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Functionalized carbon nanotubes—A boon in treating brain diseases

Rajkumar Ghosh[1], Jagabandhu Bag[2], Aparna Datta[2*], Arup Pramanick[2], Isa Hassan Abubakar[3]

[1]Department of Pharmacy, Birbhum Pharmacy College, Birbhum, India.
[2]Department of Pharmaceutical Technology, NSHM Knowledge Campus, Kolkata, India.
[3]School of Allied Health and Sciences, Sharda University, Noida, India.

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ABSTRACT

Typical intrinsic properties of carbon nanotube (CNTs) like one-dimensional structure with very high mechanical strength, high thermal and electrical conductivity, high aspect ratio, high surface area, ability to conjugate with functional groups, and elevated surface functionalizing capacity have made it a nanostructure of choice to be manipulated for drug delivery for the past two decades. The human brain restricts movements and or entry of ions, molecules, and cells between the blood and the brain because of the presence of the blood–brain barrier. As a result, administering drug molecules of choice to the brain under disease condition become constrained. Surface functionalized CNTs can render themselves efficient as drug carriers to the neurons, for extreme conditions like Alzheimer's disease, glioblastoma, Parkinson's disorder, brain stroke, brain tumor, etc. This review discussed in detail the advancement achieved so far in delivering drug molecules to the brain using CNT as the carrier and related management of toxicity so that a safer dose delivery can be made.

INTRODUCTION

Delivering drugs to the brain remained a challenge until recently when carbon nanotube (CNT) came up with a modification on the surface. These nanostructures in almost single dimension are delivering way out to several challenges in delivering drugs to the brain, crossing the tight barrier. Carbon-based nanostructures are becoming increasingly relevant in the field of neuroscience owing to their many exclusive chemical and physical properties. Recently, CNT based drug delivery has spawned great interest in medicine delivery and therapeutics, where significant modification of CNT helped in vaccine delivery systems (Bianco and Prato, 2003) as well as protein transporters (Kam *et al*., 2005) apart from being drug nano-carriers. Besides other nanomaterials, CNTs have been widely used in pharmaceutical and biomedical applications such as task-specific drug transport, cancer therapy and diagnosis, imaging, and or tissue engineering. While CNTs are used as drug delivery carriers, the nanotube aids in encapsulating drugs in its hollow tube-like structure. This is so since the drugs can be bound in their inner hollow area, whereas other molecules can be fabricated to the peripheral face to render them biocompatible while targeting the site (Martincic and Tobias, 2015).

Nevertheless, it is highly desirable that the drug transporter molecule must be non-immunogenic, non-toxic, have places for attachment of diagnostic or remedial agents, be electronically or spectroscopically accessible, as well as not exhibit long-term *in vivo* piling in vital organs. Since the remarkable breakthrough of CNTs by Iijima (1991), this particular structure in the nano range has exhibited valuable properties matching the above requirements for being ultra-lightweight, low deposition, highly flexible, high aspect ratio, inert with thermal and electrical conductivity, high penetrability, ultra-strong, and multiple attachable sites, ability to get surface modified, to name a few. These attributes along with many more have attracted scientists around the globe to seek its application in the healthcare and diagnostic arena. Approximately three decades of advances in carbon nanotechnology have divulged exciting perceptions and pioneering tactics in tissue regeneration and lately for nerve tissue healing (Dvir *et al*., 2011; Place *et al*., 2009). As a result, eventually, CNT is emerging as new promising aid for the management of neurological ailments

*Corresponding Author
Aparna Datta, Department of Pharmaceutical Technology, NSHM Knowledge Campus, Kolkata, India.
E-mail: adatta75 @ gmail.com

