Conference Scene

New perspectives on nanoneuroscience, nanoneuropharmacology and nanoneurotoxicology

6th Annual Conference of The Global College of Neuroprotection and Neuroregeneration. Focus on Nanomedicine: Nanoneuropharmacology and Nanoneurotoxicity

Vienna, Austria, 1–4 March 2009

The 6th GCNN Annual meeting was held in the Hilton Hotel, Vienna, Austria, on 1–4 March, 2009. In this conference a special focus was given to 'Nanomedicine: Nanoneuropharmacology and Nanoneurotoxicity' on 2–3 March, 2009. The 'Nanomedicine Focus' was organized by M Robertson (London, UK) and H Shanker Sharma (Uppsala, Sweden) and was divided into three sessions on 2 March, followed by one session on 3 March. In total, 11 speakers participated in these sessions covering more than 5 h of intensive presentations intermingled with lively discussions from the audience.

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Recent advancements in nanoscience resulted in a new focus on the role of nanoparticles (NPs) in the CNS [1,2]. NPs have been used in the past to deliver drugs at the target site into the brain [1–3]. However, NP-induced neurotoxicity is still a new subject that requires suitable attention among neuroscientists [4]. Thus, the concept of nanoneuroscience is fast emerging as a new subject to tackle all NP-related effects on the CNS [3]. Keeping these views in mind, a session on nanomedicine with a special emphasis on nanoneuroscience, nanoneuropharmacology and nanoneurotoxicity was developed in this conference series [4]. The nanomedicine session was attended by medical researchers, students, representatives from industry, scientific publishers, as well as observers from the EU consortium on Nanosciences, Brussels, Belgium and the European Office of Aerospace Research and Development (EOARD), London, UK. Local and international media and the national TV channels and newspapers of Austria, Romania and Sweden also covered this event.

The first session on nanomedicine was chaired by H Shanker Sharma and M Robertson, in which three speakers presented their novel results. In an introductory remark, Robertson presented details about the journal *Nanomedicine* and discussed the need to strengthen this newly developing subject for the betterment of human healthcare.

The first speaker was J Räty from Amsterdam, The Netherlands, who presented his findings on the use of nanomedicine in gene therapy from trials to products [5]. As gene therapy is matured from clinical trials to the first commercial products, understanding the mechanisms of gene delivery requires immediate attention. In this context, our understanding on the development of viral vectors, and the enhancement of their transduction efficiency, biodistribution and viral safety are the key issues. Thus, researchers are encouraged to identify viral biodistribution using noninvasive imaging modalities such as MRI, SPECT and PET in relation to gene therapy. These clinically relevant imaging systems could monitor viral transgene expression, as well as viral particle biodistribution. The author highlighted the use of gene therapy products that are currently available or near market approval based on p53 expression (Gendicine™ and Advexin™), conditionally replicative adenoviruses (Oncorine™) and thymidine kinase plus ganciclovir therapy (Cerepro®), for enhanced healthcare.

G Tosi from Modena, Italy, described the use of engineered polylactide-co-glycolide (PLGA) NPs as drug-delivery tools for the CNS using animal models. The major problem in nanodrug delivery to the CNS is the presence of the blood–brain barrier (BBB) [6,7]. Recently, the use of NPs made of PLGA, and modified with a simil-opioid sequence and different glycosidic moieties to reach the CNS by crossing the BBB has been demonstrated [8]. Using modified PLGA with different glycol-heptapetides (glucose, lactose, xylose and mannose as sugar moieties with a single [P] or a triple

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sequence of heptapeptides [3P]) the author showed that intravenously administered PLGA NPs labeled with a fluorescent dye could cross the BBB, as seen using confocal microscopy. However, nanodelivered analgesics using a PLGA method produced different effects with regard to the intensity and duration, depending on their surface modification. Thus, when 3P-PLGA NPs were used, a sudden maximum analgesic effect was seen, followed by a fast decrease over time when compared with other modifications. This indicates that nanodelivery depends on the surface properties of the NP.

Sharma examined the effects of chronic NP exposure on spinal cord trauma-induced pathophysiology in a rat model. As the influence of NPs on the pathophysiology of spinal cord injury (SCI) is not well known, the author examined the effects of silica dust (SiO_2) and copper (Cu) NPs on acute cord pathology. SiO_2 NPs, or silica dust, and Cu NPs from gunpowder could be easily inhaled from the environment by soldiers engaged in the Gulf War and could influence their health and/or brain function [9]. The NPs could be transported to the brain via inhalation and may induce neurotoxicity themselves or modify the pathology following additional trauma or injury to the CNS [10]. Using an animal model, the author exposed rats to Cu (50–60 nm) or SiO_2 NPs (40–50 nm) by administering them intraperitoneally (50 mg/kg) once daily for 7 days (50 mg/kg). The NP-treated and saline-treated control rats were then subjected to SCI on the eighth day. In these animals, the blood–spinal cord barrier permeability, edema formation and neuronal damages were examined 5 h after injury. In addition, the functional outcome following SCI was also compared between NP- and saline-treated rats. The results show that untraumatized rats treated with Cu or SiO_2 NPs for 7 days did not exhibit any significant alteration in behavior on rotarod performances or on capacity angle tests compared with saline-treated animals. However, subjection of these NP-treated animals to SCI resulted in a profound deterioration in motor function compared with the control group. Furthermore, the magnitude of the blood–spinal cord barrier breakdown to Evans blue and radioiodine tracers following SCI in NP-treated animals was much more aggravated when compared with saline-treated traumatized rats. The edema formation and cord pathology was also exacerbated in NP-treated injured animals compared with the saline-treated control group. Pretreatment with SiO_2 induces a more profound cord pathology after SCI compared with Cu NP treatment. These novel observations suggest that exposure to NPs enhances the sensitivity of the spinal cord to injuries and the magnitude and severity of SCI depends on the type of NP used. Whether NP treatment will also alter the pharmacotherapeutic effects of neuroprotective agents in SCI is unknown and is currently being investigated in the author's laboratory.

