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Gold nanoparticles prevent cognitive deficits, oxidative stress and inflammation in a rat model of sporadic dementia of Alzheimer's type☆

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ABSTRACT

Alzheimer's disease (AD) is the most common form of neurodegenerative dementia in the aged brain. Even though its etiology is unknown, factors such as neuroinflammation, mitochondrial dysfunction, formation of reactive oxygen species (ROS), and impaired insulin signaling may play a role. We used a sporadic AD model in rats generated by intracerebroventricular–streptozotocin (i.c.v.–STZ) injection to test the therapeutic effect of gold nanoparticles (GNPs). We tested the null hypothesis that there would be no difference between the STZ + GNPs group and the STZ group in the analyzed markers. We observed that STZ-induced impairment in mitochondrial ATP production, neuroinflammation, and oxidative stress were all prevented by GNP treatment. Moreover, while STZ induced deficits in both spatial and recognition memory, GNPs prevented this effect. These results suggest that GNPs may be considered as a potential treatment for dementias.

1. Background

Alzheimer's disease (AD) is the most common form of progressive neurodegenerative dementia in the aged brain [1]. AD affects millions of people and has become a major medical and social issue in developing societies [2]. AD has an unknown etiology, genetic factors are enrolled in few cases, however, most cases are associated with the sporadic AD, which has neuroinflammation, oxidative stress and impaired on insulin signaling as factors associated [2,3].

Evidence from several studies have inferred a close association between inflammatory mediators such as nuclear factor k-light-chain-enhancer of activated B cells (NF-κB) and interleukin-1 (IL1)-β and the AD due to the cyclooxygenase (COX)-2-mediated reciprocal regulation of IL-1β and amyloid (A)β between glial and neuronal cells [4,5]. Furthermore, mitochondrial dysfunction and oxidative stress play a prime role in the pathogenesis of neurodegenerative disease [6]. Defects in mitochondrial function and ROS production generate oxidative stress in neuronal cells which triggers the release of cytochrome *c* from mitochondria leading to neurodegeneration [7,8]. Brain cells are particularly vulnerable to oxidative damage because of high oxygen consumption, substantial polyunsaturated fatty acid content, and the fact that this organ has a limited ability to combat oxidative stress [9,10]. Oxidative damage to lipids (lipid peroxidation) and proteins (protein carbonyl formation) can lead to structural and functional disruption of the cell membrane, inactivation of enzymes, and finally, cell death [11].

Animal models of AD have been developed and they are characterized by neuroinflammation and oxidative stress [12]. Animal model of sporadic AD can be generated by intracerebroventricular–streptozotocin (ICV–STZ) injection. The disruption of insulin signaling by ICV–STZ leads to impaired cognition performance [13], a decrease in cerebral glucose and mitochondrial metabolism [14,15], and oxidative stress [16,17]. The insulin-resistant brain state is thought to play a pivotal role in the pathogenesis of neurodegenerative disorders including AD. While animal models of AD have been used to test a potential of numerous compounds to treat or prevent the disease progression, the actual advancements in the treatment of AD patients are scarce [18].

Over the past few decades, inorganic nanoparticles, whose structures exhibit significantly novel and distinct physical, chemical, and biological properties, have elicited much interest given their biological and pharmaceutical potential [19]. Gold nanoparticles (GNPs) have been actively investigated in a wide variety of biomedical applications because of their biocompatibility and easy conjugation to biomolecules [20]. Commonly, GNPs are employed as a carrier in drug delivery

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systems, including delivery into the brain [21–23]. However, the effect of the use of GNP per se in the brain in neurodegenerative disease is unknown.

GNPs have also received a great deal of attention as anti-inflammatory agents because of their ability to inhibit the expression of NF-κB and subsequent inflammatory reactions [24,25]. Another study demonstrated that the GNPs blocked NF-κB activation by interacting with cys-179 of IKK-β and inhibiting the production of pro-inflammatory cytokines, such as TNF-α and IL-1β [24]. Furthermore, studies using GNPs showed anti-inflammatory (decrease of pro-inflammatory cytokines) and antioxidant effects in dermal and muscle injury models [26–28].

Considering that therapeutic options for AD are limited and that many promising agents have failed in clinical trials, it may be interesting to explore the therapeutic potential of GNPs in AD. The aim of the present study was to analyze the effect of GNP treatment on cognitive function, neuroinflammation and oxidative stress using a sporadic AD model.

