Exacerbation of amyloid beta peptide and Tau protein in the cerebrospinal fluid following Traumatic Brain Injury at Hot Environment

Neuroproetction by TiO2 nanowired delivery of cerebrolysin with tau antibodies

[1]**Hari Shanker Sharma***, [2]José V Lafuente, [3]Dafin F Muresanu[a], [4]Rudy J Castellani, [5]Mark A Smith, [6]Ala Nozari, [7]Ranjana Patnaik, [8]Z Ryan Tian, [9]Asya Ozkizilcik[b], [10]Herbert Mössler[a], [1]Aruna Sharma

[1]International Experimental CNS Injury & Repair (IECNSIR), Laboratory of Cerebrovascular Research, Dept. of Surgical Sciences, Anesthesiology & Intensive Care Medicine, Uppsala University Hospital, Uppsala University, SE-75185 Uppsala, Sweden Email: Aruna.Sharma@surgsci.uu.se; Sharma@surgsci.uu.se;
[2]Dept of Neurosciences, University of Basque Country, Bilbao, Spain
[3]Dept. Clinical Neurosciences, University of Medicine & Pharmacy, Cluj-Napoca, Romania; [a]''RoNeuro'' Institute for Neurological Research and Diagnostic, 37 Mircea Eliade Street, 400364, Cluj-Napoca, Romania
[4]University of Maryland, Dept. of Pathology, Baltimore, MD, USA
[5]Case Western Reserve Medical University, Dept. of Pathology, Cleveland, OH, USA
[6]Anesthesiology, Massachusetts General Hospital, Harvard University, Boston MA, USA
[7]School of Biomedical Engineering, Dept. of Biomaterials, Indian Institute of technology, Banaras Hindu University, Varanasi, India
[8]Dept. Chemistry & Biochemistry & [9]Biomedical Engineering, University of Arkansas, Fayetteville, AR, USA
[10]Ever NeuroPharma, Oberburgau, Austria

ABSTRACT

Military personnel are prone to develop Alzheimer's disease (AD) during their lifetime. This is attributed largely due to mild traumatic brain injury, stress, hyperthermia and exposure to nanoparticles or chemical fumes during combat operations. All these conditions could induce breakdown of the blood-brain barrier (BBB) and edema formation. BBB breakdowm will allow plasma amyloid beta protein (AβP) to enter into the brain parenchyma and lead to slowy developing AD. Clevage of amyloid precursor proetin (APP) leads to tau phosporylation that is toxic to neurons and participate in AD process. In present innovation we demonstrate that concussive head injury (CHI) results in increased AβP and tau proetins in the cerebrosoinal fluid (CSF) of rats. This effect is exacerbated in hot environment (HE). As a result, brain pathology, BBB breakdown and edema formation are also exacerbated after CHI in HE.
It appears that TiO2-nanwored delivery of cerebrolysin together with antibodies to tau remarkably reduced AβP and tau concetrations in the CSF and induced neuroprotection. This indicates that nanodelivery of cerebrolysin with tau antibodies has supeior neuroprotective ability in CHI, not reported earlier.

Keywords: Concussive head injury, Alzheimer's Disease, Brain Pathology, Hot environment, Cerebrolysin, TiO2 nanowired delivery, tau antibodies, Neuroprotection

1 INTRODUCTION

Military personnel are highly vulnerable to Alzheimer's disease (AD) [1]. This is because of the fact that severe stress of trauma, sleep deprivation, hyperthermia or combat stress causes increased deposition of amyloid beta peptide (AβP) in the cerebrospinal fluid (CSF) and in brain parenchyma [1-3]. Military personnel are often exposed to high environmental temperature in desert areas during combat operations. Since traumatic brain injury (TBI) is quite common in combat operation, a possibility exists that hot environment (HE) could affect pathophysiology of TBI. However, effects of HE on TBI are not well known. Previous reports show that hyperthermia duirng TBI could worsen the pathological outcome. However, TBI induced pathophysiological outcome following HE still require further investigation. TBI induces increase in amyloid beta peptide (AβP) and tau protein in the brain and in the cerebrospinal fluid (CSF). AβP and tau are responsible for neuronal, glial and microvascular damages leading to breakdown of the blood-brain barrier (BBB) and vasogenic edema formation. However, effects of HE on AβP and tau are unknown. In present investigation we examined whether TBI could induce an increased production of

