control culture void of AMG silver grains. **f.** Electron micrograph of a J774 macrophage exposed to metallic silver for 24 hours. AMG silver grains located in lysosome-like structures in the cytoplasm (arrows). n: nucleus. Scale bars: a-e, 20 µm; f, 1 µm.

possibility of chemical dissolution of metals and most likely other insoluble non-phagocytoseable (>20 µm) foreign bodies in the organism (Ziats et al., 1988; Wälivaara et al., 1994; Lopes et al., 2006; Jakobsen et al., 2007; Larsen et al., 2007).

The presence of AMG amplified silver-sulphur nanocrystals in the macrophages of the present study reveals that silver ions are liberated from metallic silver surfaces in the same way as gold ions from a metallic gold surface (Larsen et al., 2007). With AMG the released silver ions can be followed from the dissolution membrane and into the dissolucytotic macrophages themselves.

The bio-dissolution of metallic silver is much faster than is the case with metallic gold (Larsen et al., 2007). The fast and heavy presence of the first AMG silver grains in lysosomes of the attacking macrophages just a few minutes after they have contacted the metallic surface strongly suggests that precaution should be observed when placing metallic silver into living organisms.

The AMG amplifiable silver seen in the lysosomes and in the dissolution membrane is most likely in the form of silver–sulphur nanocrystals, as only three chemical forms of silver are known to ignite the AMG amplification process, i.e. silver atoms, silver sulphide and silver selenide molecules. As selenium is present in an insignificant amount in our set-up, silver must bind chemically as the very stable silver-sulphide molecules that accumulate as silver-sulphur nanocrystals (Danscher, 1981; Danscher and Stoltenberg, 2006).

When the amount of silver-sulphur nanocrystals increases in the lysosomal vesicles the AMG enhancement will make them merge, causing the appearance of rather big AMG grains. This process can even continue when lysosomal vesicles are placed close to each other. In such cases the confluencing process can result in larger AMG grains covering two or more vesicles. That this is what takes place has been proved by analyzing EM pictures of the process (Fig. 3; Holm et al., 1988; Danscher and Stoltenberg, 2006).

The sensitivity of the AMG technique is so immense that, at least theoretically, one silver ion replacing a hydrogen ion in a sulphydryl group will ignite the amplification process. Therefore, more studies are needed to establish whether the extracellular AMG particles represent silver enhanced silver – sulphide radicals or include more silver atoms and sulphur atoms, being in fact tiny silver-sulphur nanocrystals, i.e less than 0.4 nanometer.

We believe that cyanide ions are involved in the liberation of gold ions, resulting in the production of aurocyanide. The aurocyanide is most likely produced using thiocyanate generated during the oxidative burst of polymorphnuclear leucocytes and macrophages (Graham and Dale, 1990; Rudkowski et al., 1990; Graham and Kettle, 1998; Ferre and Claria, 2006).

