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APPLICATION OF CARBON NANOTUBES FOR CONTROLLED RELEASE OF GROWTH FACTORS OR ENDOCANNABINOIDS: A BREAKTHROUGH IN BIOMEDICINE

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Carbon nanotubes (CNTs), the nanostructures with immense potential in various scientific fields such as the regenerative medicine, have emerged as innovative nanosreservoirs with multimodal functionality and application in theranostic settings. The superior mechanical properties, high thermoelectrical conductivities, or improved solubility and biocompatibility have made CNTs as suitable candidates for biosensing, high-resolution imaging, tissue engineering, and delivery of a variety of compounds with poor solubility or short half-life. These advanced nanovectors which promote neuronal growth and functional connectivity, have shown great theranostic potential in the central nervous system disorders. Several pioneering works have shown the ability of CNTs for controlled release of drugs or growth factors into the brain. Over the last decade, the neurotrophic and metabotrophic effects of nerve growth factor, brain-derived neurotrophic factor and endocannabinoid system and their involvement in the mechanism of action of a wide variety of drugs have been the focus of intense research. In order to overcome the rapid degradation and/or non-specific distribution of nerve growth factor or endocannabinoids, conjugation with CNTs has led to the prolonged effects of these modulating factors. Based on their unique properties, the appropriate application of functionalized CNTs may indeed revolutionize the current biomedical interventions that has been highlighted in the present review. ***Biomed Rev*** **2016; 27: 41-49**

Key words: nanotechnology, advanced nanovectors, theranostic settings

INTRODUCTION

Treatment of neurological disorders has remained as one of the most challenging areas of medicine. Traumatic injuries to the central nervous system (CNS) including the spinal cord injury (SCI) and traumatic brain injury (TBI) may lead to the progressive or irreversible damages. The currently available drugs do not reverse the condition and the neurosurgical approaches are usually associated with various complications and remarkable costs. Furthermore, application of the artificial transplants or transplanted organs necessitates the lifelong immunosuppression (1). During the last few decades, nanotechnology has emerged as a rapidly developing interdis-

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ciplinary approach with a vast discovery area in the basic or clinical research. Indeed, application of this highly advanced technology has expanded our knowledge about the molecular mechanisms of the neurological disorders. The highly efficient nanotech-based vehicles have been designed to transport a wide variety of molecules across the blood-brain barrier (BBB) (2) which prevents the entrance of large molecules or hydrophilic drugs into the brain (3). Among the precisely engineered nanomaterials, carbon nanotubes (CNTs) have emerged as the innovative nanostructures with multimodal functionality and application in theranostic settings. CNTs with high surface reactivity, electrothermal conductivity, biocompatibility, and intrinsic diagnostic capability have been successfully used for biosensing, high-resolution and non-invasive imaging, tissue engineering, and efficient delivery of drugs or biomolecules (4-6). Moreover, CNT-neurotrophin conjugates are able to promote neurite outgrowth and connectivity of neuronal networks (3, 5).

In recent years, neurotrophic and metabotrophic effects of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and endocannabinoid system (eCBs) and their involvement in the pathogenesis and therapy of neurodegenerative and cardiometabolic diseases have been the focus of intense research (7-13). Meanwhile, rapid degradation, poor solubility, or non-specific distribution of NGF or endocannabinoids (14, 15) may negatively affect their effectiveness that necessitates the development of suitable carriers to provide longer lasting effects for these modulators. In this respect, application of CNTs has shown promising results that will be discussed herein.

CARBON NANOTUBES: THE GENERAL ASPECTS

The strongest materials yet discovered, CNTs are categorized as single- and multi-walled CNTs (SWCNTs and MWCNTs, respectively). Unlike their tiny diameters, the length of CNTs may be extended to hundreds of micrometers. Such an extraordinary length-to-diameter ratio may result in a precise detection of biological or chemical compounds and high drug loading (16). Because of their small size, CNTs may be easily distributed and react with living cells (17). Due to the highly reactive surface, CNTs bind to a wide variety of molecules including the drugs, genes, proteins, and markers (18) that might be of great theranostic significance. In the neuronal tissue or muscles, CNTs have been used as stimulants or sensors (19). CNTs may also be used as the glucose sensors because of their ability to control far-infrared luminescence (20) and suppress the bacterial proliferation (21) that might be useful in infectious diseases. At initial stages in cancers, CNTs are able to detect the expression of biomarkers and inhibit tumour growth by enhancement of permeability and retention of anticancer agent (22). CNT composites with high drug encapsulation efficiency have been used for prolonged drug release (23). Interestingly, real-time monitoring of drug delivery is possible due to the intrinsic spectroscopic properties of CNTs (24).