AβP and tau in HE. Furthermore to understand the role of tau in neuropathology we infused tau antibodies [Anti-Tau antibody [E178, ab32057) together with cerebrolysin (a known neuroprotective agent) in the CSF after TBI with HE.

In this innovation, we demostrate that conussive head injury (CHI) enhances AβP and tau deposition in the CSF that is further aggravated when CHI is inflicted at hot environment (HE). Since CHI and/or AD results in decrease of several neurotrophic factors, it appears that exogenous supplement of a balanced composition of various neurotrophic factors and active peptide fragments i.e., cerebrolysin may be beneficial in both AD and CHI. Thus, we used TiO2 nanowired cerebrolysin for effective delivery to brain with tau antibodies to neutralise neurotoxic activity of tau in vivo to enhance neuroprotection in a rat mode of CHI at HE and at normal temperature. Our studies showed a superior neuroprotection in CHI with co-administration of nanowired cerebrolysin with tau antibodies.

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 performed according to the Guidelines & Care for Laboratory Animals as described by National Institute of Health and approved by Local Institutional Ethics Committee.

2.1 Concussive head injury

CHI was inflicted by dropping a weight of 114.6 g from 20 cm height on the exposed parietal skull bone (see Fig. 1) in Equithesin anaesthetized rats. This weight and height causes an impact of 0.224 N on the right parietal bone surface. CHI was inflicted in rats either acclimatized at 21-23°C (room temperature) or exposed to 34°C for 4 h per day for 2 weeks in biological oxygen demand incubator (BOD, relative humidity 45-47 %, wind speed 20-25 cm/sec). (see Fig. 1) [1,2].

2.2 AβP and Tau protein assay

AβP levels were measured in CSF by sandwich ELISA using Thermo Fisher commercial Kit (KHB3481) Ab9 (anti-Aβ1–40) as the capture antibody and 4G8 (anti-Aβ17–24) as the detection antibody. Tau proetin levels were assayed in CSF using Thermo Fisher commercial kit (KHB0041) according to standard protocol [3,4].

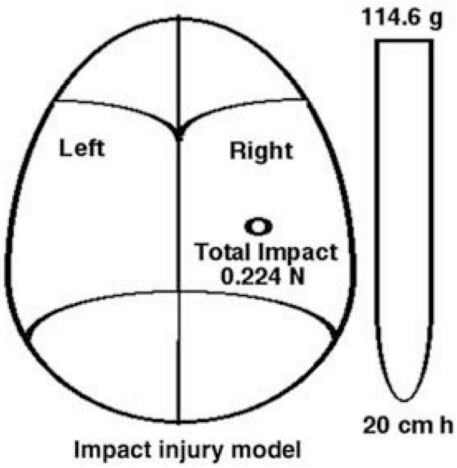

Fig. 1. Concussive head injury (CHI) model in the rat.

2.3 Brain Pathology

Brain pathology was analyzed using BBB breakdown, edema formation neurinal injury using standard protocol. The blood-brain barrier (BBB) breakdown to Evans blue radioiodine ($^{[131]}$-Iodine) was examined after intravenous administration (100 µCi/kg) 5 min before the end of the experiment [1-5]. Brain edema was determined using regional water content by wet and dry weights of the brain samples [3,6]. In separate groups of animals, neuronal changes were studied using histopathological examination of Nissl of H&E staining.

