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# Silver and titanium dioxide nanoparticles alter oxidative/inflammatory response and renin–angiotensin system in brain

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ABSTRACT

The study was designed to examine the effects of silver AgNPs, 20 nm) and titanium dioxide (Aeroxide® P25 $TiO_2$NPs, 21 nm) nanoparticles on brain oxidative stress parameters, its antioxidant potential and brain renin–angiotensin system (RAS) *in vivo*. The analysis was performed 28 days after single dose injection of $TiO_2$NPs and AgNPs (10 or 5 mg/kg body weight, respectively). The AgNPs, but not $TiO_2$NPs, administration resulted in decreased lipid and cholesterol peroxidation. Antioxidant enzymes gene expression and/or activity were changed differently for $TiO_2$NPs and AgNPs group. The $TiO_2$NPs decreased aromatase gene expression, and glutathione peroxidase and reductase activities. In AgNPs group the sodium dismutase 1 and glutathione reductase mRNA levels were decreased as opposed to their activities. Both NPs altered the expression of brain RAS genes (angiotensinogen, renin, angiotensin I converting enzyme 1 and 2), but only $TiO_2$NPs caused similar changes on protein level. The expression of amyloid beta precursor protein gene was not altered by any kind of injected NPs. The $TiO_2$NPs were more potent modulator of gene expression in the brain than AgNPs, despite the two times lower dosage. These results suggest that AgNPs and $TiO_2$NPs exposure may modulate the brain function, but with different strength.



*Abbreviations:* ACTB, beta actin; APP, amyloid β (A4) precursor protein; APx, ascorbic acid peroxidase; ARO, aromatase; ANG, angiotensin; ACE, angiotensin converting enzyme 1; ACE2, angiotensin converting enzyme 2; AGT, angiotensinogen; BBB, blood–brain barrier; BW, body weight; CAT, catalase; COX-2, cyclooxygenase 2 (or PTGS2, prostaglandin-endoperoxide synthase 2); GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPX, glutation peroxidase; GSH, reduced glutathione; GSR, glutation reductase; GSSG, oxidized glutathione; GSTP1, glutathione S-transferase pi 1; HMOX1, heme oxygenase 1; IL, interleukin; *iv.*, intravenous; MDA, malondialdehyde; NMs, nanomaterials; NPs, nanoparticles; NO, nitric oxide; NOAEL, no observable adverse effect level; NOS, nitric oxide synthase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PBS, phosphate-buffered saline; PPIC, peptidylprolyl isomerase C; REN, renin; RAS, renin–angiotensin system; ROS, reactive oxygen species; SOD, superoxide dismutase; TAS, total antioxidant status; TBARS, thiobarbituric acid reactive substances; TNFα, tumor necrosis factor α.

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## 1. Introduction

Nanotechnology is a rapidly developing field of science and technology focused on materials with the nanoscale dimension. In the recent years a number of applications based on the use of nanomaterials (NMs) in everyday life has increased dramatically. Nowadays, NMs are found in variety of products, such as household items, cosmetics, food packaging systems, food additives and biosensors (Savolainen et al., 2010; Philbrook et al., 2011; Kim and An, 2012). NMs are also used in many fields of biology and medicine, including drug and gene delivery, tissue engineering, detection of protein and pathogens and cancer treatment (Yang et al., 2012b). Due to the widespread use of NM, and nanoparticles (NPs) in particular, it is important to consider the health consequences that nanotechnology and engineered NPs can bring upon people. NPs can enter living organisms by inhalation or injection (Yildirimer et al., 2011). The intravenous (*iv.*) administration of NPs seems particularly important, since there is a growing evidence that NPs can be a promising platform for molecular diagnostics (biosensing and bioimaging) and/or therapy (Loo at al., 2005; Zhou et al., 2011; Larguinho and Baptista, 2012). However, once present in the circulation, due to their small size, NPs are able to cross blood–brain barrier (BBB) and accumulate in brain (Dziendzikowska et al., 2012; Lima et al., 2012).

As silver and titanium NPs (AgNPs and $TiO_2$NPs) are among the most commonly used NPs in nanotechnology (http://www.nanotechproject.org; Bouwmeester et al., 2011; Munger et al., 2014), an increasing number of studies is now focused on explaining their impact on human health and the environment (Gaillet and Rouanet, 2015). It was already shown that AgNPs pass through BBB (Tang et al., 2009; Sharma et al., 2010), and our previous study demonstrated a long time deposition of AgNPs in the brain after a single *iv.* injection (Dziendzikowska et al., 2012). Also van der Zande et al. (2012) showed that 28-day oral exposure to AgNPs caused the long retention of NPs in the brain. Accumulation of $TiO_2$NPs (5 nm) in the brain was reported by Ma et al. (2010) and Ze et al. (2014c). On the other hand, Shinohara et al. (2014) did not observed accumulation of 21 nm $TiO_2$NPs in the brain after *iv.* injection, likely due to the very quick NPs clearance. Whether or not, the NPs accumulate in the brain, their widespread presence in our everyday life rises a question of their effect on brain function. The effects of $TiO_2$NPs on central nervous system were recently reviewed by Czajka et al. (2015).