Regarding the potential toxicity of CNTs, inflammatory reactions may occur in the case of structural defects of CNTs. Meanwhile, these reactions are usually mild and short-lasting as CNTs are rapidly eliminated from the body due to their small size (25). In general, the efficiency and adverse effects of CNTs, like other nanomaterials, depend on their mode of application. In a 2-year bioassay, MWCNTs have shown no carcinogenicity (26). Regarding the cortical neurons, pluronic-coated CNTs not only did not induce degeneration *in vitro* or *in vivo*, but also reduced pluronic toxicity (27). In the clinical settings, carbon-based biomaterials have been used for a long time without serious adverse reaction (28). In general, surface modification of CNTs make them more soluble, bioactive, and biocompatible leading to the reduced cytotoxicity of these nanostructures (29).

APPLICATION OF CARBON NANOTUBES IN REGENERATIVE MEDICINE: THE FOCUS ON NEUROLOGICAL DISORDERS

The limited efficacy of the current treatment strategies against the neurodegenerative disorders may adversely affect patient's compliance and quality of life. For instance, silicon implants in the cerebral cortex or neuroprosthetics may be surrounded by astroglial scars leading to a significantly reduced efficiency of electrical stimulation (30). Furthermore, polymer-based nerve growth conduits may be associated with limitations due to their low biocompatibility or unfavourable physicochemical properties (31). These problems have evoked tremendous efforts towards the development of novel therapeutic approaches to improve neural repair or motor functional recovery. In this respect, application of CNTs in regenerative medicine has attracted a growing interest. Development of the CNT-based devices has led to the regeneration of damaged neurons or facilitation of drug delivery across the BBB. Because of their high biocompatiblility and electroconductivity, CNT-based scaffolds are able to induce the regeneration of Schwann cells, differentiation of embryonic stem cells into the neurons, and improve neuronal performance (32- 34). In this context, interfacing the hippocampal neurons with CNTs has led to

the strong potentiation of spontaneous synaptic activity. Furthermore, culturing of cortical neurons on CNT clusters resulted in the formation of a well-organized neural network (35) suggesting the suitability of CNTs for neural applications. Interestingly, the interaction between neurons and CNTs may lead to the activation of intracellular signalling cascades. For instance, CNTs-induced neurite outgrowth is associated with the activation of extracellular signal-regulated kinase and phospholipase C signalling pathways, respectively (36).

The ability of CNTs to modulate the electrochemical events in the neural networks has represented them as the attractive nanodevices for neuromodualtion. In Alzheimer's disease which is the most common cause of severe memory problems in the elderly, the currently available treatments have shown limited efficacy. Because of the cholinergic deficit, the suitable treatment options should increase the cholinergic neurotransmission (37). Since acetylcholine has a short half-life and does not readily cross the BBB (37), CNTs may be used as the carriers of acetylcholine. In an experimental model of Alzheimer's disease, acetylcholine-loaded SWCNTs have restored the cognitive function, while, free acetylcholine showed no effect (38). MWCNTs have been used as the high-resolution probes for visualizing the amyloid-β (Aβ) fibrils (39). Furthermore, CNT-based biosensors provide the possibility for real-time detection of Aβ in human serum with lower limit of detection as compared to the enzyme-linked immune sorbent assay (40).

In Parkinson's disease, one of the most common neurodegenerative disorders worldwide, the currently available drugs alleviate the symptoms but do not affect disease progression (41). Furthermore, infusion pumps or skin patches may be associated with various complications (42). Since the conventional techniques for deep brain stimulation (DBS) are usually associated with multiple limitations such as high electrical current needs, large size of electrodes, and lack of feedback monitoring of brain electrical activity (43), CNTs might be attractive nanomaterials for DBS due to their prolonged stability and ability for real time neuromonitoring, neuromodulation, and large charge storage (44).

In multiple sclerosis (MS), a debilitating autoimmune disease of CNS which is associated with myelin degradation and cognitive impairment, disease modifying agents including the immunosuppressants, monoclonal antibodies, or high-dose corticosteroids do not stop the disease process (45). Because of their ability to record or stimulate the neural activity, CNTs might be promising theranostic candidates in MS. In this respect, CNT-neuron hybrid networks have been shown to improve the neuronal network connectivity and performance (46). In epilepsy, one of the most prevalent neurological disorders which is associated with abnormal electrical activity within the brain, the conventional antiepileptic drugs are usually associated with various side effects and do not effectively reduce the seizure severity or frequency (47). CNTs due to their unique properties have been represented as suitable carriers for drug delivery into the brain (48).