like Parkinson's disease, Alzheimer's disease (AD), and ischemic stroke (Folch *et al*., 2016; Kakkar and Dahia, 2015; Komane *et al*., 2016). Effective application of CNTs in delivering drugs *in vivo* has been reported in many diseases and disorders like rheumatoid arthritis, osteoporosis, bone implants, and cancer (Malarkey and Parpura, 2007). Nonetheless, for the successful application of CNTs in neurological disorders, very limited preclinical studies have been performed (Fabbro *et al*., 2013). Investigations on fullerene derivatives have shown a promising role of this as neuroprotective agents (Dugan *et al*., 2001). For instance, reports for central nervous system (CNS) protection in rats against chronic alcohol were rendered by nanostructures of hydrated C60 fullerene (C60HyFn) (Dugan *et al*., 1997). Additionally, neurotransmitter metabolism has been suggested by the indirect participation of C60HyFn in the same report. There are several studies that have demonstrated that multiple synergistic mechanisms are offered by fullerene derivatives that can be employed for AD treatment (Kerna *et al*., 2020; Podolski *et al*., 2007; Tykhomyrov *et al*., 2008; Vorobyov *et al*., 2015). Because of their exceptional physical properties added to the recently discovered interface capacity with neuronal circuits and membranes, synapses, CNT-based techniques are deemed very appealing to enhance neuron healing after brain injury (Fabbro *et al*., 2013).

It is an established fact that aqueous solutions do not dissolve CNTs in their natural or unaltered state. Hence, the requirement of surface modification is an alternative to handle this kind of issue. Surface functionalization and chemical modification improve aqueous solubility, making them more useful for treating neurological diseases (Georgakilas *et al*., 2002). Because of its very high aspect ratio, studies have shown that the use of pure CNTs causes an inflammatory response along with a loss in cell viability. Furthermore, CNT aggregation caused by *van der Waals* interactions has a negative impact on cellular responses and causes pulmonary toxicity *in vivo* (Fisher *et al*., 2012; Liu *et al*., 2013). The above-mentioned limitations can be addressed through chemical modification. As a result, many of the neuro-therapeutic compounds never reach the market since they are unable to cross the blood–brain barrier (BBB) (Bicker *et al*., 2014). The growth configuration of neurons on as-grown functionalized multi-walled nanotubes has been investigated by Mattson *et al*. (2000) which came up with encouraging reports. Later, Pantarotto *et al*. (2004) in another study revealed that the internalization of nanotubes with fluorescent tagging had no obvious toxicity effects on cells; however, the uptake mechanism could not be identified. Paracellular transport across the BBB tight junctions can be improved via the functionalization of CNTs since these tight barriers allow certain particles only in the range of <20 nm (Chenthamara *et al*., 2019). CNTs offered a wide variety of unlike molecules that could be decorated onto the surface of nanotubes, as illustrated in Figure 1. Hence, a possibility of an easily customized way of ferrying molecules into cells in a smart way. Therapeutic and biomedical uses of CNTs *in vitro* and *in vivo* for brain cells-oriented disease treatments have seen modern advances so far (Xiang *et al*., 2020).

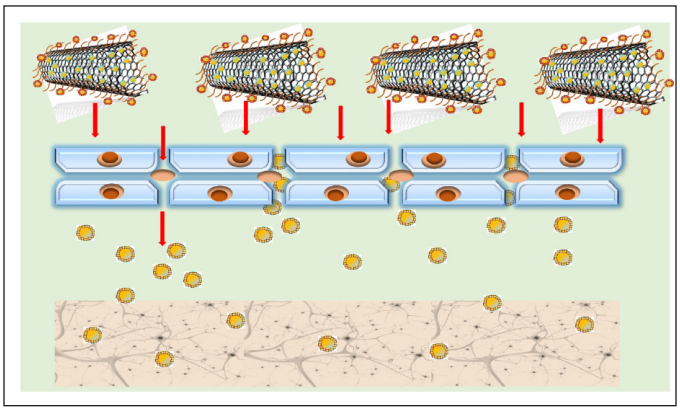

Figure 1. Functionalized CNTs allow the passage of drugs via tight barriers to the brain cells.

Studies have revealed that if the size of a particle is less than 100 nm, it gets easily transferred through BBB (Costa *et al*., 2004). Thus, nanostructures that can be brain-targeted as a part of the therapeutic approach have led to improved brain delivery. CNTs are used as a nano-carrier in treating CNS disease for their distinct physical and chemical properties, which include large surface area, better solubility in physiological solvents for its nature to functionalize, unique carrying ability of drug molecules, and biocompatibility with the neural system (John *et al*., 2015). The permeability of antitumor medication molecules through the BBB is extremely poor, which has a detrimental influence on brain tumor therapy. A patent in this regard was filed by BRAINGUARD CO Ltd. wherein a thorough discussion and implementation of CNT could be made for prophylactic or treatment of any diseases of the brain (Jeong *et al*., 2011).

