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REVIEW ARTICLE

Porcine brain microvessel endothelial cells show pro-inflammatory response to the size and composition of metallic nanoparticles

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Abstract

The purpose of the current studies was to determine if systemic exposure of various metallic nanoparticles differing in size and composition [silver (Ag-NPs, 25, 40 and 80 nm), copper-oxide (Cu-NPs, 40 and 60 nm) or gold (Au-NPs, 3 and 5 nm)] can induce the release of pro-inflammatory mediators that influence the restrictive nature of the blood–brain barrier (BBB) *in vitro*. Confluent porcine brain microvessel endothelial cells (pBMECs) (8–12 days) were treated with various metallic nanoparticles (15 μg/ml). Extracellular concentrations of pro-inflammatory mediators (IL-1β, TNFα and PGE_2) were evaluated using ELISA. pBMECs were cultured in standard 12-well Transwell® inserts, and permeability was evaluated by measuring the transport of fluorescein across the pBMEC monolayers. PGE_2 release following Cu-NP exposure was significantly increased when compared to the control. Similar results were observed for Ag-NPs but not Au-NPs. The secretion of TNFα and IL-1β was observed for both Cu-NPs and Ag-NPs but not in response to Au-NPs. The post-treatment time profiles of TNFα and IL-1β revealed that the IL-1β response was more persistent. The permeability ratios (exposure/control) were significantly greater following exposure to Cu-NPs or Ag-NPs, compared to Au-NPs. Together, these data suggest that the composition and size of NPs can cause significant pro-inflammatory response that can influence the integrity of the BBB.

Keywords

Blood–brain barrier, lipopolysaccharide (LPS), metallic-colloidal nanoparticles, neuroinflamation, neurotoxicity, porcine brain microvessel endothelial cells

History

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Introduction

Various metallic colloidal nanoparticles are small (1–100 nm in size) particles used in manufacturing hundreds of industrial and commercial products. Silver nanoparticles (Ag-NPs) are widely used in engineering, manufacturing and biomedicine. Currently, there are several consumer products that contain various silver nanoparticles owing to their anti-microbial properties. At present, copper oxide (CuO) nanoparticles (Cu-NPs) have numerous commercial applications in anti-microbial preparations, heat transfer fluids, inks, intrauterine contraceptive devices, metallic coatings, polymers/plastics, semiconductors and as lubricant additives that effectively repair worn surfaces, reducing friction and wear (Abraham et al., 1996). Recent advances in material science and biomedical engineering on gold nanoparticles (Au-NPs) offer possibilities for their applications in targeted pharmaceutical therapy or diagnostic imaging with surface modifications (proteins, ligands and antibodies). With the increased industrialization and commercialization of products containing nanomaterials, these nanoparticles will inevitably enter the environment as has been reported for other nanoparticles (Aruoja et al., 2009; Audus & Borchardt, 1987; Barone & Feuerstein, 1999; Bove et al., 2001). Exposure during shipping and handling of the materials and the disposal of consumer products containing these nanomaterials may have significant impact on the environment and eventually the human population. The potential environmental and public health consequences remain unknown. Consequently, we must understand how biological systems interact with and respond to nanomaterials since it is of importance to public health. A recent report on state of the art human risk assessment of silver compounds in consumer products suggested different approaches can be assumed in a data deficient situation (Schafer et al., 2013). One of the important point suggested during the meeting was to study the barrier penetration of nanosilver because studies have shown distribution of silver to brain and testis. Therefore, testing should be address potential neurotoxicity and reproductive toxicity of nanosilver (Schafer et al., 2013).

Present knowledge of the biological responses of colloidal nanoparticles with cerebral microvasculature and the central

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nervous system (CNS) is very limited. The cerebral microvasculature functions as a highly specialized barrier by regulating the passage of substances and thus protecting the cells of the CNS (Claudio et al., 1994; Calingasan et al., 2000; Deli et al., 1995). In fact, the primary brain microvessel endothelial cells (BMECs) isolated from cerebral cortices grow polarized cell monolayers representative of the blood–brain barrier (BBB); they can therefore serve as a suitable *in vitro* model for the evaluation of BBB molecular mechanisms, permeability and transport characteristics (Claudio et al., 1994; Calingasan et al., 2000; Deli et al., 1995; de Vries et al., 1996). Immunological, chemical or physical insult can cause a dysfunction of the BBB that results in increased permeability which has been well-correlated with the release of pro-inflammatory cytokines [tumor necrosis factor alpha (TNF) and interleukin-1 beta (IL-1β)] and several second messengers including vasodilators like prostaglandin E_2 (PGE_2) and nitric oxide, as has been validated both *in vitro* (Dobias & Bernier-Latmani, 2013; Duchini, 1996; Ergenekon et al., 2004; Fiala et al., 1997; Fisher, 2008; Franke et al., 2000) and *in vivo* (Dobias & Bernier-Latmani, 2013; Duchini, 1996; Ergenekon et al., 2004; Franke et al., 1999; Gurunathan et al., 2009; Hartung et al., 1992; Kalishwaralal et al., 2009). The activation of cerebral microvasculature, release of pro-inflammatory signals (i.e. cytokines) and BBB dysfunction can markedly affect brain functions and have been well-reviewed (Karlsson & Artursson, 1992). Therefore, determining how the cerebral microvasculature responds to nanomaterials provides a vital key to the potential neurotoxicity related to exposure, and an *in vitro* model of the BBB can serve as a suitable model.

