TiO2 nanowired delivery of a Chinese traditional medicine DL-3-n-butylphthalide (DL-NBP) induces profound neuroprotection in concussive head injury

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ABSTRACT

Our military personnel are often engaged in combat or peacekeeping operation across the World. These soldiers are prone to traumatic head or spine injury in the battlefield. Blunt head trauma is one of the most devastating injuries leading to instant death in more than 80% of the victims. Those who survive show lifetime disability. Thus, novel therapeutic strategies to treat blunt head trauma is the need of the hour. In this innovation we have explored the role of a Chinese traditional medicine DL-3-n-butylphthalide (DL-NBP) in a rat model of concussive head injury (CHI). We delivered this compound either alone or using Titanium nanowires delivery to explore the benefit of nanodrug delivery in CHI. Since CHI induces much more pronounced death and disability and so far therapeutic approaches are still elusive we showed that this traditional Chinese medicine has some values in CHI in reducing brain pathology. These neuroprotective effects of NBP are far superior when the drug was delivered using nanobiotechnology in CHI.

Keywords: DL-3-n-butylphthalide (DL-NBP), concussive head injury, brain patholoy, TiO2 nanowired drug delivery, blood-brain barrier, brain edema, neuroprotection,

1 INTRODUCTION

DL-3-n-butylphthalide (DL-NBP) a yellowish oil-like liquid (99.6% purified) is a Traditional Chinese Medicine used for various neurological ailments since ages. The basic mechanisms behind such a neuroprotective effects of DL-NBP are related to its possible strong antioxidative properties [1]. Since CHI cuased by motor vehicle accident or blunt trauma on the head during combat operations induces severe brain pathology and oxidative stress, use of NBP in such circumstances is needed [1-3]. In CHI cases rehabilitation and supportive strategies costs several millions of USD with only a mild improvement in the victims over time. Thus, we investigated DL-NBP in CHI to reduce brain pathology and behavioral dysfunction using nanotechnologies.

The DL-NBP is an extract from Chinese celery and is used in stroke patients showing marked improvement in their cognitive and mental health [1]. Since concussive head injury (CHI) is often fatal in more than 90% of cases involving our soldiers in the battlefield and the remaining 10 % of cases show lifetime disability there is an urgent need to find out better therapeutic use of novel medicine or use of available medicine in a more effective way. Keeping these views in mind we wanted to explore the role of DL-NBP in CHI using nanodrug delivery employing TiO2 nanowires in our rat model of CHI that induced profound brain pathology similar to that of clinical cases.

2 MATERIALS & METHODS

Experiments were carried out on Male Wistar Rats (200-300 g) housed at controlled ambient temperature (21±1°C) with 12 h light and 12 h dark schedule. Food and water were provided ad libitum before 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 Concussive head injury (CHI)

The CHI was induced by dropping a weight of 114.6 g on the right parietal skull bone over a distance of 20 cm in

anesthetized rats resulting an impact of 0.224 N on the skull surface [Fig. 1]. This impact induces severe brain pathology over 4 h to 24 h. [3]. The animals were allowed to survive either 8 h or 24 h aftre trauma in this study.

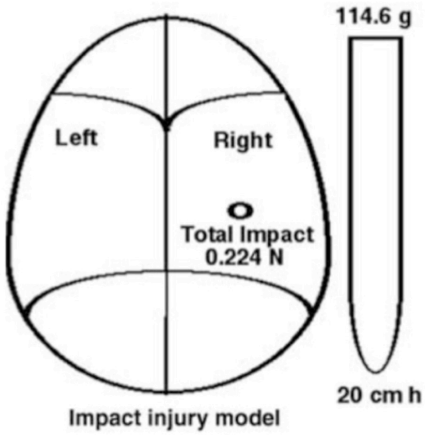

Fig. 1. Concussive head injury (CHI) model in rats

2.2 Treatment with DL-3-n-butylphthalide

DL-NBP was administered in a dose of 40 mg or 60 mg/kg, i.p. following 2 h and 4 h after injury in 8 h survival group and 8 h and 12 h after trauma in 24 h survival group. In separate group if animals DL-NBP was tagged with TiO2 nanowires using standard procedures [Ref 2; See Fig. 2] and TiO2-nanowired NBP was given in identical conditions in CHI in a dose range of 20ng or 40 mg/kg, i.p. [See Table 1].