The second session on this focal theme was chaired by RJ Andrews from NASA Ames, Moffett Field, CA, USA, and M Robertson. Andrews presented a very interesting talk on the use of nanomedicine in neuroprostheses. His talk entitled 'See no electricity, hear no electricity – but the brain speaks with electricity: sensory neuroprostheses and the neural-electrical interface' was highly appreciated by clinicians and basic researchers alike. In this keynote speech, Andrews emphasized new research on sensory neuroprostheses – cochlear or retinal implants that aim to optimize the neural–electrical interface – and discussed new strategies for enhancing neuromodulation/neuroregeneration.

Retinal prosthesis research includes tissue injury from undue charge transfer due to electrode size (smaller electrodes require smaller charge transfer), electrode composition (both material and coatings, which can affect impedance and capacitance), electrode to tissue distance and characteristics of the stimulating current (e.g., waveform, pulse width and frequency). Research on neuromodulation focuses on the use of penetrating arrays (for 3D contact), polymer coatings and carbon nanotube arrays (or carbon nanotube electrode coatings) that could greatly enhance safe charge transfer [11].

The author's research in this area shows that anatomical and/or surgical issues influence neuroprostheses development. Thus, in this emerging area, new strategies including tissue engineering and nanolevel techniques to improve neuroprostheses are needed. To this aim, the use of stem cells, neurotrophin-eluting

hydrogels and nanoscaffolds could be examined [12]. In addition, the possible benefit of using appropriate electromagnetic fields for sensory neuroprostheses will require more investigation.

GA Silva from University of California, San Diego, CA, USA, presented new approaches for imaging neural cells with functionalized quantum dots in animal models in relation to structure and function. For this purpose, semiconductor quantum dot nanocrystals were used that fluoresce at specific wavelengths suitable for biological imaging [13]. With appropriate chemical modifications, quantum dots can be conjugated to antibodies or peptides, which in turn can be utilized to specifically bind antibody–quantum dot complexes with antigens on and within the cells. Based on these principles, Silva discussed the use of quantum dot cellular imaging nanotechnology, which has clear advantages over the traditional organic fluoropore labeling and/or imaging. In addition, quantum dots are suitable for multiplexing several different specific colors for different epitopes in a single preparation. Silva also discussed in detail the optimization of quantum dot imaging protocols for neural cells, both *in vitro* and *in situ* in intact neural tissues. This will certainly help our understanding of neural cellular structure and function in *in vivo* situations.

A Campbell from Pomona, CA, USA, discussed neuroinflammation caused by the inhalation of particulate matter in animal models. The etiology and progression of neurodegenerative disorders depends on the interactions between several factors (e.g., age, environmental exposure and genetic susceptibility factors) [14]. Campbell discussed the possible link between exposure to particulate matter and enhancement of CNS proinflammatory markers [15,16]. In a genetically susceptible mouse strain, exposure to ultra-fine particles seems to be more effective at promoting inflammatory events in the CNS. Similarly, using normal rats Campbell found a selective regional variation in neuroinflammatory response following their exposure to diesel engine exhaust. Thus, the levels of the proinflammatory cytokines (TNF-α and IL-1α) were enhanced only in the striatum of diesel-exposed rats compared with other brain regions. These observations suggest that particulate matter exposure induces inflammatory responses in the brain in a very specific and selective manner.

The third session on nanomedicine was chaired by Robertson and V Fraifeld, Beer-Sheva, Israel. V Parpura from University of Alabama, Birmingham, AL, USA, demonstrated the use of carbon nanotubes (CNTs) as a good modulator of neuronal growth in cell culture studies [17]. Neurons communicate with each other by forming synapses, thus extension of the growth cone of immature neurons could control the pattern of the synapse formation. In mature neurons, the growth cone plays an important role in neurite regeneration, particularly following injury. Parpura suggests that CNTs can be used as prosthetics devices in the process of neuronal regeneration after injury. Using these CNTs as a scaffold for neuronal growth, Parpura observed that neurons grown on positively charged multiwalled nanotubes exhibited numerous growth cones, longer neurite outgrowth and successful neurite branching compared with neurons grown on negatively charged CNTs. In addition, chemically functionalized water-soluble single-walled CNT graft copolymers were able to increase the length of various neuronal processes. Since single-walled CNT-poly(ethylene glycol) could block stimulated membrane endocytosis and influx of Ca^{2+} in neurons, an extended neurite length is quite possible.

