

Nanodrug delivery by single-walled carbon nanotubes (SWCNTs) in the central nervous system induces neurotoxicity. Potential neuroprotective effects of Cerebrolysin

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ABSTRACT

Our military personnel are often inflicted with brain or spinal cord injury during combat operations caused by gunshot, missile explosions, fall or direct blunt or sharp trauma to their CNS leading to life threatening illnesses. Recently, single-walled carbon nanotubes (SWCNTs) are used for nano drug delivery in cancer therapy however; SWCNTs induced neurotoxicity is not well known. In this innovation, we used Cerebrolysin, a balanced composition of several neurotrophic factors and active peptide fragments in attenuating SWCNTs induced neurotoxicity in the rat central nervous system (CNS). These observations support the idea that co-administration of Cerebrolysin with SWCNTs is needed to enhance the neuroprotective effects of nanodrug delivery using SWCNTs as a vehicle.

Keywords: cerebrolysin, single walled carbon nanotubes, brain pathology, drug delivery, blood-brain barrier, neuroprotection

CNTs alone could have toxicological properties when administered intravenously in animals. Lung and liver toxicity was seen enhanced following administration of CNTs in rats or mice. This suggests that drug delivery using CNTs for neuroprotection may also be not safe unless the neurotoxicity of CNTs is investigated in great details. Interestingly, effects of CNTs on the central nervous system (CNS) in vivo situation are still not well examined. Thus, there is a need to understand the role of CNTs on brain pathology in normal animals.

In our previous experiments, we have observed that Cerebrolysin, a balanced composition of several neurotrophic factors and active peptide fragments markedly induces neuroprotection in various models of CNS trauma. Thus, a possibility exists that co-administration of Cerebrolysin with CNTs in vivo models may attenuate neurotoxicological properties of CNTs. Thus, in this innovation we combined Cerebrolysin treatment with SWCNTs in a rat model after their intravenous administration and examined neurotoxicity using standard procedures.

1 INTRODUCTION

Nanodrug delivery is likely to enhance the therapeutic effects of the compounds for effective treatment in brain diseases or cancer therapy. Recently, carbon nanotubes (CNTs) are employed for nanodrug delivery of anticancer agents with the hope of better treatment capabilities. Single-walled CNTs (SWCNTs) are preferred for nanodrug delivery as compared to multi-walled CNTs (MWCNTs) in most cases for their physicochemical ability to transport drugs into the biological system. [1] Although, nanodrug delivery through SWCNTs are promising in cancer cases, further studies are needed to explore neurotoxicity of CNTs as such in vivo situations. Available evidences suggest that

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 SWCNTs administration

In this investigation, we used intravenous SWCNT (outer diameter 2 to 8 nm, length 0.5 to 2.0 μm from Sigma Aldrich, USA Prod nr. 652482, concentration 1 mg/ml) in a dose of 1 μg or 5 $\mu\text{g}/\text{Kg}/\text{min}$ infused into the right jugular vein for 10 min in Equithesin (3 ml/kg, i.p.) anesthetized rats (age 15 to 18 weeks) using a Microinfusion Pump (Harvard Apparatus, Boston, MA, USA).

2.2 Cerebrolysin Treatment

In separate group of rats Cerebrolysin (Ever NeuroPharma, Austria) was co-administered in a dose of 2.5 ml or 5 ml/kg, intravenously together with SWCNT administration [2].

2.3 Parameters Measured

The following parameters were measured in animals that received SWCNT alone or with Cerebrolysin after 24 of administration.

2.3.1 Blood-brain barrier

The blood-brain barrier (BBB) leakage was measured using Evans blue albumin (EBA) and radioiodine (^{131}I -Iodine] extravasation in the brain. For this purpose the EBA (2 % of 0.3 ml/100g body weight) was administered intravenously 5 min before termination of the experiment. After washing out of intravascular tracer with 0.9 % saline perfused through heart at 90 Torr, the brain were dissected out and examined for blue staining. The tissue pieces from selected brain areas were then dissected out weighed and radioactivity determined in a Gamma Counter (Packard, USA). Before saline perfusion about 1 ml whole blood was withdrawn from cardiac puncture to determine radioactivity or EBA concentration in the whole blood. Leakage of these tracers was expressed as percentage increase in the brain over blood concentration [3].

