

Nanodelivery of cerebrolysin reduces functionalized Gold Nanoparticles induced Blood-brain barrier disruption, brain edema formation and brain pathology

Aruna Sharma^{1,3,4}, Preeti Kumaran Menon^{1,6}, Dafin Fior Muresanu^{2,3}, José Vicente Lafuente^{4,9,10},

Ala Nozari⁵, Ranjana Patnaik⁶, Asya Ozkizilcik⁷, Z Ryan Tian⁸, Herbert Mössler³, Hari Shanker Sharma^{*1,3,4}

¹International Experimental Central Nervous System Injury & Repair (IECNSIR), Dept. of Surgical Sciences, Anesthesiology & Intensive Care Medicine, Uppsala University Hospital, Uppsala University, SE-75185 Uppsala, Sweden Email: Sharma@surgsci.uu.se; Aruna.Sharma@surgsci.uu.se

²Dept. Clinical Neurosciences, University of Medicine & Pharmacy, Cluj-Napoca, Romania;

³“RoNeuro” Institute for Neurological Research and Diagnostic, 37 Mircea Eliade Street, 400364, Cluj-Napoca, Romania

⁴LaNCE, Dpt. Neuroscience, University of the Basque Country (UPV/EHU), Leioa, Bizkaia, Spain

⁵Anesthesiology, Massachusetts General Hospital, Harvard University, Boston MA, USA

⁶School of Biomedical Engineering, Dept. of Biomaterials, Indian Institute of technology, Banaras Hindu University, Varanasi, India

⁷Department of Biomedical Engineering, University of Arkansas, Fayetteville, AR, USA

⁸Dept. Chemistry & Biochemistry, University of Arkansas, Fayetteville, AR, USA

⁹Nanoneurosurgery Group, BioCruces Health Research Institute, 48903 Barakaldo, Bizkaia, Spain

¹⁰Faculty of Health Science, Universidad Autónoma de Chile, Santiago de Chile, Chile

ABSTRACT

Recently, gold nanoparticles (AuNPs) are used for drug delivery in treating several neurological disorders e.g., Alzheimer's disease (AD), Parkinson's disease, Stroke and trauma. In addition, use of AuNPs for diagnostic purposes in various diseases are also common in clinical medicine. However, the neurotoxic effects of AuNPs in vivo studies are not well explored. In this innovation we present evidences that functionalized AuNPs induced brain pathology that depends on size, dose and route of administration. These AuNPs induce breakdown of the blood-brain barrier (BBB) permeability to protein tracers causing brain edema formation and neuronal and glial cell injuries. The magnitude and intensity of brain pathology caused by AuNPs is inversely related to the size of the NPs. Interestingly, co-administration of cerebrolysin, a balanced composition of various neurotrophic factors and active peptide fragments reduces AuNPs induced brain pathology. This effect was much more pronounced when the cerebrolysin was administered using TiO₂ nanowired delivery, not reported earlier. Thus, use of cerebrolysin with or without nanowired as adjunct therapy could be used where AuNPs are used either for drug delivery or for diagnostic purposes in clinics.

Keywords: Functionalized gold nanoparticles, brain pathology, blood-brain barrier, brain edema, Cerebrolysin, nanodelivery

1 INTRODUCTION

Recently, gold nanoparticles (AuNPs) are used for drug delivery in treating several neurological disorders e.g., Alzheimer's disease (AD), Parkinson's disease, Stroke and trauma [1,2]. In addition, use of AuNPs for diagnostic purposes in various diseases are also common in clinical medicine [2]. However, the neurotoxic effects of AuNPs in vivo studies are not well explored [3]. Thus, it would be interesting to examine AuNPs neurotoxicity in vivo models in relation to size related effects on brain pathology. Furthermore, we also examined the effects one potent multimodal neuroprotective agent cerebrolysin that is a balanced composition of several neurotrophic factors and active peptide fragments on the AuNPs induced brain pathology with or without nanodelivery in a rat model.

2 MATERIALS & METHODS

Experiments were carried out on Male Wistar rats (200-250 g body weight) housed at controlled room temperature (21±1°C) with 12 h light and 12 h dark schedule. Food and tap water were supplied *ad libitum* before the experiment. All the experiments were carried out according to the Guidelines & Care for Laboratory Animals as described by National Institute of Health and approved by Local Institutional Ethics Committee.

