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Letter

A carrier protein is essential for the action of silver nanoparticles in an animal experiment

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ABSTRACT — Many environmental chemicals caused rat hyperactivity, a key feature of attention deficit hyperactivity disorder (ADHD). These were confirmed by many epidemiological studies, showing the correlation in their exposure levels in patients with ADHD. The nanomaterials have been difficult to be evaluated health risk because of its physicochemical nature. In this study, we examined the possibility that silver nanoparticle might be ADHD related chemical. Oral exposure of rat pups to silver nanoparticles (3mg/kg) with 1% bovine serum albumin as a carrier protein at 5 days old increased 1.3~1.4 fold spontaneous motor activity while without carrier proteins, it failed to do so. Thus, dispersion of silver nanoparticle solution by carrier proteins was necessary to examine the toxicological action of silver nanoparticles in the animal experiment.

Key words: Silver nanoparticle, Carrier proteins, ADHD

INTRODUCTION

Nanotechnology has brought revolution in science (Feynman, 1960). In particular, nanotechnology has been rapidly developed, producing many types of nanomaterials, which have a wide range of applications in various fields such as biology, medicine, and bioengineering. Since nanoparticles have novel physical and chemical properties, they have different ones from the conventional materials. Thus, more widespread exposure to humans and to the environment presents unknown potential risks (Oberdörster, 2009; Oberdörster *et al*., 2013).

Silver nanoparticles (AgNPs) have been extensively used as antimicrobial agents in cosmetic, textile, and food industries as well as disinfectants for medical devices and for coating home applications (Chen and Schluesener, 2008; Wijnhoven *et al*., 2009). Therefore, it is very important to evaluate the health risk of AgNPs, using animal experiments.

In this study, we examined the effects of AgNP on a neonatal central nervous system in the rat.

MATERIALS AND METHODS

Materials

Chemicals and dynamic light scattering measurement

AgNPs (10 nm) and bovine serum albumin were purchased from Sigma-Aldrich (Tokyo, Japan). Particle size was analyzed using dynamic light scattering (ELSZ-2, Otsuka Electronics Co., Osaka, Japan).

Animals and treatments with chemicals

Pregnant Wistar rats were obtained from Clea Japan (Tokyo, Japan). They were maintained in home cages and fed with a standard laboratory chow (MF diet, Oriental Yeast Corp., Tokyo, Japan) and distilled water *ad libitum* at 22°C on a light-dark cycle (12 hr/12 hr) for at least one week. Typically, about 50 male pups were born from 10

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pregnant rats and 5-7 pups were randomly housed. They were weaned at 3 weeks of age.

Silver nanoparticles were suspended in olive oil in the presence (Fig. 1A) and the absence (Fig. 1B) of 1% bovine serum albumin. The chemical (3 mg/kg/10 µL/rat pup) was orally administered into 5-day-old pups. Control rats were done with vehicle alone (10 µL) in the same way. Animals received humane care according to the National Institute for Environmental Studies guidelines.

Measurements of spontaneous motor activity

Spontaneous motor activity of rats (8 weeks of age) was individually measured in a home cage with a Supermex system (Muromachi Kikai, Tokyo, Japan), as described previously (Masuo *et al.*, 1997, 2002; Ishido *et al.*, 2002, 2004a, 2004b, 2004c, 2004d, 2007, 2011, 2017). In this system a sensor detects the radiated body heat of an animal. A Supermex sensor head consists of paired infrared pyroelectric detectors. This system detects any object with a temperature at least 5°C higher than background within a cone-shaped area with a 6 m diameter and a 110° vertex. The sensor monitors motion in multiple zones of the cage through an array of Fresnel lenses placed above the cage and movement of the animal in the *X, Y,* and *Z* axis can be covered. We measured the activity counted by this system during 15 min for 22-24 hr and maintained on a 12 hr light:dark cycle. Food and water were fully given at the beginning of counting and rats were never disturbed in any way. Ten rats were recorded concurrently.

Statistics

Statistical analyses were carried out using the StatView Ver. 5.0 software (SAS Institute, Cary, NC). Effects of neonatal AgNPs on spontaneous motor activity measured for every 2 hr were statistically analyzed by ANOVA of repeated measure. The total activities in nocturnal phase were analyzed by Student's *t*-test.

RESULTS AND DISCUSSION

We examined the effects of oral administrations of 3 mg/kg AgNPs including 1% bovine serum albumin on the developing rat brain. Control rats were given olive oil alone. AgNPs increased motor activity through the nocturnal phase of the light-dark cycle. The effects of AgNP on spontaneous motor activity were significant ($p < 0.0001$ by ANOVA). Total spontaneous motor activities in the dark periods (12 hr) of the nanoparticle-treated rats were 1.3~1.4 times higher than vehicle-treated control rats ($p < 0.001$, Student's *t* test) (Fig. 1A). Without any carrier proteins, AgNPs failed to increase the spontaneous motor activity (Fig. 1B).

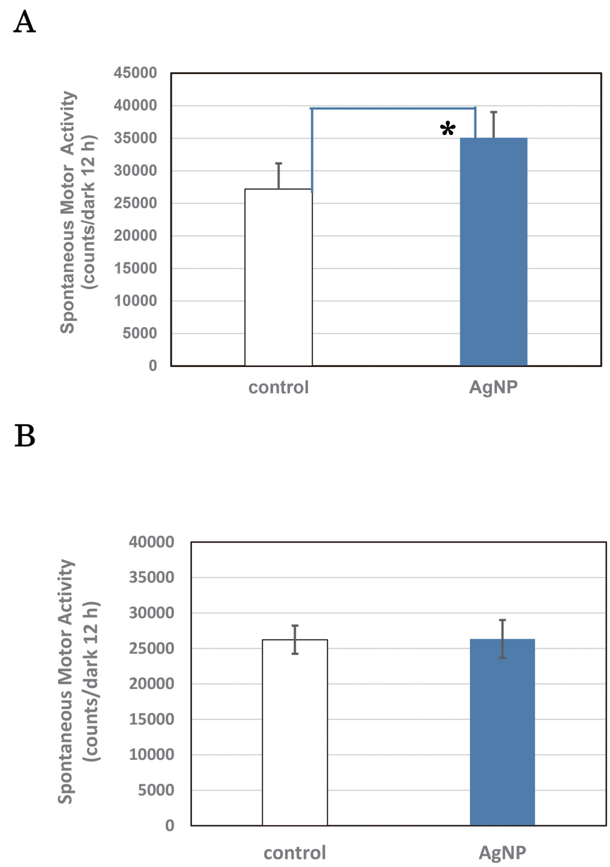

Fig. 1. Effects of carrier proteins in particle preparations on central nervous actions of AgNPs in the neonatal pups. AgNP (3 mg/kg) was dissolved in olive oil with (*A*) or without (*B*) 1% bovine serum albumin, followed by orally administered to rat pups at 5 days of age. Their spontaneous motor activity was measured with Supermex[R] at 8 weeks of age. Asterisks denote significant differences in their spontaneous motor activity between untreated rats (control) and AgNP-treated rats (AgNP).

There have been the potential risks of products by nanotechnology, including AgNPs, for health as well as for the environment due to the lack of toxicological data. Therefore, it is required to evaluate the possible toxicity of the nanoparticles using *in vivo* animal experiments.

On the basis of physics, nanomaterials have been considered to have a quantum nature, which is called 'Kubo effects'. It tends to be easily agglomerated, leading to be beyond a nano size. Thus, it is very important to disperse the nanomaterials by such as carrier proteins, seen in this study.

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Conflict of interest---- The author declares that there is no conflict of interest.

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