It is a well-founded speculation to suggest that silver

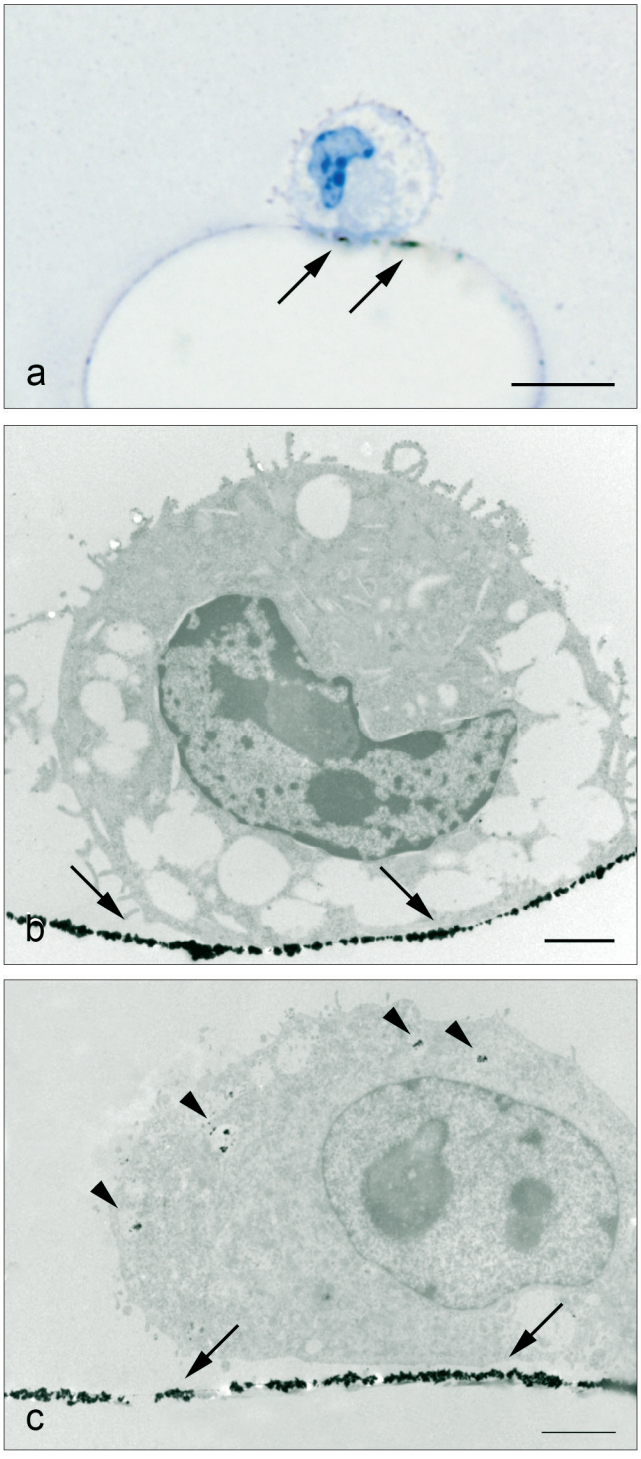

Fig. 3. The process of dissolucytosis. **a.** Light micrograph of the dissolution membrane (arrow) between a J774 macrophage and the space after a silver grid dropped out under the preparation procedure. Slice thickness 3 µm. Scale bar: 10 µm. **b.** Electron micrograph of a J774 macrophage exposed to silver grids and the intervening dissolution membrane containing black silver enhanced silver-sulphur nanocrystals (arrows). Scale bar: 2 µm. **c.** Electron micrograph of AMG silver grains in lysosome-like structures (arrowheads) in a macrophage and the dissolution membrane (arrows). Scale bar: 2 µm.

ions are liberated by the same chemical processes as gold ions (Danscher, 2002; Larsen et al., 2007).

We know that some of the silver ions react with sulphide ions or sulphydryl radicals in the dissolution membrane, causing the creation of silver-sulphur radicals or silver-sulphide molecules that accumulate in AMG detectable silver-sulphur nanocrystals (Danscher, 1981).

The speed of dissolucytotic liberation and subsequent uptake of silver ions by macrophages is remarkable compared to the relatively slow liberation and uptake of gold ions from metallic gold surfaces, where only 5% of the macrophages revealed AMG grains after 24 hours (Larsen et al., 2007). In contrast almost all the dissolucytotic macrophages in the present study were loaded one hour after being attached to the silver surface. This difference in dissolucytotic speed between gold and silver is probably due to the fact that silver is easily oxidized while gold is almost impossible to oxidize (Russel and Hugo, 1994).

Our finding that a few sparsely or even unstained macrophages could be seen attaching to the silver grids after 24 hours of exposure is most likely due to the macrophages leaving the dissolution membrane after being loaded with silver thus making room for other macrophages on the grid.

It has been known for some time that even at low doses silver influences the metabolism of cultured macrophages (Wataha et al., 2002). Therefore the heavy load of silver nanoparticles seen after 24 hours must affect the normal function of the cells. We did not evaluate what impact the bio-released silver ions had on the survival of the macrophages, nor if it influenced functional parameters, as the aim of the study was to reveal whether silver ions are released from the surface of metallic silver by dissolucytosis or not.