Following SCI, permanent paralysis occurs because of the inability of axons to regenerate across the lesion. Application of neuroprotective agents or stem cell therapy does not usually result in a functional repair (49, 50), while, post-SCI administration of SWNT-polyethylene glycol has promoted tissue repair in the experimental SCI (51). In brain injuries, transplantation of neural stem cells into the injured region may be associated with multiple limitations because of their low survival rate (52). CNTs as biocompatible substrates or scaffolds by promoting the differentiation and maturity of neural stem cells provide improved function of damaged nerve tissues (53). Application of CNTs have been shown to reduce the levels of inflammatory markers and promote the recovery from stroke (54, 55). Moreover, CNT-mediated siRNA delivery and gene silencing of neuronal tissues has successfully promoted functional motor recovery from brain ischemic insult (56).

CONJUGATION OF CARBON NANOTUBES WITH GROWTH FACTORS OR ENDOCANNABINOIDS: IMPROVED TREATMENT OUTCOMES

Growth factors, the endogenous polypeptides which regulate the cellular proliferation, migration and differentiation, have been presented as leading therapeutic candidates in neural tissue engineering (57). Because of the rapid degradation or non-specific distribution after systemic administration, the outcome of growth factor-based therapies largely depends on their delivery mode. In this respect, advanced growth factor delivery systems have been designed among which the functionalized CNTs proved to be particularly promising for controlled release of growth factors (58). For targeted killing of cancer cells, epidermal growth factor-directed CNT-drug conjugates have shown efficiency both *in vitro* and *in vivo* (59).

Carbon nanotubes loaded with GDNF, a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons, increase the integration of transplanted embryonic dopaminergic neurons into the striatum and promote neuronal survival (60). Indeed, the promotion of neurite outgrowth and synaptogenesis by neurotrophin-coated CNTs has attracted a

growing interest (61). The prototypic member of the neurotrophin family, nerve growth factor (NGF), which plays a regulatory role in the survival, differentiation, and maintenance of the functions of specific populations of neurons and mediates the therapeutic effects of a wide variety of CNS drugs (8-13), is trophic for the cholinergic neurons which are critically involved in the cognitive processes (62). NGF has also shown therapeutic potential in the neurological disorders such as SCI and Alzheimer's disease (63, 64). In dorsal root ganglia or PC12 cells, CNTs-NGF complex is able to promote the neuronal outgrowth (65, 66). In an *in vitro* model of ischemic stroke, aminated MWCNTs have been presented as efficient nanocarriers for NGF which provide a sustained concentration and longer lasting effects for this neurotrophin (67) that might be of therapeutic significance against the disorders associated with NGF deficiency. In the latter study, MWCNTs-NGF complex dose-dependently attenuated the oxidative stress via the reduction of MDA; a marker of lipid peroxidation, tissue injury, and free radical generation (68), and elevation of the activities of antioxidant enzymes including the SOD; an enzyme which is implicated in the cell protection against the oxidative damage, and CAT; the scavenger of hydrogen peroxide which is a cell-permeable oxidizing agent (69). Since the neurotrophic factors promote the expression of antioxidant proteins and the loss of neurotrophic support may lead to the development of various disorders in the central or peripheral nervous system (14, 57), therefore, prolonged suppression of oxidative stress by CNTs-NGF complex might be of great therapeutic value against the neurological disorders.

Based on the protective effects of NGF against the cerebral insults and forebrain ischemia (70), the ability of CNTs to provide a sustained concentration and longer lasting effects for NGF may be beneficial against the cellular dysfunction due to the acute or chronic form of neural injury. Interestingly, both NGF and CNTs modulate the synaptic plasticity (5, 8-13, 27), therefore, application of NGF-CNTs complex appears as a promising treatment option against the disorders which are associated with abnormal synaptic plasticity.