2.1 Administration of AuNPs in vivo

We examined the effects of moderate doses of AuNPs of 3 different sizes (5 nm, 10 nm and 40 nm) administered either through intraperitoneally (10 mg/kg, i.p.), intravenously (5 mg/kg, i.v.), intracarotidly (2 mg/kg, i.c.a.) or intracerebroventricularly (20 µg in 20 µl, i.c.v.) in rats (Age 20 to 25 weeks).

2.2 Blood-brain barrier function

Blood-brain barrier (BBB) breakdown to Evans blue albumin (EBA 3 ml/kg, i.v.) and ¹³¹I-iodine (100 µCi/kg, i.v.) was examined 24 h after AuNPs administration in the brain using standard procedures. In brief, the BBB breakdown was examined using Evans blue (EB) and ¹³¹I-iodine leakage across the brain microvessels after intravenous administration of these tracers (EBA 2 % solution 3 ml/kg, and radioiodine 100 µCi/kg) 5 min before the end of the experiment [4,5]. At the end of the experiment, the intravascular tracer was washed out with 0.9% saline and the brains were removed dissected weighted and radioactivity was determined in a Gamma counter. Before perfusion with saline, about one ml whole blood was obtained from cardiac puncture to analyze whole blood radioactivity. EBA dye entered into the brain was measured colorimetrically as described earlier.

2.3 Brain Edema formation

Brain edema was determined using regional water content by wet and dry weights of the brain samples [5,6]. For this purpose, brains were removed and dissected immediately and weighed to record wet weight of the sample. After that tissue samples were placed in an oven at 90°C for 72 h to record dry weight of the sample. Brain water content was calculated from the differences between wet and dry weight. Volume swelling was calculated from the differences in water content between control and experimental group.

2.4 Neural and Glial cell injury

In separate groups, neuronal changes were studied using histopathological examination of Nissl or Haematoxylin & Eosin (HE) staining [5-7]. Immunohistochemistry of glial fibrillary acidic protein (GFAP) was used to detect astrocytic dysfunction according to standard procedures.

2.5 TiO₂-nanowired delivery of Cerebrolysin

Cerebrolysin (CBL, Ever NeuroPharma, Austria) was tagged with TiO₂ nanowires according to standard protocol [4,6,7]. The TiO₂ nanowired Cerebrolysin (2.5 ml/kg, i.v., NWCBL) was co-administered with AuNPs.

2.6 Normal Cerebrolysin Delivery

For comparison, we used normal delivery of cerebrolysin in identical condition to evaluate neuroprotective effects of cerebrolysin in AuNPs induced brain pathology [6].

2.7 Statistical Analyses

ANOVA followed by Dunnett's test for multiple group comparison with one control was used to analyze statistical significance of the data obtained. A p-value less than 0.05 was considered significant.

3 RESULTS

3.1 Brain Pathology of AuNPs

Marked BBB breakdown in several brain areas e.g., cerebral cortex, hippocampus, cerebellum, thalamus, hypothalamus and brain stem was seen following administration of 5 to 10 nm AuNPs irrespective of the routes of administration after 24 h [Fig. 1].

However, intracarotid administration exhibited more profound leakage of these tracers in the ipsilateral side whereas i.c.v. administration showed leakage of tracers on the dorsal surface of the ipsilateral hemisphere. Brain edema and neuronal injuries were tightly correlated with BBB breakdown. Activation of astrocytes and neuronal damages were also evident in the areas showing BBB leakage. Interestingly, there was an inverse relationship between AuNPs size and brain damage.

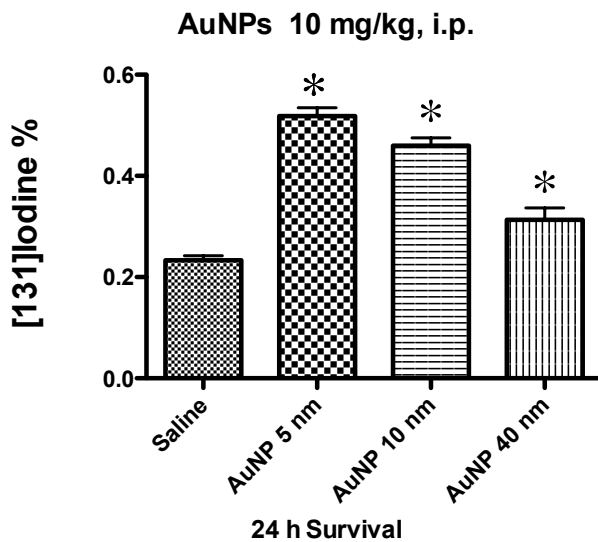


Fig. 1. Blood-brain barrier (BBB) leakage of radioiodine [¹³¹Iodine] in the cerebral cortex after AuNPs administration. The magnitude BBB breakdown appears to be size related and exhibit inverse relationship between size and intensity of iodine leakage.