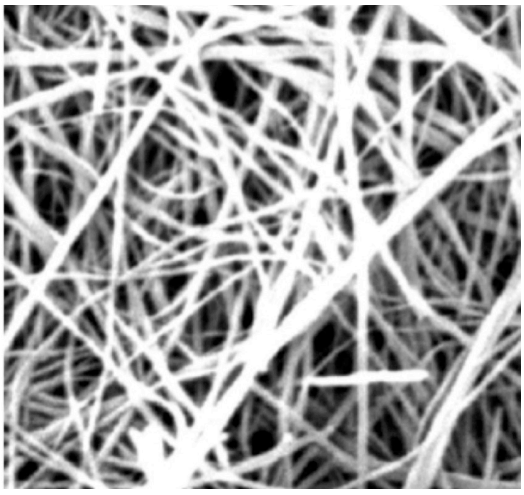

Fig. 2. TiO2 nanowired for tagging DL-3-n-butylphthalide

2.3 Parameters measured

The following parameters were measured in untreated or NBP terated traumatized rats and the data were compared with saline treated control groups under identical conditions.

2.3.1 Blood-brain barrier

The blood-brain barrier (BBB) leakage was measured using Evans blue albumin (EBA, 2% of 0.3 ml/100g i.v.) and radioiodine ($^{[131]}$-Iodine], 10 μCi/100g i.v.) extravasation in the brain [2,3]. After washing out of intravascular tracer with 0.9 % saline through heart the brain were dissected out and examined for blue staining. After that tissue pieces from selected brain areas were then dissected and radioactivity determined in a Gamma Counter (Packard, USA). Leakage of these tracers was expressed as percentage increase in the brain over blood concentration [2,3].

2.3.2 Brain Edema formation

The brain edema was measured using brain water content. Desired tissue pieces from brain were dissected out and weighed to determine their wet weight. After that these tissue pieces were kept in an oven maintained at 90° C for 72 h to obtain their dry weight. The percentage water content was calculated from the differences between wet and dry weight of the samples [2,3].

2.3.3 Neuronal injury

Neuronal injury was evaluated using Nissl or Haematoxylin & Eosin (HE) staining using standard histopathological techniques [3]. For this purpose, animals were perfused in situ with 4 % buffered paraformaldehyde preceded with a brief saline rinse though cardiac puncture. Coronals sections of the brain were the cut passing through the hippocampus and embedded in paraffin. About 3 μm thick sections were cut and stained with HE or Nissl using commercial protocol [3]. The number of damaged or distorted neurons in specific anatomical brain areas were counted manually.

2.4 Statistical analyses

ANOVA followed by Dunnett's test for multiple group comparison with one control group 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 induced Neurotoxicity

We observed a progressive increase in the BBB breakdown from 4 h after CHI that continued to enhance by 200 % at 8 h, 400 % after 12 h and 600 % at 24 h after injury. The neuronal damages were also showed massive increase by 8 h after CHI (+4 to 6 fold) followed by +7 to +8 fold at 12 h and +9 to +10 fold at 24 h in the cortical and subcortical areas. There was a voluminous increase in brain swelling by 1 % at 4 h, 2 % at 8 h, 4% at 12 h and 6 % at 24 h as compared to intact controls.Leakage of Evans blue and radioioidine across the BBB was also increased by 4 to 8 fold in CHI group as compared to saline treated controls [Table 1].

3.2 NBP induced Neuroprotection

In separate group of rats with CHI, we administered NBP (40 or 60 mg/kg, i.p.) 2h and 4 h after injury in 8 h survival group and 8 h and 12 h after trauma in 24 h survival group. We found that NBP in 40 mg was able to reduce BBB breakdown, brain edema formation and brain pathology after 8 h CHI, whereas, 60 mg dose of NBP was needed for identical neuroprotection in 24 h CHI group. Interestingly, TiO2 Nanowired NBP requires only 20 mg/kg in 8 h group and 40 mg/kg NBP in 24 h group for effective neuroprotection indicating a potential enhanced effectiveness of DL-NBP following TiO2 nanowired delivery in CHI [See Fig. 3, Table 1].

Neural injury, neuronal loss, distorted neurons; perineuronal edema and sponginess of the neuropil were also markedly reduced by TiO2-nanowired NBP at 12 and 24 h with lower doses of the compound (20 and 40 mg respectively) as compared to normal NBP drug [See Fig. 3].

The left uninjured half exhibited better neuroprotection by TiO2-Nanowired NBP as compared to right injured side.

Also reduction in leakage of Evans blue, radioiodine and volume swelling of the brain correlated well with decreased neuronal injury in TiO2-nanowired NBP treated CHI group (Result not shown).