2. Materials and methods

2.1. Synthesis and characterization of GNP

The GNPs were synthesized and characterized as described Turkevich [29] with minor modifications. Tetrachloroauric acid ($HAuCl_4$) was acquired from Sigma-Aldrich (St. Louis, MO, USA), and sodium citrate ($Na_3C_6H_5O_7$), a reducing agent and stabilizer, was acquired from Nuclear (Diadema, SP, Brazil). Briefly, 100 mL of 0.50 mM tetrachloroauric acid solution was maintained under reflux and stirred mechanically at 700 rpm. Then, a sodium citrate solution (5 mL; 63.5 mM) was added. The reactional medium was maintained under reflux and stirring by 20 min. Then, it was cooled and the pH was adjusted to physiologic pH with a buffer solution. The solution was centrifuged at 13,000 rpm for 15 min; the supernatant was removed to eliminate the excess of sodium citrate and washed twice with ultrapure water and dispersed in a saline solution prior use. The UV–vis spectrum shows a SPR (surface plasmon resonant) with $\lambda_{max} = 528$ nm and was registered using a Shimadzu instrument (model UV-1800; Shimadzu Corp., Kyoto, Japan). X-ray diffraction analysis was performed using a Shimadzu LABX model XDR-6000 diffractometer with Cu Kα radiation ($\lambda = 1.54056$ Â, 30 kV, 30 mA). The scan rate was 2°/min from 20 to 80°. It was possible to calculate the mean particle diameter using the Scherrer's equation on the signal from the X-ray diffractogram at $2\theta = 38°$ (major relative intensity) and by transmission electron microscopy (TEM) analysis, which was performed using a JEOL Titan 80–300 kV [30]. The Au concentration was measured by atomic absorption spectroscopy employing an Atomic Absorption Spectrometer (Varian model AA 240Z; Varian Medical Systems, Inc., Palo Alto, CA, USA) and the value obtained for solution was 2.5 $mg \cdot L^{-1}$.

2.2. Animals

Wistar male rats (n = 30 per group, 250–300 g) were obtained from our breeding colony. The animals were kept at 12-h light/dark cycle with free access to food and water. All experimental procedures were in accordance with the Brazilian Guidelines for the Care and Use of Animals for Scientific and Didactic Purposes (DOU 27/5/13, MCTI, p. 7) and approved by the local ethical committee (Protocol no. 69/2014-1). The animals were randomly divided into 4 groups as follows: Group 1: administration of vehicle i.c.v. (Sham), Group 2: administration of streptozotocin i.c.v. (STZ), Group 3: streptozotocin i.c.v. + GNPs 48 h (STZ + GNPs 48 h), Group 4: vehicle i.c.v. + GNPs 48 h (Sham + GNPs 48 h).

2.3. Sporadic AD model and GNP treatment

The animals were anesthetized by intraperitoneal (i.p.) injection of ketamine (80 mg/kg body weight) and xylazine (20 mg/kg body weight) and underwent i.c.v. surgery procedures as previously described. The STZ (3 mg/kg) was injected i.c.v. (2 μL/hemisphere) bilaterally into the lateral ventricle. Sham animals were injected with saline [31].

The intraperitoneal GNP treatment was initiated 48 h following administration of STZ, and the rats were given a dose of 2.5 mg/kg body weight with 20 nm particle size. GNP administration frequency was every 48 h until the twenty-first day after stereotactic surgery. The brain bioavailability and safety of this treatment has been shown previously (unpublished data).

2.4. Object recognition

After 21 days of treatment, recognition memory was analyzed using the object recognition task. On the first day (day 1), the rats were randomly placed into individual square wooden boxes (90 × 90 × 90 cm) that were positioned on the floor of a soundproof and diffusely illuminated room for 10 min. On day 2, the rats were familiarized with two identical plastic objects that were placed 10 cm away from the walls of the cage. Each testing session lasted 5 min, and the time spent exploring each object was analyzed. Following the trial, the rats were returned to their home cages for 24 h and were then placed in the arena for a new object recognition test. In this test, one of the objects was replaced with a new object that differed in shape, color and texture. The time of exploration of the two objects was recorded and the discrimination ratio was defined as follows: TN / (TN + TF), [TN = time spent exploring the new object; TF = time spent exploring the familiar object]. All of the objects and the arena were thoroughly cleaned with 10% ethanol between the trials to remove any residual odors. Each exploration was defined as an act in which the rat would approach the object with its nose (within 2 cm), sniff, and touch the object with the tip of its nose and/or with its paws. If the rat only stood next to the object or on top of it, this was not considered as exploratory activity.

2.5. The Barnes Maze

Another group of animals performed the spatial memory task after the GNP treatment. The Banes Maze is a 90-cm-high circular platform with a diameter of 120 cm and an escape hole measuring 5 cm in diameter. This hole leads to a small chamber attached underneath the platform. There are 19 other imitation holes circled around the maze in order to distract the animals from the real hole. The imitation holes look like the escape hole, but do not lead to an escape chamber. The Maze is lit using a 100 W light bulb. The rats are kept in a dark cage until the beginning of trails. Once placed in the center of the platform, the rat had 1 min to find the escape hole. The rat spent 20 s in the escape chamber and was then returned to its original housing cage. The latency to find the escape hole was recorded. After five days of learning, a probe trial was performed in which the rat was once again placed on the maze without the escape hole, now virtually looking like all the other holes. The time spent around the escape hole was recorded. After 60 s, the rat was placed in its original housing cage.

2.6. Tissue and homogenate preparation

Forty-eight hours after the last administration of GNPs, the animals were euthanized by decapitation with guillotine. The whole brain was surgically removed, immediately processed, and stored for further analyses.