2.3.2 Brain Edema formation

The brain edema formation was determined using measurement of water content in the brain. For this purpose, small tissue pieces of brain were dissected out and weighed immediately 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 [3].

2.3.3 Neuronal injury

Neuronal injury was evaluated using Nissl or Haematoxylin & Eosin (HE) staining on paraffin sections using standard histopathological techniques [3]. For this purpose, animals were perfused in situ with 4 % buffered paraformaldehyde preceded with a brief saline rinse through cardiac puncture. After in situ fixation, the brain were removed and kept in the same fixative for 24 h. On the 2nd day coronal sections of the brain were cut passing through the hippocampus and the blocks were embedded in paraffin using standard procedures. About 3 μm thick sections were cut and stained with HE or Nissl using commercial protocol [3].

The sections were examined under an Inverted Carl-Zeiss Microscope and the images were recorded using a digital Olympus camera [8]. The number of damaged or distorted neurons in designated anatomical areas was 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 SWCNTs induces Neurotoxicity

Intravenous administration of SWCNT in rats induced dose dependent breakdown of the BBB permeability to EBA and radioiodine after 24 h of administration (Table 1). At this time significant increase in brain water content was also observed in the cerebral cortex. This effect of SWCNT on brain edema formation was also dose dependent. Neuronal injuries were prominent in the cortex after SWCNT administration. The magnitude and intensity of neuronal damage is dose related (Table 1).

Thus, neuronal loss, distorted neurons, perineuronal edema and sponginess of the neuropil are quite frequent in the cortex after SWCNT administration (Fig. 1). A general expansion of the neuropil supporting edema formation is also clearly evident in SWCNT treated animals.

However, SWCNT administration did not alter apparent behavioral dysfunction or sensory motor disturbances within 24 h in these animals (Result not shown).

Table 1. SWCNTs induced neurotoxicity and neuroprotection by Cerebrolysin co-administration

Expt. Type	Control	SWCNT 24 h	SWCNT 24 h	SWCNT 24 h+	SWCNT 24 h+
		1 μ g	5 μ g	Cerebrolysin 1 μ g +2.5 ml	Cerebrolysin 5 μ g+5 ml
EBA mg%	0.24 \pm 0.06	0.89 \pm 0.04**	1.27 \pm 0.08**#	0.36 \pm 0.08*a	0.34 \pm 0.06*#b
¹³¹ Iodine %	0.30 \pm 0.08	1.03 \pm 0.09**	1.98 \pm 0.07**#	0.45 \pm 0.08*a	0.44 \pm 0.05*#b
Brain Edema	74.76 \pm 0.18	75.84 \pm 0.14**	76.73 \pm 0.16**#	74.89 \pm 0.14a	75.06 \pm 0.13#b
Neuron Injury	2 \pm 3	89 \pm 15**	158 \pm 16**#	8 \pm 4*a	6 \pm 4*#b

Values are Mean \pm SD of 5 to 6 rats, * $P < 0.05$, ** $P < 0.01$ from control, # $P < 0.05$ from SWCNT 1 μ g, a $P < 0.05$ from SWCNT alone, b $P < 0.05$ from SWCNT 5 μ g. For details see text.

3.2 Cerebrolysin induces Neuroprotection

Co-administration of Cerebrolysin 2.5 ml/kg, i.v. together with 1 μ g SWCNT and 5 ml/kg, i.v. with 5 μ g SWCNT significantly attenuated neurotoxicity in these rats as seen at 24 h after administration (Fig. 1, Table 1). A significant reduction in EBA and radioiodine leakage following Cerebrolysin treatment was seen in rats after SWCNT administration (Table 1). This effect of Cerebrolysin appears to be dose related. Thus, with SWCNT 5 μ g, 5 ml dose of Cerebrolysin was superior to that of 2.5 ml Cerebrolysin administration (Results not shown).

Interestingly, reduction in the BBB breakdown was also well correlated with marked reduction in brain edema formation in Cerebrolysin treated group (Table 1).

Neuronal injury showed a significant reduction in Cerebrolysin treated animals following SWCNT administration (Table 1). Thus, in Cerebrolysin treated animals several healthy neurons with distinct nucleus containing nucleolus are seen in many areas of the brain. The number of neurons is also higher in identical areas of neuropil in Cerebrolysin treated animals after SWCNT administration (Fig. 1). Neuropil was most preserved in Cerebrolysin treated rats after SWCNTs administration as seen using ultrastructural studies (Fig. 2).