Indeed, previous studies have noted that, when compared to 80 nm silver nanoparticles, 25 and 40 nm silver nanoparticles activate rat BMECs involving the significant release of pro-inflammatory mediators (PGE_2, TNFα and IL-1β) associated with morphology changes correlated to increased BBB permeability (Mark et al., 2001). In other studies, gold nanoparticles (Au-NPs) (3, 5, 7, 10, 30 and 60 nm) demonstrated only mild activation of rat BMECs and permeability changes without altering cellular morphology or the homeostatic baselines of pro-inflammatory signals (PGE_2, TNFα and IL-1β) (Mayhan, 2002). From this standpoint, insight into how the cerebral microvasculature responds is of keen importance in assessing potential neurotoxicity risks. In the current study, primary cultured porcine brain microvessel endothelial cells (pBMECs) were used as *in vitro* model system to evaluate pro-inflammatory response and permeability changes following exposure to various metallic colloidal NPs Figure 1. To our knowledge, the involvement of pro-inflammatory mediated changes in pBMEC integrity and increased permeability after exposure to various colloidal nanoparticles has not been reported previously.

The characterization of various metallic colloidal nanoparticles

The physiochemical properties of the various colloidal nanoparticles were characterized in detail and reported elsewhere (Mark et al., 2001; Mayhan, 2002; Murdock et al., 2008) whereas a summary is presented in Table 1.

The effects of nanoparticles on the release of prostaglandin E_2

The release of prostaglandin E_2 (PGE_2) from the pBMEC monolayers was evaluated at various time intervals (0–8 h) following exposure to NPs of various size and composition, and the data are presented as mean ± SD concentration per

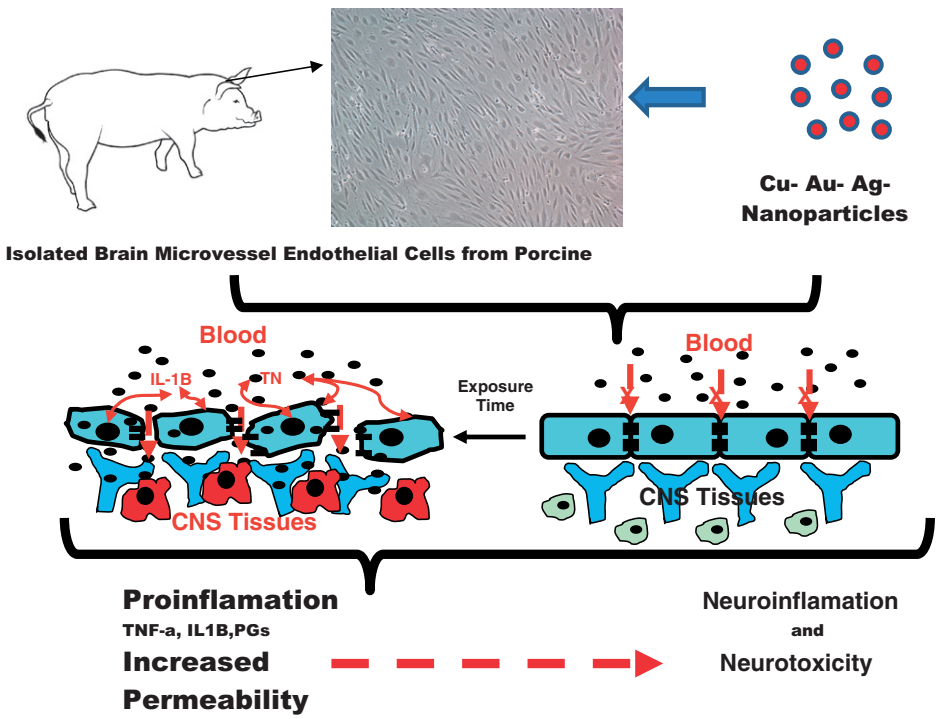

Figure 1. Study design schematic of autocrine or paracrine signaling of known proinflammatory cytokines and vasodilators involved in increased permeability of the cerebral microvasculature following activation or injury.

Table 1. Characterization summary for the various colloidal nanoparticles.

Particle	TEM Size distribution (nm)	DLS Z-average diameter (nm)	DLS PdI	LDV Zeta potential ζ (mV)	LDV Electrophoretic mobility *U* [μmcm/(Vs)]	Reference
Ag 25 nm	28.3 ± 9.6					Franke et al. (1999)
DI H_2O		106	0.379	−44.2	−3.46	
BMEC Media		393	0.473	NA	NA	
Ag 40 nm	47.5 ± 5.6					
DI H_2O		54.9	0.122	−46.0	−3.6	
BMEC Media		73.9	0.191	NA	NA	
Ag 80 nm	102.2 ± 32.8					
DI H_2O		177	0.048	−29.5	−2.32	
BMEC Media		166	0.060	NA	NA	
Au 3 nm	2.9 ± 1.1					Gurunathan et al. (2009)
DI H_2O		20.1	0.209	−22.0	−1.72	
BMEC Media		36.3	0.74	NA	NA	
Au 5 nm	5.3 ± 0.6					
DI H_2O		12.9	0.301	−37.4	−2.93	
BMEC Media		34.6	0.473	NA	NA	
Cu 40 nm	Large agglomerates					Hartung et al. (1992)
DI H_2O	NA	NA	NA	−47.6	NA	
BMEC Media		NA	NA	NA	NA	
Cu 60 nm	Large aggrlomerates					
DI H2O	NA	NA	NA	−36.6	NA	
BMEC Media		NA	NA	NA	NA	

NA, not available.

mg total cellular protein [Figure 2: (A) Cu-NPs (40 and 60 nm), (B) Ag-NPs (25, 40 and 80 nm) or (C) Au-NPs (3 or 5 nm)]. The exposure of 40 nm Cu-NPs produced significantly increased extracellular levels of PGE_2 when compared to control as early as 2 h post-exposure. In contrast, at 2-h post-exposure, the 60-nm Cu-NPs produced only slightly elevated levels of PGE_2 similar to LPS treated monolayers, but cells treated with both 40 and 60 nm Cu-NPs showed significantly elevated levels of extracellular PGE_2 by 4 h post-exposure similar to LPS treated cells. These significantly elevated levels of PGE_2 persisted for the duration of the experiment period (8 h).