2.4 Nanowired delivery of Cerebrolsyin and Tau antibodies

Standard procedures were used to tag cerebrolsyin and tau antibodies [Anti-Tau antibody [E178, ab32057) to TiO2 nanowires as described earlier. TiO2-nanowired cerebrolysin (5 ml/kg, i.v.) together with 50 µl 1:20 tau antibodies i.c.v. were admonistered 4 h after CHI at room tempearture or following heat exposure under identical conditions [6,7]. Brain pathology was evaluated in each group after drug and antibodies treatment 24 h after CHI (as above).

2.5 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 CHI induces AβP and tau protein elevation in CSF at HE

Exposure to HE alone did not result in BBB breakdown, edema formation or changes in AβP or tau levels. The AβP and tau levels in CHI group after heat exposure increased by 2- to 6-fold in the CSF (control AbP ng/ml 0.23±0.04; TBI 0.82±0.05; TBI+HS 2.34±0.12; Control tau pg/ml 20±2; TBI 34±6; TBI+HS 76±8).

3.2 CHI and brain pathology at HE

CHI in HE animals resulted in about 2-to 3-fold higher breakdown of the BBB to Evans blue albumin and radioiodine ($^{[131]}$-I) and neuronal, glial and axonal damage as compared to CHI in rats at room temperature after 24 trauma.

A representative example of neuonal damage after CHI at room temperature (23°C) and at HE (34°C) is shown in Fig. 2. Loss of neurons, perineuronal edema and sponginess of neuropil is clearly seen aftre CHI at 34°C as compared to identical trauma at 23°C (Fig. 2).

3.3 TiO2 Cerebrolysin with tau antibodies and Brain pathology in CHI

Treatment with cerebrolysin (5 ml/kg, i.v.) together with 50 µl 1:20 tau antibodies i.c.v. 4 h after injury resulted in reduction of tau and AβP levels and brain pathology in CHI but failed to induce neuroprotection in HE group (results not shown). However, when TiO2-nanowired cerebrolysin was co-administered with nanowired tau antibodies in identical conditions pronounced neuroprotection was observed in HE group with CHI (Fig. 2). Also the tau and AβP levels were considerably reduced (AβP ng/ml 0.33±0.08; tau pg/ml 18±6) in the CSF of HE rats after CHI.

This treatment was also effective in reducing BBB breakdown, brain edema formation, neuonal injuries (Fig. 2) following CHI at hot environment.

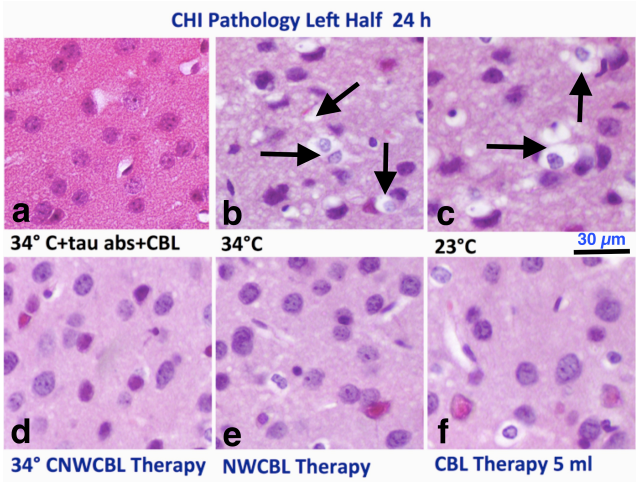

Fig. 2. Low power light microscopy showing neuronal distortion and damages following concussive head injury (CHI) at 23°C (c) and their exacerbation at 34°C (b). Perineuronal edema (arrows) and loss of neurons are prominent at 34°C (b) after CHI as compared to identical injury at 23°C (c). Normal cerebrloysin (CBL) induces neuroprotection after CHI at 23°C (f) whereas TiO2 nanowired cerebrolysin (NWCBL, d,e) induced superior neuroprotection after CHI at 34°C (d,e). Co-administration of NWCBL with tau antibodies (abs) induces most marked neuroprotection in CHI at 34° C (a). Nissl stain on 3-µm thick paraffin sections. Bar = 30 µm.