3.2 Neuroprotective effects of TiO₂ Cerebrolysin (NWCBL)

Interestingly, co-administration of cerebrolysin (2.5 ml/kg, i.v.) using TiO₂ nanowired delivery significantly reduced the BBB breakdown, edema formation and brain damage following AuNPs administration to any route after 24 h.

Thus, treatment with NWCBL resulted in profound reductions in the BBB breakdown to EBA and radioiodine in several brain regions. Also the neuronal and glial cell damages were markedly reduced.

3.3 Neuroprotective effects of normal Cerebrolysin

Normal cerebrolysin infusion required higher doses (5 ml/kg, i.v.) to reduce BBB breakdown, brain edema formation and cell injuries following AuNPs administration under identical conditions.

AuNP 5 nm Neurotoxicity 24 h

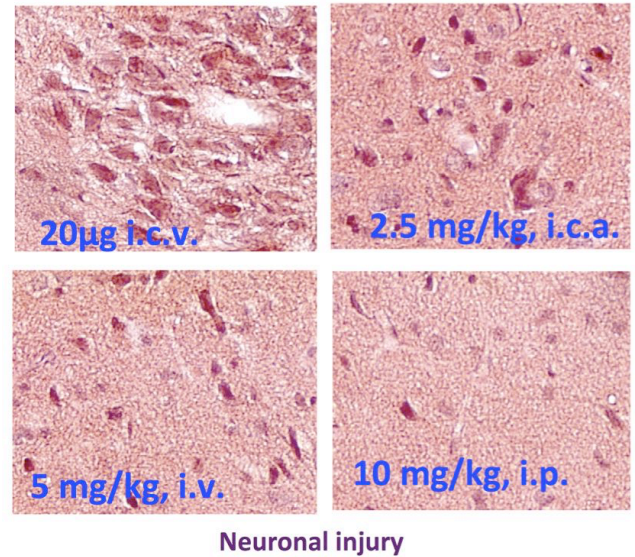


Fig. 2. Representative example of AuNPs (5 nm) induced neuronal damages following in vivo administration in rats through various routes. Neuronal damages were much more pronounced after intraventricular (i.c.v) injection followed by intracarotid (i.c.a), intravenous (i.v) and intraperitoneal (i.p) route. H&E stain on 3-µm thick paraffin sections (x 40).

Using higher doses of cerebrolysin significant reductions in the BBB breakdown and brain edema formation was observed. The cell changes were also less apparent than untreated group after AuNPs administration. observed.

4 DISCUSSION

Our novel findings demonstrate that AuNPs when administered in vivo could cause significant brain pathology. This effect of AuNPs appears to size and dose related. Thus, smallest size of AuNPs (5 nm) in our hands exhibited the most marked brain pathology followed by 10 nm and 40 nm sizes of the AuNPs. This suggests that small sized AuNPs has the most devastating effects on brain pathology.

Another important findings of this investigation shows that route of administration of AuNPs is also very important in causing brain pathology in vivo situations. Thus, direct administration into the brain compartment (i.c.v) resulted in the highest number of neuronal damages followed by internal carotid artery (i.c.a) and intravenous (i.v)

injections. Intraperitoneal (i.p.) injection showed the least damage although the damage was significantly higher than the saline control group. These observations indicate that AuNPs depending on their dose and route of administration could induce brain pathology.

It appears that AuNPs induced breakdown of the BBB plays important roles in brain edema formation and brain pathology [4-6]. This idea is further supported by the fact that neuronal and glial cell damages were most prominent in the areas exhibiting EBA leakage. Leakage of EBA and radioiodine indicates extravasation of proteins into the brain microfluid environment. Since EBA and radioiodine when administered into the systemic circulation they bind with serum proteins, largely albumin. Thus, leakage of these tracers across the BBB represents serum protein extravasation. Obviously, serum protein leakage into the brain extracellular environment leads to vasogenic edema formation and subsequent neuronal and glial cell injuries.

This is further apparent from the findings that cerebrolysin either alone or co-administered with TiO₂ nanowires is able to thwart BBB breakdown to proetin tracers caused by AuNPs injection. In cerebrolysin treated group brainedema and cell injuries were also much less evident.