The effects of nanoparticles on the extracellular concentrations of cytokines

The time release profiles of TNFα and IL-1β were determined at various time intervals (0–8 h) in pBMEC monolayers in response to NPs of various sizes and composition, and the data are presented as mean ± SD concentration per mg total cellular protein [Figures 3 and 4: (A) Cu-NPs (40 and 60 nm), (B) Ag-NPs (25, 40 and 80 nm) or (C) Au-NPs (3 or 5 nm)]. Significant amounts (∼2-fold) of TNFα were released from the pBMEC following treatment with both 40 and 60 nm Cu-NPs and LPS at 4-h post-exposure (Figure 3A). However, only the 40-nm Cu-NPs and LPS remained significantly elevated at 6 h. The pBMECs treated with either sized (40 or 60 nm) Cu-NPs returned levels similar to that of the control at 8-h post-exposure, while treatment with a known neurotoxicant LPS to monolayers further increased to ∼7-fold. Conversely, the TNFα response following exposure to the smaller (25 and 40 nm) Ag-NPs was elevated at 4 h and remained elevated throughout the experimental period (Figure 3B) – whereas exposure to the 80-nm Ag-NPs produced results similar to those found in the control monolayers throughout the observed experimental period (Figure 3B). Similarly, basal levels of TNFα following exposure to small Au-NPs remained unremarkable over the observed experimental time frame (Figure 3C). On the other hand, the extracellular levels of IL-1β were significantly elevated after 2 h for 60-nm Cu-NPs and LPS when compared to control monolayers (Figure 4A). By 4-h post-exposure, treatment with both sized Cu-NPs significantly elevated (∼3-fold) the extracellular levels of IL-1β similar to LPS when compared to control, and the levels remained elevated throughout the experiment (8 h) to a maximal amount of ∼10-fold. Similarly, the smallest Ag-NPs (25 nm) increased the release of IL-1β at 4-h post-exposure, which further increased to a maximal magnitude (∼10-fold) by 8 h (Figure 4B). A significant IL-1β response was only observed at 8 h following exposure to 40 nm Ag-NPs (Figure 4B). Changes in the levels of IL-1 β in response to 80 nm Ag-NPs were unremarkable throughout the course of the experiment (Figure 4B). Similarly, unremarkable changes in the basal levels of IL-1β were observed following exposure to Au-NPs (Figure 4C).

The exposure effects of various sizes and composition of NPs on the permeability in pBMEC

The permeability effects were determined by evaluating the transport of fluorescein across pBMEC monolayers following apical (blood-side) exposure to NPs of various sizes and composition, and the data are presented as mean ± SD [Figure 5: (A) Cu-NPs (40 and 60 nm), (B) Ag-NPs (25, 40 and 80 nm) or (C) Au-NPs (3 or 5 nm)]. The exposure of both 40 and 60 nm Cu-NPs significantly increased the permeability of fluorescein across the pBMEC monolayers over the 90-min experimental time frame. The magnitude of the effect was

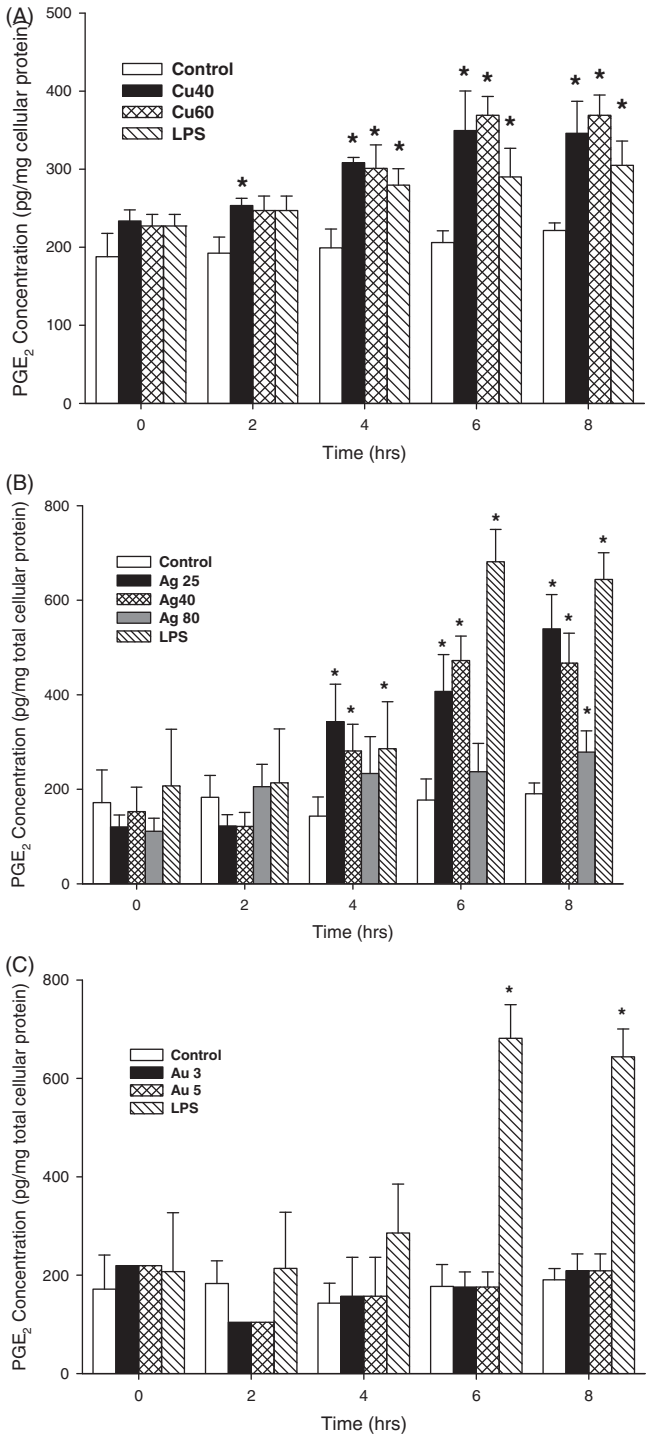

Figure 2. The effects of various metallic colloidal nanoparticles on the release of Prostaglandin E_2 (PGE_2) from PBMEC. Triplicate cell monolayers were treated with nanoparticles (15 μg/ml) of various size and composition: (A) control (media alone) (open bars), 40 nm Cu-NPs (closed black bars), 60 nm Cu-NPs (cross hatch bars), LPS (diagonal bars); (B) control (media alone) (open bars), 25 nm Ag-NPs (closed black bars), 40 nm Ag-NPs (cross hatch bars), 80 nm Ag-NPs (closed gray bars) or LPS (diagonal bars); (C) control (media alone) (open bars), 3 nm Au-NPs (closed black bars), 5 nm Au-NPs (cross hatch bars) or LPS (diagonal bars). The data are presented as means ± SD, $n = 3$. *Considered statistically significant $p < 0.05$.