In conclusion: Macrophages in culture are able to release silver ions from the surface of metallic silver. The process by which the bio-liberation takes place is similar to what was recently found with metallic gold. We call this process dissolucytosis and have described how the dissolution membrane spaced between the adhering macrophages and the silver surface stores the chemical processes that lead to oxidation of silver atoms to silver ions. The process was found to be fast compared to the bio-release of gold ions. Further studies are needed to evaluate whether the liberated silver ions harm the macrophages.

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References

Aaseth J., Olsen A., Halse J. and Hovig T. (1981). Argyria-tissue deposition of silver as selenide. Scand. J. Clin. Lab. Invest. 41, 247-251.

Bach A., Schmidt H., Böttiger B., Schreiber B., Böhrer H., Motsch J., Martin E. and Sonntag H.G. (1996). Retention of antibacterial activity and bacterial colonization of antiseptic-bonded central venous catheters. J. Antimicrob. Chemother. 37, 315-322.

Brett D.W. (2006). A discussion of silver as an antimicrobial agent: alleviating the confusion. Ostomy Wound Manage. 52, 34-41.

Brunk U.T., Dalen H., Roberg K. and Hellquist H.B. (1997). Photo-oxidative disruption of lysosomal membranes causes apoptosis of cultured human fibroblasts. Free Radic. Biol. Med. 23, 616-626.

Danscher G. (1981). Light and electron microscopic localization of silver in biological tissue. Histochemistry 71, 177-186.

Danscher G. (2002). In vivo liberation of gold ions from gold implants. Autometallographic tracing of gold in cells adjacent to metallic gold. Histochem. Cell Biol. 117, 447-452.

Danscher G. and Stoltenberg M. (2006). Silver enhancement of quantum dots resulting from (1) metabolism of toxic metals in animals and humans, (2) in vivo, in vitro and immersion created zinc-sulphur/zinc-selenium nanocrystals, (3) metal ions liberated from metal implants and particles. Prog. Histochem. Cytochem. 41, 57-139.

Ellermann-Eriksen S., Rungby J. and Mogensen S.C. (1987). Autointerference in silver accumulation in macrophages without affecting phagocytic, migratory or interferon-producing capacity. Virchows Arch. (B). 53, 243-250.

Ferre N. and Claria J. (2006). New insight into the regulation of liver inflammation and oxidative stress. Mini Rev. Med. Chem. 6, 1321-1330.

Fox C.L. Jr., Rappole B.W. and Stanford W. (1969). Control of pseudomonas infection in burns by silver sulfadiazine. Surg. Gynecol. Obstet. 128, 1021-1026.

Gamelli R.L., Paxton T.P. and O'Reilly M. (1993). Bone marrow toxicity by silver sulfadiazine. Surg. Gynecol. Obstet. 177, 115-120.

Graham G.G. and Dale M.M. (1990). The activation of gold complexes by cyanide produced by polymorphnuclear leucocytes-II. Evidence for the formation and biological activity of aurocyanide. Biochem. Pharmacol. 39, 1697-1702.

Graham G.G. and Kettle A.J. (1998). The activation of gold complexes by cyanide produced by polymorphonuclear leukocytes. III. The formation of aurocyanide by myeloperoxidase. Biochem. Pharmacol. 56, 307-312.

Hartmann M., Guttmann J., Müller B., Hallmann T. and Geiger K. (1999). Reduction of the bacterial load by the silver-coated endotracheal tube (SCET), a laboratory investigation. Technol. Health Care 7, 359-370.

Holm I.E., Andreasen A., Danscher G., Pérez-Clausell J. and Nielsen H. (1988). Quantification of vesicular zinc in the rat brain. Histochemistry 89, 289-293.

Jakobsen S.S., Danscher G., Stoltenberg M., Larsen A., Bruun J.M., Mygind T., Kemp K., and Soballe K. (2007). Cobalt-chromium-molybdenum alloy causes metal accumulation and metallothionein up-regulation in rat liver and kidney. Basic Clin. Pharmacol. Toxicol. 101, 441-446.

Kristiansen S., Ifversen P. and Danscher G. (2008). Ultrastuctural

localization and chemical binding of silver ions in human organotypic skin cultures. Histochem. Cell Biol. 130, 177-184.