CNTS FOR GLIOBLASTOMA

Intrinsically, CNTs are hydrophobic in nature. As such this keeps them agglomerated and is one of the limitations in cellular uptake and or penetration. Surface functionalization of these nanostructures resolves this issue to a great extent. Conjugated CNT with cytosine–guanosine dinucleotide (CpG), short single-stranded synthetic DNA molecules) has established counteraction on the immunosuppressive setting when tested in intracranial GL261 gliomas in tumor-bearing mice. Hence, low doses of CpG-modified CNTs could boost immunity against tumors and also eliminate gliomas (malignant brain tumors) at low doses (Zhao *et al*., 2011). In another study, polyethylene glycosylated (PEGylated) multi-walled CNTs were surface modified with Angiopep-2 (a peptide that shows a high brain penetration capability) to overcome the BBB. Such surface-decorated CNTs were capable of distributing in brain cells and later get accumulated in the target tumor. The nature of CNTs offered exceptionally high surface area and significantly high loading capacity of doxorubicin, an anticancer drug. This functionalized CNT was able to deliver drugs to the cancer-affected cells of the brain after crossing the BBB (Ren *et al*., 2012).

Poor penetration and low permeability of delivery systems across the BBB and into the tumor tissue limits the therapeutic effect of glioma. In an attempt to overcome these two barriers, several proposals were made where Angiopep-conjugated drug-loaded CNTs could target glioma treatment. This assembly successfully targeted the glioma cells by traversing across BBB typically through lipoprotein receptor-related protein receptor-mediated trans-cytosis (Kafa *et al*., 2016).

In another study, –COOH functionalized single-walled carbon nanotube (SWCNT) had levodopa attached to the carrier system. This could successfully be delivered to PC12 cells (cell line obtained from a pheochromocytoma of the adrenal medulla of rats) and evaluate its effect on normal neuronal cells *in vitro*. This was a pH-dependent release of levodopa (LD) and -COOH modified LD attached SWCNT (SWCNT-COOH-LD) that affected the cell viability of PC12 cells negatively (Tan *et al*., 2015). Here, the surface decorated CNTs-based drug delivery could successfully be delivered to the nervous system.

CNTS FOR ALZHEIMER DISEASE

More than 10% of the world's population over the age of 65 years is affected by Alzheimer disease (AD). Owing to the low accuracy and or expensive neuroimaging and neuropsychological investigations, early diagnosis of AD is still confusing. CNTs are becoming a promising nano-engineered technology for biomedical applications (Dugan *et al*., 1997; Vorobyov *et al*., 2015; Wang *et al*., 2014). Kim *et al*. (2020) reported that clinically precise and ultra-sensitive detection of several AD main biomarkers like Aβ40, Aβ42, p-tau181, and t-tau can be done using densely linearly associated CNTs in the blood plasma of human beings. Low coefficient of variation, close to <6%, femtomolar-level limit of detection, and a high degree of recovery of more than 93.0% were obtained in a study with meticulously packed and unidirectional CNT sensor arrangement that demonstrated high precision, sensitivity, and accuracy. Evidence for the treatment of AD was obtained by coating berberine-loaded MWCNTs (multi-walled CNTs) with phospholipids and polysorbate. The produced optimum formulation had a particle size of 186 nm exhibiting 68.6% absorption of the drug along with 96% release of the drug within a span of 16 hours (Kim *et al*., 2020). The study revealed that from day 18 to day 20, specially coated MWCNTs showed tremendous improvement in the memory performance of the subjects by reducing amyloid-induced AD. This was established further by standard biochemical markers in brain soft tissue. Neurite outgrowth for therapeutic application in nerve regeneration was promoted when Li *et al*. (2017) created a neural scaffold based on uniformly dispersed MWCN-hydrogel nanowires with tunable structural porosity. The newly fabricated MWCNTs with a noticeable electrical spur showed potential efficacy in promoting neurite extension for nerve regeneration, according to the findings of Guo *et al*. (2017).