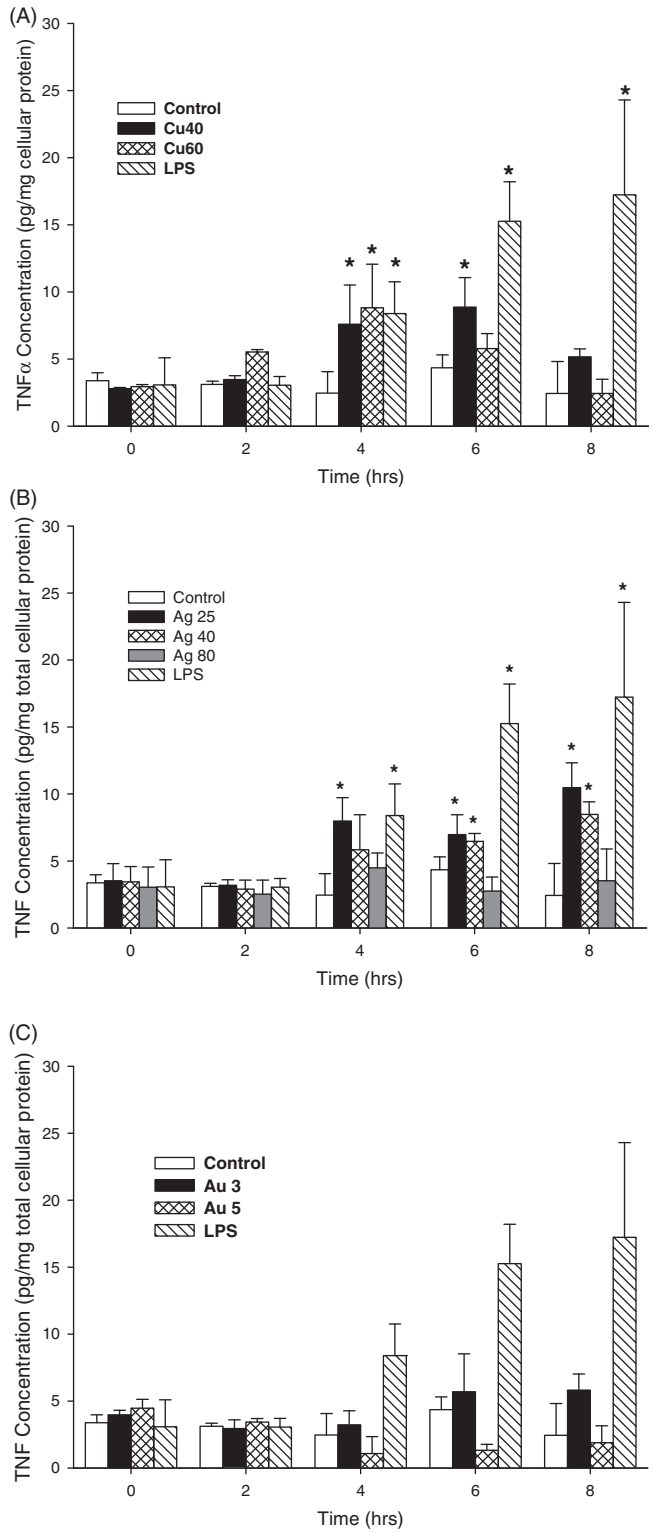

Figure 3. The effects of various metallic colloidal nanoparticles on the release of tumor necrosis factor alpha (TNFα) from pBMEC. Triplicate cell monolayers were treated with nanoparticles (15 μg/ml) of various size and composition: (A) control (media alone) (open bars), 40 nm Cu-NPs (closed black bars), 60 nm Cu-NPs (cross hatch bars), LPS (diagonal bars); (B) control (media alone) (open bars), 25 nm Ag-NPs (closed black bars), 40 nm Ag-NPs (cross hatch bars), 80 nm Ag-NPs (closed gray bars) or LPS (diagonal bars); (C) control (media alone) (open bars), 3 nm Au-NPs (closed black bars), 5 nm Au-NPs (cross hatch bars) or LPS (diagonal bars). The data are presented as means ± SD, $n = 3$. *Considered statistically significant $p < 0.05$.