Free radical formation and oxidative stress induction are currently considered as a major potential mechanism of antimicrobial activity of AgNPs and photocatalytic properties of $TiO_2$NPs (von Moos and Slaveykova, 2014). However, this is also main molecular mechanism of nanotoxicity. An *in vitro* and *in vivo* studies showed that exposure to NPs, including AgNPs and $TiO_2$NPs, stimulated reactive oxygen species (ROS) generation, oxidative stress induction leading to serious cellular damage and degradation of the membrane structure of the cells, both *in vitro* and *in vivo* (Yildirimer et al., 2011; Kim and Ryu, 2013). Skalska et al. (2015) reported that exposure of adult Wistar rats to AgNPs led to ultrastructural changes in synapses including synaptic degeneration, especially in the hippocampus. The negative influence of $TiO_2$NPs on rat primary cultured hippocampal neurons was also stated by Hong et al. (2015) and Sheng et al. (2015). In the brain $TiO_2$NPs and AgNPs are said to induce ROS generation and suppress antioxidant defense system elements causing damage to cellular components, DNA breaks, lipid and protein peroxidation (Kim and Ryu, 2013; Czajka et al., 2015). Oxidative stress plays an important role in the induction and enhancement of inflammation. Nanoparticles can elicit a spectrum of tissue responses including immune cell activation. Several *in vitro* and *in vivo* AgNPs and $TiO_2$NPs exposure studies demonstrated also that exposure to these nanoparticles induced proinflammatory response in brain, including increased levels of tumor necrosis factor α (TNFα) and interleukin (IL) − 1β in mice through the stimulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway, nitric oxide synthase (NOS) activity and nitric oxide (NO) production (Xue et al., 2012).

Inflammation and increased ROS generation has been implicated in the pathogenesis of several neurodegenerative and cerebrovascular disorders, as well as in brain aging. Hence, this work is focused on the effects of $TiO_2$NPs or AgNPs on a renin–angiotensin system (RAS) in brain tissue with possible mechanism connected with oxidative stress. The hypothesis to be tested was that increased by NPs injection oxidative stress leads to the imbalance of RAS components in the brain. RAS is a systemic regulatory axis involved in the control of blood pressure and volume homeostasis. A key player in the systemic RAS is renin (Ren), a kidney-derived enzyme that cleaves an angiotensinogen protein (Agt) to angiotensin I (Ang I). Ang I is then converted to Ang II by angiotensin converting enzyme 1 (Ace). There is also angiotensin converting enzyme 2 (Ace2) that promotes alternative conversion of Ang II to Ang-(1–7) or Ang I to Ang-(1–9). The angiotensin regulates cardiovascular function by binding to At1 and At2 receptors on cardiac, renal and vascular cells. Despite the systemic action, it has been postulated that the RAS can operate at the tissue level in the tissue specific way, independently of the circulating RAS, and may be activated even when the circulating RAS is suppressed or normal. A functional tissue RAS has been identified in brain, kidney, heart, adipose tissue, hematopoietic tissue, gastrointestinal tract, liver, endocrine system and blood vessels (for an extensive review about RAS see Paul et al., 2006; Nguyen Dinh Cat and Touyz, 2011). The brain RAS was suggested to play an important role in cardiovascular and fluid-electrolyte homeostasis, metabolic syndrome and obesity, aging and cancer (Baltatu et al., 2011). Especially as the components of RAS do not cross BBB which emphasizes the role of local gene expression (Wright and Harding, 2011).

A noticeable aging of the European population and a higher risk of developing of neurodegenerative and circulatory disorders among the elderly people rises the question of the role of NMs, and NPs in particular, in these processes. It was shown that accumulation of NPs in the brain may induce inflammation followed by adverse long term effects (Tang et al., 2008; Kiruba Daniel et al., 2010; Klippstein et al., 2010; Krol, 2012; Su et al., 2015). Thus, taking into account possible harmful action of NPs, the aim of this study was to explore effects of $TiO_2$NPs and AgNPs *iv.* administration on oxidative stress/inflammation response and components of RAS in the brain.

## 2. Material and methods

### 2.1. Dispersion of nanoparticles

Spherical AgNPs of nominal diameter 20 ± 5 nm were provided by PlasmaChem (Berlin, Germany). Aeroxide® P25 $TiO_2$NPs with nominal size 21 nm from Degussa–Evonik (Essen, Germany) were provided courtesy of European Commission Joint Research Center depository. AgNPs and $TiO_2$NPs stock solutions were prepared by dispersion of 5 mg of nanoparticles in 800 mL of 0.9% saline solution. AgNPs and $TiO_2$NPs were sonicated on ice for 3 min using a probe sonicator (Branson, Danbury, Connecticut, USA) with 420 J/$m^3$ total ultrasound energy. Subsequently 100 mL of a 10× concentrated phosphate-buffered saline (PBS) and 100 mL of 15% bovine serum albumin (BSA) were added promptly after sonication. A detailed characteristic of used AgNPs and $TiO_2$NPs is published in Lankoff et al. (2012) (Table 1).

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