The problems of drug transport across the BBB using NPs was discussed by J Kreuter of Goethe-Universität, Frankfurt, Germany. The BBB represents an insurmountable obstacle for drug delivery to the CNS [6,7]. To overcome this barrier, drug delivery to the brain is possible using NPs. Thus, several drugs (e.g., hexapeptide dalargin, the dipeptide kyotorphin, loperamide, tubocurarine and doxorubicin) and the NMDA receptor antagonists MRZ 2/576 and MTRZ 2/596 could be transported into the brain using intravenous injection of NPs, resulting in enhanced pharmacological effects. To achieve significant transport across the BBB, coating of the NPs with polysorbate 80 (Tween® 80) plays a key role [18]. Kreuter showed that 25–40% of rats with extremely aggressive glioblastoma could survive for 6 months if treated with intravenous injection of the polysorbate 80-coated

NP preparation. On the other hand, the untreated controls died within 10–20 days. It appears that the NPs entered into the brain tissue by endocytotic uptake from the brain capillary endothelial cells followed by release of the drugs in these cells or their diffusion into the brain by transcytosis. This hypothesis is further supported by the identification of albumin NPs in the brain using transmission-electron microscopy.

On the second day of the conference, the fourth session on nanomedicine was chaired by K Martinez from Copenhagen, Denmark, and K Jain from Basel, Switzerland.

S Gelperina from Moscow, Russia, discussed the toxicological aspects of NP-induced drug delivery [12,16]. Poly(butyl cyanoacrylate) NPs coated with polysorbate 80 can easily be delivered to the brain, whereas the antitumor antibiotic doxorubicin without NPs is unable to penetrate across the BBB. On the other hand, NP-bound doxorubicin reaches the brain in a concentration sufficient enough to considerably inhibit the growth of intracranially implanted glioblastoma in rats. However, the poly(butyl cyanoacrylate) NPs are a relatively safe material because it is a biodegradable low-molecular-weight polymer that is rapidly eliminated from the body. However, its toxicological effects should be examined in more detail for further development of the nanotherapy. The author's new findings show that binding of doxorubicin to the NPs did not enhance its neurotoxicity. Furthermore, the intravenous injection of empty NPs (up to 400 mg/kg) did not show adverse toxic effects. Thus, the lower toxicity of the NP formulation is most suitable for drug delivery using NPs.

Martinez presented new results on the use of nanowires (NWs) for biosensing in mammalian cells. To achieve this, a probe is directly placed in the interior of living cells. However, this approach presents several challenges due to the micron size of the probes and the complexity of the cytoplasm and the cell membrane [19].

Using nanotechnology, fabrication of 1D nanostructures of various materials that could penetrate the cell membrane without causing significant damage is made possible [1]. The small diameter of the NWs allows them to cross the lipid bilayer of the cell membrane without causing significant injury. This makes them attractive as a tool for gaining access to the intracellular environment. Moreover, a large number of NWs per cell can be controlled and can penetrate cells simply by depositing a cell suspension on top of the array.

The author showed interesting cross-section images of living cells with embedded NWs using scanning-electron micrographs depicting the interactions between NWs and the living cells.

The session was concluded by Jain who discussed an integrated approach to neuroprotection in traumatic brain injury (TBI). The enormous complexity of the pathomechanisms of TBI mean that an integrated approach to target various pathways to interrupt the chain of events that aggravate the initial impact of injury is required [20]. For example, blast injuries caused by roadside bombs exhibit a new pattern of TBI in Iraq and Afghanistan that have not been seen in any previous wars. In such situations, neuroregeneration for lost brain tissue is needed and should be integrated with neuroprotection. Thus, in addition to neuroprotective drugs, hyperbaric oxygen, molecular therapies, biomarkers and nanobiotechnology may be combined for management of TBI. The author suggested that nanofibers could be used in conjunction with stem cells for the repair of the damaged brain. Neural stem cells could be retrovirally transduced to produce NGF and transplanted into the injured brain for marked improvement of cognitive and sensory-motor functions. Active or passive immunization (vaccination) with CNS-associated self-antigens could also promote recovery from TBI. Thus, there is an urgent need to translate these techniques into effective therapies so that promising strategies for neuroprotection in TBI in human can be introduced into clinics.

Taken together, the thematic session on nanomedicine provided many new leads on the possible development of nano-drug delivery and also highlighted the problems of nanoneurotoxicity, especially in *in vivo* situations. It appears that when NPs enter into the body, the fluid environment will induce cellular and metabolic stress that could lead to adverse cell and tissue reaction. These factors must be considered when developing new biotechnology for nano-drug delivery for therapeutic or diagnostic purposes. It is hoped that new developments

in nanoneuropharmacology and nanoneurotoxicology will take center stage in the seventh GCNN meeting in Uppsala, Sweden, on 3–6 March, 2010. All interested persons in nanoneuroscience are most welcome to actively particulate in this event.

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