The basic mechanisms by which cerebrolysin is able to thwart brain pathology following AuNPs administration could be due to strengthening of the BBB function and thereby reducing vasogenic brain edema formation [5-7]. Restoration of BBB function and reduction in brain edema are instrumental for neuronal survival [4,5]. Enhancement of neuroprotection by NWCBL may either be due to an enhanced penetration of the drug within the brain or to a slow degradation or metabolism of cerebrolysin within the brain [5,6]. Thus, co-administration of NWCBL has the superior neuroprotective activity as compared to the drug alone following AuNPs neurotoxicity, not reported earlier.

5 CONCLUSION

In conclusion, our observations are the first to show that AuNPs induced neurotoxicity is related to the size and route of administration. Furthermore our investigation showed that co-administration of nanowired cerebrolysin is far more effective in reducing AuNPs induced brain pathology. This suggests cerebrolysin could be used as adjunct therapy in clinics where AuNPs is used either for diagnostic or drug delivery for better therapeutic strategies, not reported earlier.

6 ACKNOWLEDGEMENTS

This investigation is partially supported by grants from the Air Force Office of Scientific Research (EOARD, London, UK), and Air Force Material Command, USAF, under grant number FA8655-05-1-3065; Swedish Medical Research Council (Nr 2710-HSS), IT 794/13 (JVL), Govt. of Basque Country and UFI 11/32 (JVL) University of Basque Country, Spain. Technical assistance of Mari-Anne Carlsson and Ingmarie Olsson of Uppsala University is highly appreciated. The U.S. Government is authorized to reproduce and distribute reprints for Government purpose notwithstanding any copyright notation thereon. The views and conclusions are exclusively those of the authors and should not reflect the official policies or endorsements of the Air Force Office of Scientific Research or the U.S. Government or any of the granting organizations or collaborating entities mentioned above.

7 REFERENCES

- [1] Cabuzu D, Cirja A, Puiu R, Grumezescu AM. Biomedical applications of gold nanoparticles. *Curr Top Med Chem.* 2015;15(16):1605-13. Review.
- [2] Prades R, Guerrero S, Araya E, Molina C, Salas E, Zurita E, Selva J, Egea G, López-Iglesias C, Teixidó M, Kogan MJ, Giralt E. Delivery of gold nanoparticles to the brain by conjugation with a peptide that recognizes the transferrin receptor. *Biomaterials.* 2012 Oct;33(29):7194-205.
- [3] Lasagna-Reeves C, Gonzalez-Romero D, Barria MA, Olmedo I, Clos A, Sadagopa Ramanujam VM, Urayama A, Vergara L, Kogan MJ, Soto C. Bioaccumulation and toxicity of gold nanoparticles after repeated administration in mice. *Biochem Biophys Res Commun.* 2010 Mar 19;393(4):649-55.
- [4] Sharma HS, Menon P, Lafuente JV, Muresanu DF, Tian ZR, Patnaik R, Sharma A. Development of in vivo drug-induced neurotoxicity models. *Expert Opin Drug Metab Toxicol.* 2014 Dec;10(12):1637-61.
- [5] Menon PK, Muresanu DF, Sharma A, Mössler H, Sharma HS. Cerebrolysin, a mixture of neurotrophic factors induces marked neuroprotection in spinal cord injury following intoxication of engineered nanoparticles from metals. *CNS Neurol Disord Drug Targets.* 2012 Feb;11(1):40-9. Review.
- [6] Sharma HS, Muresanu DF, Patnaik R, Stan AD, Vacaras V, Perju-Dumbrav L, Alexandru B, Buzoianu A, Opincariu I, Menon PK, Sharma A. Superior neuroprotective effects of cerebrolysin in heat stroke following chronic intoxication of Cu or Ag engineered nanoparticles. A comparative study with other neuroprotective agents using biochemical and morphological approaches in the rat. *J Nanosci Nanotechnol.* 2011 Sep;11(9):7549-69.
- [7] Sharma HS, Menon PK, Lafuente JV, Aguilar ZP, Wang YA, Muresanu DF, Mössler H, Patnaik R, Sharma A. The role of functionalized magnetic iron oxide nanoparticles in the central nervous system injury and repair: new potentials for neuroprotection with Cerebrolysin therapy. *J Nanosci Nanotechnol.* 2014 Jan;14(1):577-95. Review.

¹***Hari S Sharma**, Dr. Med. Sci. (UU), Director Int. Expt. CNS Injury & Repair (IECNISIR), University Hospital, Uppsala University, Prof. Neurobiology (MRC); Docent Neuroanatomy (UU); Frödingsgatan 12:28, SE-75421 Uppsala, Sweden, Phone & Fax: +46 18 243899, Cell Phone: +46 70 2011 801; Email: Sharma@surgsci.uu.se, hssharma@aol.com.