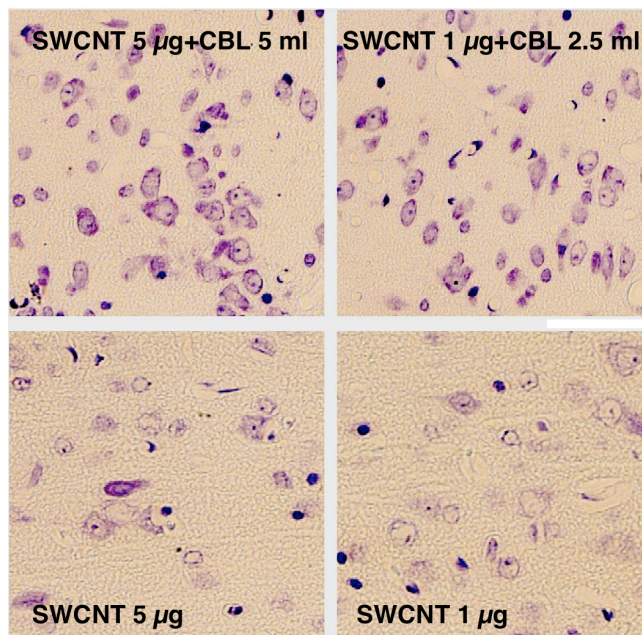


Fig. 1. Neuroprotective effects of Cerebrolysin (CBL) on Single Walled Carbon Nanotube (SWCNT) induced Neurotoxicity in the cerebral cortex of rats. Bar = 35 μ m.

4 DISCUSSION

The salient novel finding in this innovation suggests that Cerebrolysin if co-administered with SWCNT is able to prevent neurotoxicity caused by CNTs alone. This opens up new possibilities to deliver drugs using SWCNTs

together with suitable doses of Cerebrolysin to prevent toxic effects of the CNTs *per se* within the CNS.

This suggests that CNTs could be neurotoxic and nanodrug delivery based on SWCNTs or MWCNTs require suitable measured preventing toxicity of the

vehicle (CNTs) using suitable precautionary measures e.g., co-administration of Cerebrolysin. We have seen that Cerebrolysin is able to reduce neurotoxicity when administered with SWCNTs. However, this is also possible that other organ toxicity may also be reduced by a combination of SWCNTs and Cerebrolysin. However, this is a feature that requires additional investigations.

The mechanism by which CNTs induce neurotoxicity is unclear. However, available evidences suggest that oxidative stress caused by CNTs may have some role in toxicity. Cerebrolysin being a balanced composition of several neurotrophic factors and active peptide fragments could thus be able to reduce oxidative stress and induce cell membrane stability [4].

A reduction in the BBB breakdown and edema formation in Cerebrolysin treated animal following SWCNT administration is in line with this hypothesis. A reduction in edema formation and prevention of serum leakage within the neuropil could be one of the most important factors in reducing neuronal damage [3,4]. A dose related effects of Cerebrolysin suggest that the amount of neurotrophic factors reaching to the brain could be important in attenuating neurodestructive signals to induce neuroprotection.

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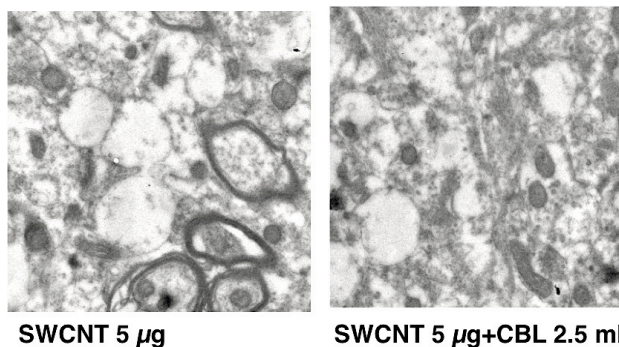
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Fig. 2. Transmission Electron Micrograph showing Neuroprotection caused by Cerebrolysin following SWCNTs administration in the rat.



needed in this direction to understand the dose related effects of cerebrolysin in neuroprotection.

5 CONCLUSION

Taken together our innovation shows that co-administration of Cerebrolysin with SWCNT is needed to reduce CNT induced neurotoxicity. Thus, future nanodrug delivery with CNTs requires adjuvant therapy of Cerebrolysin to prevent cellular damage.

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