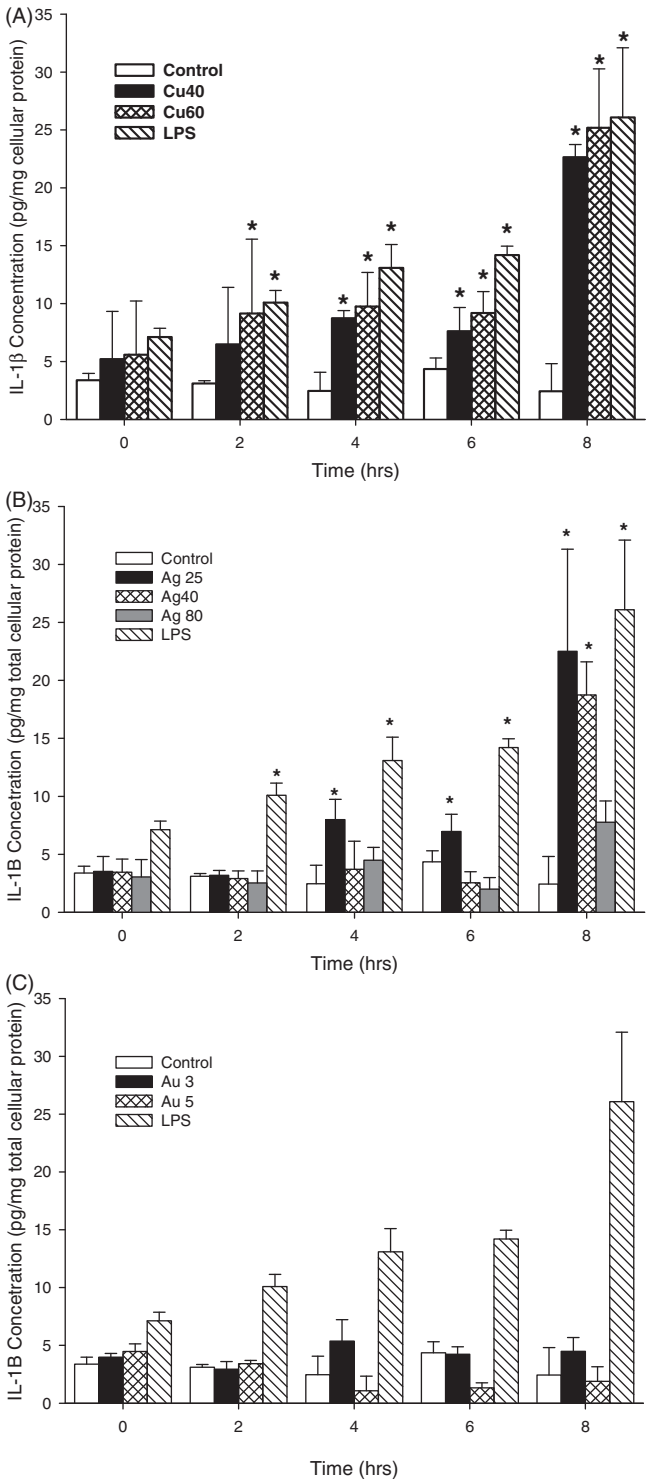

Figure 4. The effects of various metallic colloidal nanoparticles on the release of interleukin one beta (IL-1β) from pBMEC. Triplicate cell monolayers were treated with nanoparticles (15 μg/ml) of various size and composition: (A) control (media alone) (open bars), 40 nm Cu-NPs (closed black bars), 60 nm Cu-NPs (cross hatch bars), LPS (diagonal bars); (B) control (media alone) (open bars), 25 nm Ag-NPs (closed black bars), 40 nm Ag-NPs (cross hatch bars), 80 nm Ag-NPs (closed gray bars) or LPS (diagonal bars); (C) control (media alone) (open bars), 3 nm Au-NPs (closed black bars), 5 nm Au-NPs (cross hatch bars) or LPS (diagonal bars). The data are presented as means ± SD, $n = 3$. *Considered statistically significant $p < 0.05$.

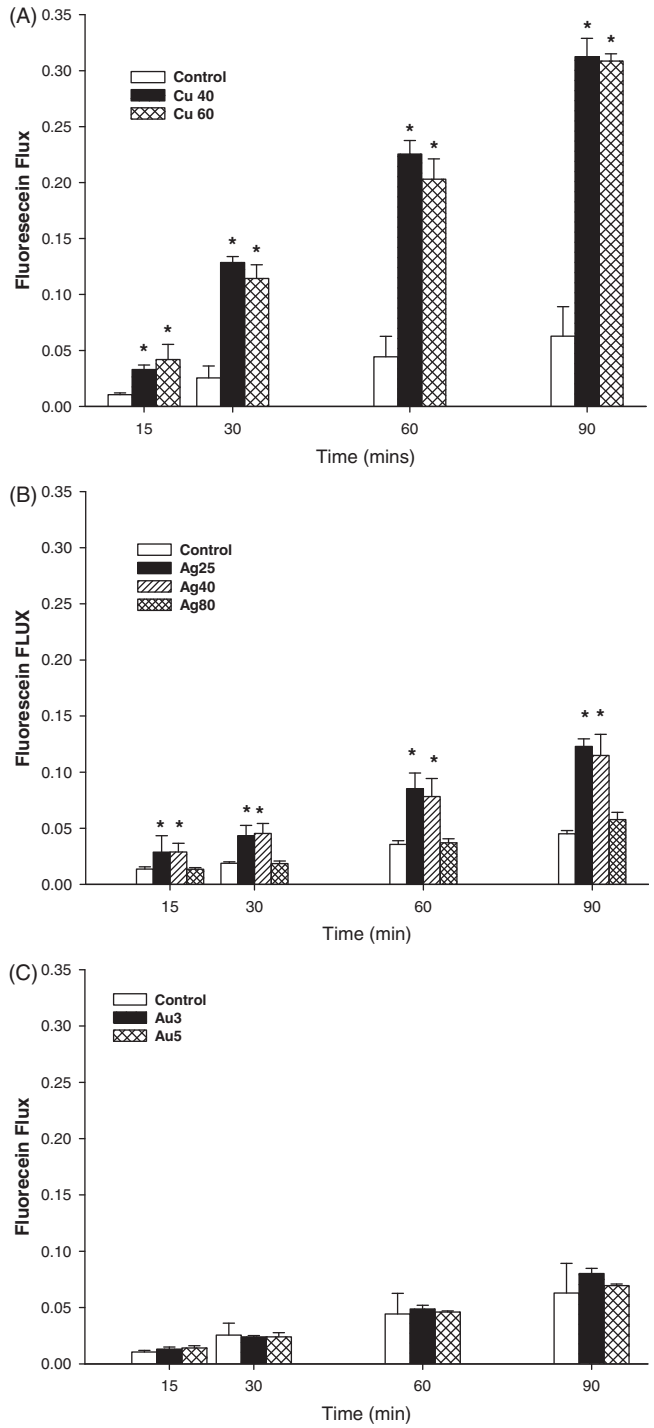

Figure 5. The effects of various metallic colloidal nanoparticles on the permeability of fluorescein across PBMEC. Triplicate cell monolayers were treated with nanoparticles (15 μg/ml) of various size and composition: (A) control (media alone) (open bars), 40 nm Cu-NPs (closed black bars), 60 nm Cu-NPs (cross hatch bars), LPS (diagonal bars); (B) control (media alone) (open bars), 25 nm Ag-NPs (closed black bars), 40 nm Ag-NPs (cross hatch bars), 80 nm Ag-NPs (closed gray bars) or LPS (diagonal bars); (C) control (media alone) (open bars), 3 nm Au-NPs (closed black bars), 5 nm Au-NPs (cross hatch bars) or LPS (diagonal bars). The data are presented as means ± SD, $n = 3$. *Considered statistically significant $p < 0.05$.