It is a well-known fact that autophagy is a manner by which a cell in its cytoplasm disrupts and destroys aged, broken, or atypical proteins and other substances. The metabolized products are then reprocessed for vital cell functions, specifically, during times of stress or starvation. It was for the first time that Xue *et al*. (2014) could establish that autophagy is noticeably weakened in primary glia from CRND8 mice and set that autophagy dysfunction and autophagic substrate clearance are upturned by SWNT with the possibility of improving autophagy in AD and suggested an innovative approach to reinstating normal autophagy activity when the lysosomal function is diminished. Another report in Alzheimer's News Today by Forest Ray in 2020 claimed that blood tests for Alzheimer's could be possible with a CNT sensor. This report revealed that surface-modified nanotubes were able to detect microscopic concentrations of Alzheimer's main protein biomarkers in blood plasma, thereby discriminating Alzheimer's patients from a healthy population with usual accuracy of 88.6%. Another similar work done by Kim *et al*. (2020) reported in Nature Communications, could identify multiple AD core biomarkers, t-tau/$A\beta_{42}$, p-tau_{181}/$A\beta_{42}$, and $A\beta_{42}$/$A\beta_{40}$ in blood plasma quantitatively for AD patients. In this study, densely arrayed CNT sensors could selectively segregate Alzheimer's patients from normal individuals with a usual sensitivity of around 90.0% and a selectivity of 90.0%. Some time back, Zhang *et al*. (2017) functionalized CNT fibers with tunable defects to act as micro-sensor for the quantitative detection of ascorbic acid levels in the brain of AD-induced rats. In this study, the measurement of oxidation of ascorbic acid served as an indirect way to establish high sensitivity and high selectivity for possible causes of interference in the brain, in studying the brain activity of AD-induced rats (Zhang *et al*., 2017). Interestingly, a thorough study by Yang *et al*. (2010) demonstrated that CNTs can pass through tight BBB for acetylcholine delivery into the brain cells of mice for AD treatment. While a safe dose of CNTs (single-walled) was 12 mg/kg, a mere 5 mg/kg Acetylcholine-loaded SWCNTs enhanced the memory and learning proficiency of the AD-induced mouse model (Yang *et al*., 2010). This study affirmed that Acetylcholine could be transported into the neuron of mice via CNT as a carrier.

CNTS TO TREAT PARKINSON'S DISORDER

Neurodegenerative illnesses of the CNS, such as Parkinson's disease in addition to AD, have received a lot of attention in recent years as a major cause of morbidity across the world. Pathognomonic indications of Parkinson's disease are the result of the death of dopaminergic cells in the substantia nigra, and hence the disparity between the cholinergic and dopaminergic systems (Stephenson *et al*., 2018). Chiefly, bradykinesia, tremor, stiffness, and postural instability are the four cardinal motor symptoms of Parkinson's disease. It is a fact that conventional drugs for handling Parkinson's disease have considerably curtailed the intensity of such symptoms thereby improving the quality of life of patients. Additionally, other medications like amantadine, biperiden and dopamine replacement therapy have been the origin of the treatment for a long. These drugs can help to reduce the progression of Parkinson's disease, but stop it (Barka *et al*., 2013). Ceasing the administration of the right medication, instead, will result in a return of signs with augmented intensity. More diversified symptoms like cognitive impairment, psychiatric problems, and autonomic dysfunction occur as the illness advances, suggesting the presence of a more widespread underlying pathophysiology, demanding the expansion of state-of-the-art therapies (Dawson *et al*., 2018). Additionally, superparamagnetic iron oxide nanoparticles, gold nanoparticles, quantum dots (QDs), nanotube derivatives, and grapheme (More *et al*., 2015) have been identified as agents that can impact the amyloid formation process in various ways. Very recently, Alimohammadi *et al*. (2021) doped nanotubes with phosphorus-forming P-CNT that could prevent the α-synuclein amyloid formation (the chief cause of Parkinson's disease). Remarkably, this study established that phosphorus-doped CNT prevented α-synuclein amyloid formation effectively hinting that this could be a possible treatment for Parkinson's disease (Alimuhammadi *et al*., 2021).