4 DISCUSSION

Continued stress in military is one of the main causes of development of mental dysfunction due to possible breakdown of the blood-brain barrier (BBB) function causing edema and cell injury [4-6]. Since military personnel during combat operation are also prone to traumatic or concussive brain injury (CHI) leading to brain pathology, it appears that trauma may further aggravate AβP deposition in the brain or CSF from plasma due to BBB dysfunction. In addition cleavage of amyloid precursor proetin (APP) by caspases results in tau phosphorylation and accumulation in the CSF as well as in the brain. Since both tau and AβP are neurotoxic, high levels of these proteins could could play critical roles in development of AD.

The key findings in this investigation clearly demonstrate that CHI at HE significantly increased the AbP and tau proetin levels in the CSF 24 h aftre trauma as compared to to identical injury at room temperature. This suggests that CHI at HE exacerbates AβP and tau deposition leading to pathological processes accelerating AD.

Another important finding of this investigation shows that when cerebrolysin that is a multimodal drug composed of several neurotrophic factors and active peptide fragments is administered togather with tau antibodies in CHI, the combination therapy signifcantly reduced the accumulation of AbP and tau proetins in the CSF even at HE and resulted in marked reductions in brain pathology.

This suggests that a reduction in AβP and tau in CSF has neuroproetctive effects in CHI. The possible mecahnisms by which cerebrolysin and tau antibodies reduce AβP and tau levels are unclear. However, it appears that cerebrolysin could strenghtem endothelial cell membrane function elading to a reduction in the BBB breakdown and edema formation. Restoration of BBB function will limit passage of plasma AβP into the brain or CSF after CHI [1-3]. Whether CHI could also elevate plasma levels of AβP is not known and require further investigation.

It appears quite likely that exogenous supplement of tau antibodies may neutralize endogenous tau activity thereby reducing neurotoxicity of tau in vivo. Thus, a combination of cerebrolsyin and tau antibodies could induce suoerior neuriproetction in CHI.

The possible reasons for greater effectiveness of cerebrolsyin and tau when delivered through TiO2 nanowred technology could be due to quick and deeper penetration of these agents into the brain or CSF as well as slow and sustained release of the compounds for long time [2,7,9]. A slow degradation or metabolism of NWCBL or tau within the brain may also be responsible for maintaining high level of CBL and tau antibodies in CHI resulting in superior neuroprotection [7,8].

5 CONCLUSION

In conclusion, our results indicate that both AβP and tau levels are somehow responsible for enhanced brain pathology in TBI following HE and nanodelivery of cerebrolysin with tau antibodies have superior neuroprotective effects, not reported earlier.

6 ACKNOWLEDGEMENTS

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; supported by Grants from the Alzheimer's Association (IIRG-09- 132087), the National Institutes of Health (R01 AG028679) and the Dr. Robert M. Kohrman Memorial Fund (MAS, RJC); Swedish Medical Research Council (Nr 2710-HSS), Göran Gustafsson Foundation, Stockholm, Sweden (HSS), Astra Zeneca, Mölndal, Sweden (HSS/AS), The University Grants Commission, New Delhi, India (HSS/AS), Ministry of Science & Technology, Govt. of India (HSS/AS), Indian Medical Research Council, New Delhi, India (HSS/AS) and India-EU Co-operation Program (RP/AS/HSS) and IT 794/13 (JVL), Government of Basque Country and UFI 11/32 (JVL) University of Basque Country, Spain, & Society for Neuroprotection and Neuroplasticity (SSNN), Romania. Technical and human support provided by Dr Ricardo Andrade from SGIker (UPV/EHU) is gratefully acknowledged. We thank Suraj Sharma, Uppsala, Sweden for computer and graphic support. The U.S. Government is authorized to reproduce and distribute reprints for Government purpose notwithstanding any copyright notation thereon. The views and conclusions contained herein are those of the authors and should not be interpreted as necessarily representing the official policies or endorsements, either expressed or implied, of the Air Force Office of Scientific Research or the U.S. Government.