Table 1. Neuroprotective effects of TiO2-nanowired delivery of DL-3-n-butylphthalide (DL-NBP) in concussive head injury (CHI)

Expt. Type	Control	CHI 12 h	CHI 24 h	CHI 24 h+ (DL-NBP) 60 mg	CHI 24 h+ TiO2-(DL-NBP) 40 mg
EBA mg%	0.24±0.06	1.86±0.11**	2.76±0.18**#	1.04±0.12*a	0.74±0.11*#b
$^{[131]}$Iodine %	0.30±0.08	1.94±0.13**	2.96±0.14**#	1.15±0.10*a	0.90±0.09*#b
Brain water %	74.76±0.18	77.64±0.24**	81.33±0.16**#	76.19±0.27a	75.06±0.13#b
Brain Swelling % *f*	0	+12**	+28**#	+8*a	+2*#b
Neuron Injury Nr	2±3	189±25**	350±36**#	98±14*a	36±14*#b

Values are Mean±SD of 5 to 6 rats, CHI = Concussive head injury; DL-NBP = DL-3-n-butylphthalide;
** P <0.05, ** P <0.01 from control, # P <0.05 from CHI 12 h, a P <0.05 from CHI 24 h, b P <0.05 from CHI+NBP. For details see text.*

4 DISCUSSION

Our observations are the first to show a new role of DL-NBP in treating CHI with regard to pathophysiology of brain injury. In addition, the nanowired delivery of NBP appears to be far superior to NBP alone in treating CHI induced brain pathology, not reported earlier.

The probable mchansims behind NBP induced neuroprotection in CHI is not well understood. However, available evidences suggests that NBP could play a key role in reducing oxidative stress, production of free radicals and generation of nitric oxide in the brain [1]. Obviosuly, a reducion in these oxidative stress parameters in CHI could be largely responsible for the neurprotective effects of NBP in this investigation [1-3]. However, to furtejr confirm this point, measurement of oxidative parameters in CHI is needed.

Another important point came out from this investigation is a better neuroprotection of the contralateral half of the brain aftre CHI [Fig. 3] aftre NBP treatment. This suggests that NBP could prevent oxidative stress in the uninjured brain much better than the directly injured side. This property of NBP could be of great clinical importantce as the drug may be able to reduce brain pathology clinical cases of CHI where uninjured side showes greater damage.

The mechanisms by which a superior neuroproetction even in lower dose by nanodelivered NBP is seen in CHI is not well understood from this investigation. However, it appears that NBP when given with TiO2 nanowired material, the drug could reach the deeper brain areas faster due to breakdown of the BBB [2]. Obciously, a greater BBB breakdown in CHI is seen in the uninjured half. Another possibility for superior effects of TiO2-nanodelivery of NBP is due to the fact that the drug could resisi biological degradation due to its tight binding with TiO2 nanowires. Thus, the drug may be acting for long time once enetered into the brain [2,3].

However, it remains to be seen whether TiO2 nanodelivery of NBP aftre CHI could also reduce behavioral and cognitive functions, a feature that is currently being investigated in our laboratory.

5 CONCLUSION

In conclusion, our observations point out a new role of NBP in terateing CHI cases in clinical situations. Furtehrmore TiO2-nanodelivery of NBP appears to be superior in nature. Thus, new studies are needed to evalute safety and relaiability of nanodelivery of NBP in clinical situations.

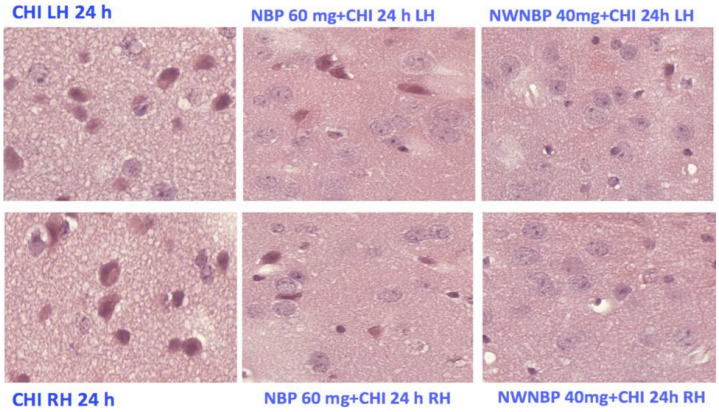

Fig. 3. Shows superior neuroprotection by TiO2-nanowired NBP at 24 h after CHI in lower doses as compared to normal NBP at identical period with higher doses. H&E stain on paraffin sections on parietal cerebral cortex. x 40.

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