CNTS AS IMAGING TOOL AND DRUG DELIVERY VEHICLES IN THE CENTRAL NERVOUS SYSTEM FOR DETECTION OF ISCHEMIC STROKE

CNT is coming up as a handy tool in tissue imaging for locating sick sites and delivering drugs to the site of action (He and Dai, 2004; Kostarelos *et al*., 2009). CNTs let imaging of the whole thick tissue and they improve visibility (Heller *et al*., 2006). CNTs are easily detectable due to their bulky resonance and increased Raman scattering characteristics (Heller *et al*., 2005). Position emission tomography, computer tomography, and magnetic resonance imaging are three imaging techniques that have helped researchers better understand how neural circuits work. Imaging is an important technique for studying both, biochemical and physiological activity in the CNS, particularly in the spinal cord and brain. Advances in technology have enhanced our acceptance of the effects of the cellular injury on the CNS. In modern days, the accuracy of neurological therapies has improved, as has the degree of invasiveness to the CNS (Nunes *et al*., 2012). Electroencephalography and magnetoencephalography are two modern CNS imaging methods. Conductors are put on the skull bone to measure electrical impulses emanating from the brain in these approaches. The skull is surgically opened to get access to the brain so that electrodes may be inserted to measure brain impulses directly. This procedure is quite intrusive, necessitating the use of a noninvasive alternative. Small interfering RNA (siRNA)-modified CNT was injected cortically into an endothelin-1-induced stroke rat model in a study. It was observed that the perceptive ability of stroke in rats increased markedly (Khuloud, 2015).

When combined with fluorescein probe-functionalized CNTs, established medicines like methotrexate have been shown to increase visibility in the body during its functioning. Figure 2 illustrates a schematic representation of the fact that when CNTs are further modified, they can reach the remote cells in the brain crossing the tight barrier, and thus this device can further be modified to be used as an imaging tool for determining the location of an ischemic stroke and treating it when tagged with suitable biomarkers. Because SWCNTs have better photostability than QDs and fluorophores, they can accomplish a longer excitation duration at a higher laser intensity (Vidu *et al*., 2014). This permits the perceptibility of the impervious tissue in the range of 700–1,400 nm. Regardless of certain merits, this technique renders a few demerits as well, such as limited sensitivity, difficulty to penetrate the BBB, and a shorter $t_{1/2}$ (half-life) after intravenous delivery (Nunes *et al*., 2012). To aid in visualizing the sick spot within a tissue, miniaturized video cameras can be encapsulated in CNTs as well and delivered orally (Beg *et al*., 2011). In a limited 1.3–1.4 m zone, Hong and the team pioneered that non-invasive brain imaging could be made possible with CNT alignment (named the NIR-IIa region). In an epifluorescence imaging mode, this method permits penetration through the intact scalp and skull, resolving cerebral vasculatures with a previously unreachable spatial resolution of sub-10 micrometers at a depth of >2 mm beyond the surface of the scalp skin. Furthermore, high temporal resolution (200 ms/frame) dynamic NIR-IIa cerebrovascular imaging was employed to indicate significantly decreased blood flow (Hong *et al*., 2014). Thus, CNT has the potential to be employed as a non-invasive imaging technique in the treatment of neurological diseases.

Recently, stereotactic surgery is emerging as an invasive process to transfer medicines and other physiologically relevant substances into the brain within therapeutic settings. This approach provides the path for direct access to a specific brain region of interest (Jacob and Hanein, 2008; Jacobs *et al*., 2014). Delivery of nanodrug for neuroprotection in chronic neurological illnesses, such as ischemic stroke, is of high significance following this mode of drug administration. This indicated that the use of nanotechnology to deliver medications over the BBB is an upcoming possibility (Barker and TRANSEURO Consortium, 2019; Sharma *et al*., 2013). The ability of nano-technological developments for neurotrophin delivery systems to trigger neurotrophin signaling for neuro-protection and neuro-regeneration brings new hope to the medical fraternity (Tan *et al*., 2012). Established evidences reveal that CNTs can carry neurotrophins to their sites of action, which are critical for the formation and function of neurons (Bardi *et al*., 2013; Lin *et al*., 2009).

A decade afore, a CNT drug delivery system was employed (Iverson *et al*., 2013) to improve CpG oligo-deoxynucleotide (short single-stranded synthetic DNA molecules that contain a cytosine triphosphate deoxynucleotide) immunotherapy in the