Table 2. Apparent permeability changes following various nanoparticle exposures.

Treatment groups	Apparent permeability coefficient (1×10^{-6} cm/s)	Apparent permeability coefficient ratio (treatment/control)
Control	0.23 ± 0.005	1.01 ± 0.024
Cu40	1.92 ± 0.071*	8.32 ± 0.351*
Cu60	1.85 ± 0.017*	7.92 ± 0.082*
Ag25	0.69 ± 0.014*	2.93 ± 0.010*
Ag40	0.61 ± 0.010*	2.61 ± 0.013*
Ag80	0.32 ± 0.028*	1.38 ± 0.024*
Au3	0.41 ± 0.131*	1.74 ± 0.034*
Au5	0.30 ± 0.042*	1.27 ± 0.091*

Calculated by: $P_{app} = \frac{1}{AC_0} \cdot \frac{dQ}{dt}$

where, dQ/dt is the flux across the cell monolayers, A is the surface area of the membrane and C_0 is the initial concentration of fluorescein. The data are presented as mean ± SD, and *Considered statistically different from control monolayers $p < 0.05$, $n = 3$.

8.32- or 7.92-fold higher than in pBMEC monolayers receiving media alone. The apparent permeability coefficient was statistically significant for both 40 and 60 nm Cu-NPs (Table 2). The apparent permeability coefficients rank orders following exposure to Cu-NPs with 40 nm was slightly greater than with 60 nm, and both were significantly greater than control (Table 2).

As the industrialization and commercialization of products containing nanomaterials are more prevalent, they may have significant impact on the environment and eventually the human population with unknown consequences. As a result, it is important to understand how biological systems interact with and respond to the inevitable exposure to such materials. The results of the current studies and other reports (Mark et al., 2001; Mayhan, 2002; Murdock et al., 2008) suggest that systemic exposure to several metallic colloidal nanoparticles activates the cerebral microvasculature through pro-inflammatory mediators that can alter the restrictive nature of the BBB.

Under normal conditions, proinflammatory mediators are important for growth and repair of the cerebral microvasculature (Saito et al., 1996). However, if over-stimulated and left unchecked, pro-inflammatory responses can cause BBB dysfunction and potential brain toxicity that can subsequently modify brain function (Gurunathan et al., 2009; Perry et al., 1997; Rahman et al., 2009; Rosas-Hernandez et al., 2009; Saito et al., 1996; Schafer et al., 2013; Shalev et al., 2009; Sharma et al., 2009a). The release of cytokines following cerebral microvasculature damage has been linked to oxidative free radical generation, cytokines and growth factors (Schafer et al., 2013; Sharma et al., 2009a; Sheikpranbabu et al., 2009; Trickler et al., 2010a,b; Trickler et al., 2012). The generation of reactive oxygen species (ROS) has been shown to be associated with toxicity following exposure to either Ag-NPs or Cu-NPs both *in vitro* and *in vivo* (Trickler et al., 2005; Ujiie et al., 2003; Vadeboncoeur et al., 2003). When introduced into the systemic blood supply, Ag-NPs or Cu-NPs can induce BBB dysfunction, astrocyte swelling and neuronal degeneration *in vivo* (Wang et al., 2009). The leakage of Evan's blue dye and radioiodine in the rat brain following intravenous (30 mg/kg) or intraperitoneal (50 mg/kg) administration of Ag-NPs or Cu-NPs has been demonstrated by Sharma et al. (2009b). This research team further suggested that the increase in the permeability of cerebral microvasculature involves ROS generation because the increased permeability was attenuated by nanowire-antioxidant therapy following chronic exposure *in vivo* (Wang et al., 2009; Wittmaack, 2011). Similarly, Cu-NPs have been shown to induce strong pro-inflammatory activation and increased permeability in rat BMECs (Murdock et al., 2008). On the other hand, previous studies have also identified that gold nanoparticles (Au-NPs) (3, 5, 7, 10, 30 and 60 nm) demonstrated only mildly activated rat BMECs and permeability changes without altering cellular morphology or the homeostatic baselines of pro-inflammatory signals (PGE_2, TNFα and IL-1β) (Mayhan, 2002). Furthermore, studies have previously shown that smaller silver nanoparticles (25 and 40 nm) produced stronger activation of rat BMECs involving the significant release of pro-inflammatory mediators (PGE_2, TNFα and IL-1β) associated with morphology changes correlated to increased BBB permeability compared to larger silver nanoparticles (80 nm) (Mark et al., 2001).

In contrast with previously reported results, Sheikpranbabu et al. (2009) reported that Ag-NPs (40–50 nm) inhibit VEGF and IL-1B induced vascular permeability and cellular proliferation in porcine retinal endothelial cells via Src dependent pathway (Weber et al., 1993). However, significant methodology differences in exposure time may have been the contributing factor contrasting these studies (6 h compared to 24 h). Indeed, Gurunathan et al. (2009) and Kalishwarala et al. (2009) clearly demonstrated that Ag-NPs are anti-angiogenic, inhibiting VEGF-induced cellular proliferation at 24 h of exposure. However, both of these reports also showed Ag-NPs inhibit the cell survival pathway PI3K/Akt, clearly enhance caspase 3 activity and induce apoptosis after 24 h of exposure in bovine retinal endothelial cells (Xui et al., 2011; Zhang et al., 2011). These reports demonstrate that the length of exposure is of considerable importance. However, species differences and the tissue origin of the cells should not be overlooked. Therefore, the current report concerning pBMECs addresses the questions arising from species differences.