7 REFERENCES

[1] Sharma HS, Muresanu DF, Lafuente JV, Nozari A, Patnaik R, Skaper SD, Sharma A. Pathophysiology of Blood-Brain Barrier in Brain Injury in Cold and Hot Environments: Novel Drug Targets for Neuroprotection. CNS Neurol Disord Drug Targets. 2016;15(9):1045-1071. Review.

[2] Sharma A, Menon P, Muresanu DF, Ozkizilcik A, Tian ZR, Lafuente JV, Sharma HS. Nanowired Drug Delivery Across the Blood-Brain Barrier in Central Nervous System Injury and Repair. CNS Neurol Disord Drug Targets. 2016;15(9):1092-1117. Review

[3] Ozkizilcik A, Williams R, Tian ZR, Muresanu DF, Sharma A, Sharma HS. Synthesis of Biocompatible Titanate Nanofibers for Effective Delivery of Neuroprotective Agents. Methods Mol Biol. 2018;1727:433-442.

[4] Sharma HS, Muresanu DF, Lafuente JV, Patnaik R, Tian ZR, Ozkizilcik A, Castellani RJ, Mössler H, Sharma A. Co-Administration of TiO2 Nanowired Mesenchymal Stem Cells with Cerebrolysin Potentiates Neprilysin Level and Reduces Brain Pathology in Alzheimer's Disease. Mol Neurobiol. 2018 Jan;55(1):300-311.

[5] Johanson C, Stopa E, Baird A, Sharma H.Traumatic brain injury and recovery mechanisms: peptide modulation of periventricular neurogenic regions by the choroid plexus-CSF nexus. J Neural Transm (Vienna). 2011 Jan;118(1):115-33. doi: 10.1007/s00702-010-0498-0. Epub 2010 Oct 10. Review.

[6] Patnaik R, Sharma A, Skaper SD, Muresanu DF, Lafuente JV, Castellani RJ, Nozari A, Sharma HS. Histamine H3 Inverse Agonist BF 2649 or Antagonist with Partial H4 Agonist Activity Clobenpropit Reduces Amyloid Beta Peptide-Induced Brain Pathology in Alzheimer's Disease. Mol Neurobiol. 2018 Jan;55(1):312-321.

[7] Sharma A, Menon PK, Patnaik R, Muresanu DF, Lafuente JV, Tian ZR, Ozkizilcik A, Castellani RJ, Mössler H, Sharma HS. Novel Treatment Strategies Using TiO-Nanowired Delivery of Histaminergic Drugs and Antibodies to Tau With Cerebrolysin for Superior Neuroprotection in the Pathophysiology of Alzheimer's Disease. Int Rev Neurobiol. 2017;137:123-165.

[8] Sharma HS, Sharma A. Nanowired drug delivery for neuroprotection in central nervous system injuries: modulation by environmental temperature, intoxication of nanoparticles, and comorbidity factors. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2012 Mar-Apr;4(2):184-203.

[9] Feng L, Sharma A, Niu F, Huang Y, Lafuente JV, Muresanu DF, Ozkizilcik A, Tian ZR, Sharma HS. TiO_2-Nanowired Delivery of DL-3-n-butylphthalide (DL-NBP) Attenuates Blood-Brain Barrier Disruption, Brain Edema Formation, and Neuronal Damages Following Concussive Head Injury. Mol Neurobiol. 2018 Jan;55(1):350-358.

[1]***Hari S Sharma**, Dr. Med. Sci. (UU), Director Int. Expt. CNS Injury & Repair (IECNSIR), 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, harishanker.sharma@ehu.eus