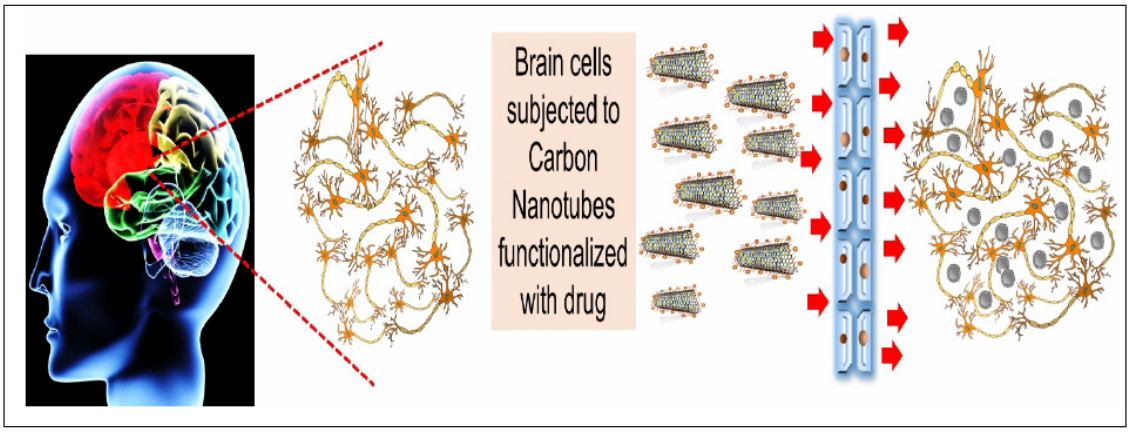

Figure 2. Brain cells when exposed to surface modified CNTs, they can deliver the drug molecules, imaging elements or antibodies at the respective sites in the brain, crossing the tight junction.

treatment of glioblastoma. Polyethylene glycol (PEG) was used to functionalize SWCNTs, which was then conjugated with a CpG oligonucleotide. Toll-like receptors (TLR) family members are intracellular receptors that identify carbohydrates, lipids, peptides produced by microbes, and nucleic acid structures. SWCNTs were conjugated with CpG. The study delineated that there was an augmentation of the acceptance of CpG in intracranial gliomas, *in vivo*. CpG inspired TLR in the glial cells to impede tumor development in glioma models (Zhau *et al*., 2011).

CNT-based neurotherapy might be particularly beneficial in the treatment of a variety of neurological disorders, as well as ischemic stroke. SWNCNT decorated with amine groups through amidation reaction improves neuronal ischemia damage tolerance. Neurons are preserved from damage and their functions are restored without the use of therapeutics when amine-modified SWCNTs are used (Lee *et al*., 2011). In a mouse model with induced endothelin-1 stroke, Al-Jamal and coworkers established the efficiency of amine-MWCNTs in delivering small interfering RNA (siRNA) that reduced apoptosis at the wounded site and encouraged retrieval of functioning motor neurons (Al-Jamal *et al*., 2011).

CNT-BASED TUMOR-TARGETED DELIVERY SYSTEMS

Several studies have stated that surface-modified CNTs can navigate through cell membranes passively whereas, many such movements are done actively like phagocytosis or endocytosis (Cauduro *et al*., 2017; Li *et al*., 2017). Functionalization of CNTs with suitable ligands apart from single or multiple active agent(s) and or biomarkers enables these carriers to locate and get hooked to tumors or cancerous cells effectively (Kaur *et al*., 2017; Yang *et al*., 2017). (Lee *et al* 2011), established that the high superficial energy of the positively charged SWNTs provided a favorable environment. This, in other words, is a source for neuronal regeneration, wherein the neuroprotective effect was realized due to a reduction in apoptosis, inflammation, and glia activation (Lee *et al*., 2011). In another recent report, improved cell penetration in addition to cellular uptake of CNTs than free mangiferin allowed 55% apoptosis for conjugated mangiferin on a CNT-PEG platform when tested against 21% for plain mangiferin against the U-87 cells (Harsha *et al*., 2019).

The BBB is extremely selective, allowing only a few compounds in the bloodstream to enter the CNS (Guo *et al*., 2017; Lee *et al*., 2017). The existence of the BBB makes it difficult to transfer medications to the brain for the management of tumors and other neurological illnesses, such as stroke. It hinders therapeutic molecule distribution into the CNS, resulting in fewer than 1% of the supplied medicine being delivered to the CNS by intravenous injection (Bjartmarz and Rehncrona, 2017; Zhang *et al*., 2017). This careful selection of chemicals that enter the brain is critical for maintaining CNS homeostasis. It also protects the CNS against external invaders such as poisons, viruses, bacteria, and other undesired substances (Barka *et al*., 2019; Dawson *et al*., 2018; Ji *et al*., 2010; Zhang *et al*., 2017). In the future, neuroprotection might be accomplished in chronic neurological illnesses by using nano-drug delivery.