With respect to the responses in the cerebral microvasculature previously discussed, there are lines of supporting evidence. The current studies clearly indicate that metallic colloidal nanoparticles interact with the porcine *in vitro* model of the BBB in a manner similar to the rat model previously reported (Mark et al., 2001; Mayhan, 2002; Murdock et al., 2008). With respect to cerebral microvessel endothelial cell permeability, exposure to either sized (40 or 60 nm) Cu-NPs produced more prominent increases in permeability when compared to exposure to Ag-NPs (Figure 5A and Table 2). The change in permeability increased with the smaller Ag-NPs (25 or 40 nm) when compared to the larger Ag-NPs (80 nm) (Figure 5B and Table 2) whereas changes in permeability observed following exposure to Au-NPs were unremarkable compared to either Cu-NPs or Ag-NPs (Figure 5C and Table 2). Previous independent studies in rat BMEC have also shown that exposure to Cu-NPs has greater effects on the permeability than Ag-NPs (Mayhan, 2002).

Summary and conclusion

The current report and other published work (Mark et al., 2001; Mayhan, 2002; Murdock et al., 2008) provide compelling evidence that exposure to Cu-NPs or Ag-NPs significantly results in increased cerebral microvessel permeability, while Au-NPs appear least likely to increase cerebral microvessel permeability. Smaller Ag-NPs produce stronger inflammatory responses correlated with increased cerebral microvessel permeability, whereas the effects produced by the larger Ag-NPs are much less pronounced. Together, the current results with pBMECs correlate well with the previous results in rat BMECs.

Declaration of interest

The authors have no conflict of interest. This research was supported in part by an appointment to the Postgraduate Research Participation Program with the U. S. Air Force Research Laboratory at the National Center for Toxicological Research/FDA (Jefferson, AR) administered by the Oak Ridge Institute of Science and Education (Oak Ridge, TN) through an interagency agreement between the U.S. Department of Energy, U. S. Air Force Research Laboratory/RHPB and the U. S. Food and Drug Administration. Further, the authors are responsible for the content and writing of the manuscript and do not necessarily reflect the position of the U.S. Government or FDA, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

References

Abraham CS, Deli MA, Joo F, et al. (1996). Intracarotid tumor necrosis factor-alpha administration increases the blood-brain barrier permeability in cerebral cortex of the newborn pig: Quantitative aspects of double-labelling studies and confocal laser scanning analysis. Neurosci Lett 208:85–88.

Aruoja V, Dubourguier HC, Kasemets K, Kahru A. (2009). Toxicity of nanoparticles of CuO, ZnO and TiO_2 to microalgae *Pseudokirchneriella subcapitata*. Sci Total Environ 407:1461–1468.

Audus KL, Borchardt RT. (1987). Bovine brain microvessel endothelial cell monolayers as a model system for the blood-brain barrier. Ann N Y Acad Sci 507:9–18.

Barone FC, Feuerstein GZ. (1999). Inflammatory mediators and stroke: New opportunities for novel therapeutics. J Cereb Blood Flow Metab 19:819–834.

Bove K, Neumann P, Gertzberg N, Johnson A. (2001). Role of ecNOS-derived NO in mediating TNF-induced endothelial barrier dysfunction. Am J Physiol Lung Cell Mol Physiol 280:L914–L922.

Claudio L, Martiney JA, Brosnan CF. (1994). Ultrastructural studies of the blood-retina barrier after exposure to interleukin-1 beta or tumor necrosis factor-alpha. Lab Invest 70:850–861.

Calingasan NY, Huang PL, Chun HS, et al. (2000). Vascular factors are critical in selective neuronal loss in an animal model of impaired oxidative metabolism. J Neuropathol Exp Neurol 59:207–217.

Deli MA, Descamps L, Dehouck MP, et al. (1995). Exposure of tumor necrosis factor-alpha to luminal membrane of bovine brain capillary endothelial cells cocultured with astrocytes induces a delayed increase of permeability and cytoplasmic stress fiber formation of actin. J Neurosci Res 41:717–726.

de Vries HE, Blom-Roosemalen MC, van Oosten M, et al. (1996). The influence of cytokines on the integrity of the blood-brain barrier in vitro. J Neuroimmunol 64:37–43.

Dobias J, Bernier-Latmani R. (2013). Silver release from silver nanoparticles in natural waters. Environ Sci Technol 47:4140–4146.

Duchini A. (1996). The role of central nervous system endothelial cell activation in the pathogenesis of hepatic encephalopathy. Med Hypotheses 46:239–244.

Ergenekon E, Gucuyener K, Erbas D, et al. (2004). Cerebrospinal fluid and serum vascular endothelial growth factor and nitric oxide levels in newborns with hypoxic ischemic encephalopathy. Brain Dev 26: 283–286.

Fiala M, Looney DJ, Stins M, et al. (1997). TNF-alpha opens a paracellular route for HIV-1 invasion across the blood-brain barrier. Mol Med 3:553–564.

Fisher M. (2008). Injuries to the vascular endothelium: Vascular wall and endothelial dysfunction. Rev Neurol Dis 5:S4–S11.

Franke H, Galla H, Beuckmann CT. (2000). Primary cultures of brain microvessel endothelial cells: A valid and flexible model to study drug transport through the blood-brain barrier in vitro. Brain Res Brain Res Protocol 5:248–256.

Franke H, Galla HJ, Beuckmann CT. (1999). An improved low-permeability in vitro-model of the blood-brain barrier: Transport studies on retinoids, sucrose, haloperidol, caffeine and mannitol. Brain Res 818:65–71.