Larsen A., Stoltenberg M. and Danscher G. (2007). In vitro liberation of charged gold atoms: autometallographic tracing of gold ions released by macrophages grown on metallic gold surfaces. Histochem. Cell Biol. 128, 1-6.

Leaper D.J. (2006). Silver dressings: their role in wound management. Int. Wound J. 3, 282-294.

Li W., Yuan X., Nordgren G., Dalen H., Dubowchik G.M., Firestone R.A. and Brunk U.T. (2000). Induction of cell death by the lysosomotropic detergent MSDH. FEBS Lett. 470, 35-39.

Lopes F.A., Morin P., Oliveira R. and Melo L.F. (2006). Interaction of Desulfovibrio desulfuricans biofilms with stainless steel surface and its impact on bacterial metabolism. J. Appl. Microbiol. 101, 1087-1095.

Maki D.G., Cobb L., Garman J.K., Shapiro J.M., Ringer M. and Helgerson R.B. (1988). An attachable silver-impregnated cuff for prevention of infection with central venous catheters: a prospective randomized multicenter trial. Am. J. Med. 85, 307-314.

Modak S.M. and Fox C.L. Jr (1973). Binding of silver sulfadiazine to the cellular components of Pseudomonas aeruginosa. Biochem. Pharmacol. 22, 2391-2404.

Petering H. (1976). Pharmacology and toxicology of heavy metals: Silver. Pharma. Ther. A. 1, 127-130

Ramstedt M., Houriet R., Mossialos D., Haas D. and Mathieu H.J. (2007). Wet chemical silver treatment of endotracheal tubes to produce antibacterial surfaces. J. Biomed. Mater. Res. B. Appl. Biomater. 83, 169-180.

Rudkowski R., Graham G.G., Champion G.D. and Ziegler J.B. (1990). The activation of gold complexes by cyanide produced by polymorphnuclear leucocytes – I: The effects of aurocyanide on the oxidative burst of polymorphnuclear leucocytes. Biochem. Pharmacol. 39, 1687-1695.

Rungby J. and Danscher G. (1983a). Localization of exogenous silver in brain and spinal cord of silver exposed rats. Acta Neuropathol. 60, 92-98.

Rungby J. and Danscher G. (1983b). Neuronal accumulation of silver in brains of progeny from argyric rats. Acta Neuropathol. 61, 258-262.

Rungby J., Hultman P. and Ellermann-Erikson S. (1987a). Silver affects viability and structure of cultured mouse peritoneal macrophages and peroxidative capacity of whole mouse liver. Arch. Toxicol. 59, 408-412.

Rungby J., Slomianka L., Danscher G., Andersen A.H. and West M.J. (1987b). A quantitative evaluation of the neurotoxic effect of silver on the volumes of the components of the developing rat hippocampus. Toxicology 43, 261-268.

Russell A.D. and Hugo W.B. (1994). Antimicrobial activity and action of silver. Prog. Med. Chem. 31, 351-370.

Sampath L.A., Chowdhury N., Caraos L. and Modak S.M. (1995). Infection resistance of surface modified catheters with either short-lived or prolonged activity. J. Hosp. Infect. 30, 201-210.

Wadhera A. and Fung M. (2005). Systemic argyria associated with ingestion of colloidal silver. Dermatol. Online J. 11, 12.

Wataha J.C., Lockwood P.E., Schedle A., Noda M. and Bouillaguet S. (2002). Ag, Cu, Hg and Ni ions alter the metabolism of human monocytes during extended low-dose exposures. J. Oral Rehabil. 29, 133-139.

Wälivaara B., Aronsson B.O., Rodahl M., Lausmaa J. and Tenqvall P. (1994). Titanium with different oxides: in vitro studies of protein adsorption and contact activation. Biomaterials 15, 827-834.

Zhao M., Eaton J.W. and Brunk U.T. (2000). Protection against oxidant-mediated lysosomal rupture: a new anti-apoptotic activity of Bcl-2? FEBS Lett. 485, 104-108.

Ziats N.P., Miller K.M. and Anderson J.M. (1988). *In vitro* and *in vivo* interactions of cells with biomaterials. Biomaterials 9, 5-13.

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