In both the CNS and the PNS, neurotrophins are required for the growth and activity of neurons. CNTs can be used to transport them into the brain. The usage of CNTs as a delivery tool for handling CNS pathology is established on structural features such as improved solubility in biological solvents due to surface modification, large surface area, capability to get easily adapted with drug molecules, and biocompatibility with neural systems (He and Dai, 2004). Soligo *et al*. (2021) confirmed that modification of MWCNTs leading to electro-conductivity could employ neuroprotectivity via variation of a prime neurotrophic agent and improve neurodegeneration-related gliosis.

Due to the extreme conditions laid by the BBB while reaching the brain cells, there are hardly a few FDA-approved CNT-mediated therapeutic agents for serious brain-related diseases like Parkinson's Disease and AD (Pardridge, 2020). Interestingly, there are almost 10 smart drugs like Durvalumab, Ponatinib, Epitinib succinate, Bavituximab, Temozolomide with procarbazine and cilengitide, and many more that are approved by USFDA and are under various stages of clinical trials. Although there are multiple challenges like the adaptation of the formulation by large companies for commercial production, manufacturing cost, no consistency of data regarding toxicity tests, intra- and inter-batch variation, variation in *in vivo–in vitro* co-relationship and many more common men is eagerly waiting to adapt these extremely sophisticated and smart drugs once they are in the market (Kumar *et al*., 2021).

PHOTO THERMAL BRAIN TUMOR THERAPY

CNTs bear a unique property to absorb near-infrared (NIR) radiation. This property was utilized by Santos *et al*. (2014) to convert it to heat, thereby leading to photo-thermal-related brain tumor treatment. The team delivered a report and convinced many that a permutation of intra-tumoral SWNT insertion and NIR radiation in athymic glioblastoma-bearing mice not only diminished tumor growth relative to SWNTs but also suppressed tumor recurrence for up to 80 days (Santos *et al*., 2014). The anticancer activity of polyvinylpyrrolidone-coated (PVP-G) and SWCNTs were studied by Markovic *et al*. (2011). The study revealed that PVP-G had better photo-thermic sensitivity and imparted apoptotic and necrotic death *in vitro* by means of caspase activation/DNA fragmentation and cell membrane damage via partial thromboplastin time test (PTT) in U251 glioma cells (Markovic *et al*., 2011).

TOXICITY

It is a well-known fact that BBB offers restricted access to any particle that seems foreign material to get access to the brain. While finding an alternative to passing through this tight junction, contact with CNTs interrupts this delicate equilibrium resulting in cytotoxicity (Costa *et al*., 2004). So far, there have been no reports that consider brain toxicity to CNT being administered smartly. Interestingly, there are indications that approved CNTs are biodegraded inside human brain cells by human (myeloperoxidase) MPO and hydrogen peroxide (H_2O_2) present therein. In a study related to this fact, Kagan *et al*. (2010) confirmed via Raman spectroscopy that MPO stimulated SWNT to decompose into simpler substances via a pathway that supports the production of spontaneous hypochlorite that oxidizes parts of the CNT wall structure. A serious observation of this study was that PEG used as coating material (to amplify *in vivo* bioavailability)

did not impede this process (Bhattacharya *et al*., 2014; Kagan *et al*., 2010).

CONCLUSION AND PROSPECTS

So far, there is enough evidence that CNTs have prospective use as medication delivery vehicles to targeted brain sites. Although functionalized CNTs bring a ray of hope in the smart delivery and access to the brain crossing the BBB, there is at present no conformity about the related animal models that could be utilized to consider the short- and long-term impact of CNT exposure to biological cells and tissues. Hence, it becomes vital that distinct procedures and guidelines can be provided by the right authority, so that the results and their understanding as well as analysis remain unaltered by dissimilarity in testing methods.

AUTHOR'S CONTRIBUTION

Rajkumar Ghosh and *Jagabandhu Bag* drafted the manuscript. *Aparna Datta* conceptualized and finally gave approval to the work. The figures were also contributed by her. *Arup Pramanick* contributed to the necessary corrections. *Isa Hassan Abubakar* made the final revision of the manuscript. The final draft was checked and agreed upon by all the authors.

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CONFLICT OF INTEREST

The authors declared no conflicts of interest.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

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