According to a recently published report, functionalized CNTs prolong the regulatory action of NGF on the endocannabinoid system (eCBs) (71). It has been shown that CNTs-NGF complex induces a long-lasting enhancement of brain 2-arachidonoylglycerol (2-AG) content indicating the efficiency of this nanostructure to provide a sustained concentration of NGF. Furthermore, the implication of 2-AG in the mechanism of action of NGF has been demonstrated (71) that might be of great therapeutic significance in the neurological disorders. Indeed, identification of the eCBs (a group of neuromodulatory lipids and their receptors) which is implicated in a wide variety of physiological and pathological processes (72), has provided new insights into the mechanisms underlying the pathophysiology of various diseases that may result in the development of novel treatment strategies. The endocannabinoids, anandamide and 2-AG, are produced on-demand from the membrane lipid precursors and release from postsynaptic neurons. They are ligands of two types of G protein-coupled receptors, cannabinoid CB_1 and CB_2, which are predominantly located in the central nervous system and immune cells, respectively (73). In the mammalian brain, CB_1 receptors are highly expressed in the areas which control emotional, cognitive, sensory and motor functions. Endocannabinoids by the activation of presynaptic CB_1 receptors act as retrograde synaptic messengers and inhibit the release of the excitatory and inhibitory neurotransmitters (74). Following different types of diseases, enhancement of the activity of eCBs may result in the therapeutic effects. In this sense, development of the cannabinoid receptor agonists, anandamide uptake blockers, or selective inhibitors of endocannabinoid degradation, has triggered increasing research efforts (75, 76). Based on the involvement of eCBs in the survival signaling pathways and neural plasticity and its modulatory effects on the neurodegenerative and neuroinflammatory processes (72-74), malfunctioning of the eCBs may contribute to the etiology of neurological disorders. In this context, pharmacological manipulation of this system might be of therapeutic significance in the neurological problems as there are reports suggesting the therapeutic potential of the eCBs in multiple sclerosis, stroke, Alzheimer's disease, spinal cord injury, and epilepsy (77-81).

In an *in vitro* model of stroke, anandamide-CNTs complex has shown sustained protective effects as compared to anandamide alone which its therapeutic potential may be negatively affected by its short half-life or poor solubility (82). Aminated CNTs have been represented as suitable carriers for anandamide which provide sustained concentration for this cannabinoid leading to the longer-lasting effects against the ischemic insult induced by oxygen-glucose deprivation. Anandamide-CNTs complex by suppressing the oxidative stress and increasing the cell viability has been suggested as a valuable therapeutic agent against the ischemic stroke or other neurodegenerative pathologies (82).

Moreover, conjugation of endocannabinoids with CNTs has led to the sustained therapeutic effects in gastrointestinal

disorders. In a rat model of colitis, 2-AG-CNTs complex has been designed in order to improve the pharmacological profile of 2-AG. This nanocomplex by providing a sustained concentration of 2-AG showed promising therapeutic effects through the anti-inflammatory and antioxidant mechanisms (83). Based on the high biocompatibility of CNTs and their ability for controlled drug delivery, anandamide-CNTs complex *via* the antioxidant mechanism has shown prolonged gastroprotective effects in an experimental model of gastric ulcer (unpublished data).

CONCLUSION

Implication of NGF in the molecular mechanisms of autoimmune and cardiometabolic disorders and a wide variety of psychotropic agents as well as its therapeutic potentials in neurological disorders have made this neurotrophin as an attractive candidate for theranostic settings. Meanwhile, NGF similar to other neurotrophic factors does not significantly penetrate the BBB and may be rapidly degraded. Therefore, the clinical significance of NGF depends on the development of suitable carrier systems which elevate the stability and retention of NGF in the target organ. Over the last decade, increasing research efforts have been attracted towards the eCBs which is the modulator of many cellular and physiological functions and neuroinflammatory or neurodegenerative processes. This ubiquitous signaling system may be a promising target for drug discovery particularly in disorders for which no effective therapeutic or prophylactic regimens are currently available. However, the poor solubility or short half-life may negatively affect the effectiveness of cannabinoids.

The outstanding breakthroughs in nanotechnology have provided the opportunities to develop more sophisticated delivery systems to improve the treatment outcomes. In this respect, CNTs have emerged as one of the most attractive candidates due to their outstanding properties including the biomechanical stability, capacity to integrate with neurons, re-establishment of synaptic connections, neuromonitoring, neuromodulation, and controlled drug delivery. These nanostructures have been represented as promising carriers for NGF or cannabinoids which provide longer lasting effects for these modulators that might be of great theranostic significance in a wide variety of pathological conditions including the neurological or gastrointestinal disorders. It appears that application of biofunctionalized CNTs will be considered as a major part of the next generation of therapeutic strategies in biomedicine.

Conflict of interest statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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