Gurunathan S, Lee KJ, Kalishwaralal K, et al. (2009). Antiangiogenic properties of silver nanoparticles. Biomaterials 30:6341–6350.

Hartung HP, Jung S, Stoll G, et al. (1992). Inflammatory mediators in demyelinating disorders of the CNS and PNS. J Neuroimmunol 40: 197–210.

Kalishwaralal K, Banumathi E, Ram Kumar Pandian S, et al. (2009). Silver nanoparticles inhibit VEGF induced cell proliferation and migration in bovine retinal endothelial cells. Colloids Surf B Biointerfaces 73:51–57.

Karlsson J, Artursson P. (1992). A new diffusion chamber system for the determination of drug permeability coefficients across the human intestinal epithelium that are independent of the unstirred water layer. Biochim Biophys Acta 1111:204–210.

Mark KS, Trickler WJ, Miller DW. (2001). Tumor necrosis factor-alpha induces cyclooxygenase-2 expression and prostaglandin release in brain microvessel endothelial cells. J Pharmacol Exp Ther 297: 1051–1058.

Mayhan WG. (2002). Cellular mechanisms by which tumor necrosis factor-alpha produces disruption of the blood-brain barrier. Brain Res 927:144–152.

Murdock RC, Braydich-Stolle L, Schrand AM, et al. (2008). Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. Toxicol Sci 101:239–253.

Perry VH, Anthony DC, Bolton SJ, Brown HC. (1997). The blood-brain barrier and the inflammatory response. Mol Med Today 3: 335–341.

Rahman MF, Wang J, Patterson TA, et al. (2009). Expression of genes related to oxidative stress in the mouse brain after exposure to silver-25 nanoparticles. Toxicol Lett 187:15–21.

Rosas-Hernandez H, Jimenez-Badillo S, Martinez-Cuevas PP, et al. (2009). Effects of 45-nm silver nanoparticles on coronary endothelial cells and isolated rat aortic rings. Toxicol Lett 191:305–313.

Saito K, Suyama K, Nishida K, et al. (1996). Early increases in TNF-alpha, IL-6 and IL-1 beta levels following transient cerebral ischemia in gerbil brain. Neurosci Lett 206:149–152.

Schafer B, Brocke JV, Epp A, et al. (2013). State of the art in human risk assessment of silver compounds in consumer products: A conference report on silver and nanosilver held at the BfR in 2012. Arch Toxicol 87:2249–2262.

Shalev H, Serlin Y, Friedman A. (2009). Breaching the blood-brain barrier as a gate to psychiatric disorder. Cardiovasc Psychiatry Neurol 2009:278531 (1–7).

Sharma HS, Ali SF, Hussain SM, et al. (2009a). Influence of engineered nanoparticles from metals on the blood-brain barrier permeability, cerebral blood flow, brain edema and neurotoxicity. An experimental study in the rat and mice using biochemical and morphological approaches. J Nanosci Nanotechnol 9:5055–5072.

Sharma HS, Ali SF, Tian ZR, et al. (2009a). Chronic treatment with nanoparticles exacerbate hyperthermia induced blood-brain barrier breakdown, cognitive dysfunction and brain pathology in the rat. Neuroprotective effects of nanowired-antioxidant compound H-290/51. J Nanosci Nanotechnol 9:5073–5090.

Sheikpranbabu S, Kalishwaralal K, Venkataraman D, et al. (2009). Silver nanoparticles inhibit VEGF- and IL-1beta-induced vascular permeability via Src dependent pathway in porcine retinal endothelial cells. J Nanobiotechnol 7:8 (1–12).

Trickler WJ, Lantz SM, Murdock RC, et al. (2010a). Brain microvessel endothelial cells responses to gold nanoparticles: In vitro pro-inflammatory mediators and permeability. Nanotoxicology 5: 497–492.

Trickler WJ, Lantz SM, Murdock RC, et al. (2010b). Silver nanoparticle induced blood-brain barrier inflammation and increased permeability in primary rat brain microvessel endothelial cells. Toxicol Sci 118: 160–170.

Trickler WJ, Lantz SM, Schrand AM, et al. (2012). The effects of copper nanoparticles on rat cerebral microvessel endothelial cells. Nanomedicine (London) 7:835–846.

Trickler WJ, Mayhan WG, Miller DW. (2005). Brain microvessel endothelial cell responses to tumor necrosis factor-alpha involve a nuclear factor kappa B (NF-kappaB) signal transduction pathway. Brain Res 1048:24–31.

Ujiie M, Dickstein DL, Carlow DA, Jefferies WA. (2003). Blood-brain barrier permeability precedes senile plaque formation in an Alzheimer disease model. Microcirculation 10:463–470.

Vadeboncoeur N, Segura M, Al-Numani D, et al. (2003). Pro-inflammatory cytokine and chemokine release by human brain microvascular endothelial cells stimulated by Streptococcus suis serotype 2. FEMS Immunol Med Microbiol 35:49–58.

Wang J, Rahman MF, Duhart HM, et al. (2009). Expression changes of dopaminergic system-related genes in PC12 cells induced by manganese, silver, or copper nanoparticles. Neurotoxicology 30: 926–933.

Wittmaack K. (2011). In search of the most relevant parameter for quantifying lung inflammatory response to nanoparticles exposure: Particle number, surface area, or what? Environ Health Prospect 2011: 187–194.

Weber SJ, Abbruscato TJ, Brownson EA, et al. (1993). Assessment of an in vitro blood-brain barrier model using several [Met5]enkephalin opioid analogs. J Pharmacol Exp Ther 266:1649–1655.

Xui Z, Ma J, Alvarez P. (2011). Differential effects of common ligangs and molecular oxygen on antimicrobial activity of silver nanoparticles versus silver ions. Environ Sci Technol 45:9003–9008.

Zhang W, Yao Y, Sullivan N, Chen Y. (2011). Modeling the primary size effects of citrate-coated silver nanoparticles on their ion release kinetics. Environ Health Sci Technol 45: 4422–4428.