HIGHLIGHTS

Dendrimer nanocarriers drug action: perspective for neuronal pharmacology

General aspects: Currently, several strategies have been used to overcome common problems found in pharmacological therapies, such as insolubility, reduced bioavailability and low specificity of drugs. One of the most promising alternatives proposed is the use of dendrimeric drug nanocarriers, polymeric chemical structures of diverse nature that contain, transport and deliver the desired drug in biological systems. One of the dendrimers that has been most successfully used is based on polyamidoamine (PAMAM). PAMAM are organized from a central molecule of ethylenediamine that gives way to expansive growing layers (generations) terminating in a surface of primary amines that are positively charged at physiological pH. This architecture determines the presence of intramolecular cavities that allow the encapsulation of drugs and their release (**Figure 1**). Alternatively, drugs can also be associated to the dendrimer surface. Among the main advantages of PAMAM dendrimers is their high solubility, stability and efficient encapsulation of different drugs, in addition to its easily modifiable surface. This latter feature allows the linking of several chemical groups and molecules to the surface amino groups in order to improve their properties, such as surface charges, encapsulation capacity and drug delivery, ligand linkage to reach a specific target tissue, among other applications (Svenson, 2009). The versatility of the PAMAM dendrimers has demonstrated to be useful in studying the action of several drugs of high biomedical impact. Thus, anticancer, anti-inflammatory and antimicrobial agents, among others, have been tested with promising results (Svenson, 2009). However, the pharmaceutical use of such systems in neuropathology is a field that is yet to be explored.

Cell internalization: It is generally accepted that a major advantage for using dendrimers as carriers is their ability to enter the loaded drug into the cell. Thus, they can circumvent problems not only for insolubility or permeability of some drugs, but also allow their distribution to intracellular targets. Indeed, one of the main issues to investigate is their mechanism of action for entry into the cytoplasm (**Figure 2**). There is evidence suggesting that the composition, size and ionic charges of the dendrimers are relevant not only for the internalization mechanism induced, but also for the kinetics of endocytosis and for intracellular processing mechanisms of these polymers. Using specific inhibitors for clathrin and caveolin mediated endocytosis and micropinocytosis, it has been established that the composition of surface charges of PAMAM affects the internalization pathway. The evidence shows that anionic dendrimers are internalized by caveolin mediated process, while the cationic and neutral dendrimers appear to be taken up by a caveolin and clathrin independent process in A549 cells (Perumal et al., 2008). However, colocalization studies with specific endocytic pathway markers in HeLa cells show that the cationic dendrimers are internalized by clathrin mediated endocytosis and micropinocytosis (Albertazzi et al., 2010), which demonstrate that the process is dependent of the cellular type, too. The chemical versatility of PAMAM dendrimers allows the linkage of chemical groups to interact with plasma membrane proteins inducing their endocytosis in specific tissues or cells. Even when endocytosis appears to be the main mechanism of dendrimer internalization, it cannot be discarded that passive diffusion could have a role in that process. To precisely analyze all these points is of major importance in order to focus dendrimeric polymer studies on clinical pharmacology applications.

Cytotoxicity: One of the main aspects to be considered in relation to the use of nanotechnologies applied in biological problems are the toxicity levels. It has been widely recognized that the presence of positive charges, provided by amino groups on the surface of PAMAM dendrimers, means increased cytotoxicity levels, which is also determined by the polymer size and surface composition. On the contrary, it has been demonstrated that dendrimers containing only neutral or anionic surface groups are less toxic (Lee et al., 2005). In this regard, and like other nanopolymers, autophagy process would have a major role in the overall cellular response to such molecules (Wang et al., 2014). The properties of the plasma membrane of cells treated with PAMAM dendrimers could be affected. In electrophysiological experiments, it was determined that PAMAM G5 increased the influx of Na^+ in hippocampal neurons by a mechanism that needs to be studied further (Nyitrai et al., 2013b). Another important aspect related to this issue is the biodegradability of these polymers. Indeed, PAMAM dendrimers are hydrolytically degraded only under harsh conditions because of their stable amide backbone, and hydrolysis proceeds slowly at physiological conditions (Lee et al., 2005). However, there is no strong experimental evidence about the mechanisms by which nanoparticles are degraded and eliminated. Enzymatic activity cannot be discarded considering that amide bonds of PAMAM dendrimers are similar to the ones present in other biodegradable molecules like proteins. To overcome toxicity problems and improve the properties of dendrimers in general, it is possible to covalently modify their surface linking molecules to the amino groups. A common modification is the addition of polyethyleneglycol molecules (PEG) of different sizes with a demonstrated reduction in toxicity. Several research groups have addressed these issues making it clear the great potential of "pegylated" PAMAM in biomedical applications. Formulations based on pegylated PAMAM dendrimers with have shown an increased anticancer drug encapsulation and bioavailability with a reduction in cytotoxicity. These kinds of complexes were internalized by endocytic pathways, delivering the drug inside lysosomal vesicles with a demonstrated tumor accumulation (Zhu et al., 2010). Other chemical modifications have been used to decrease the cytotoxicity of dendrimers. For example, PAMAM with 4-carbomethoxy pyrrolidone surface groups (PAMAM-pyrrolidone) shows a significative decrease of cytotoxicity in Chinese hamster fibroblasts (B14), embryonic mouse hippocampal cells (mHippoE-18) and rat liver derived cells (BRL-3A). Moreover, this modification prevents the increase of intracellular ROS levels and mitochondrial membrane potential alterations caused by PAMAM in this cell lines (Janaszewska et al., 2013). In general, as describes above, cytotoxicity of the positive charged amines is not a limitation since it could be reduced easily with chemical modifications, making nanocarrier systems based on PAMAM dendrimers a biocompatible and versatile tool.

Neurobiological applications: Nowadays, one of the most

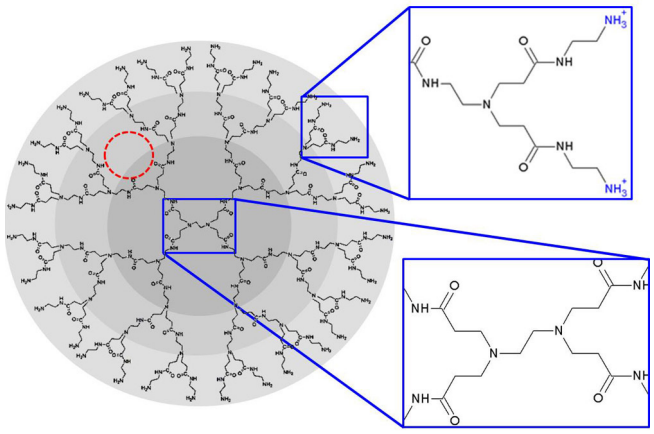

Figure 1 Schematic representation of the chemical structure of polyamidoamine (PAMAM) dendrimers.
The main details are highlighted: The blue squares show the surface amino groups protonated at physiological pH and the ethylendiamine central core; the red circle indicates the space where drugs could be encapsulated. The different generations of polymer layers are marked in tones of grey.

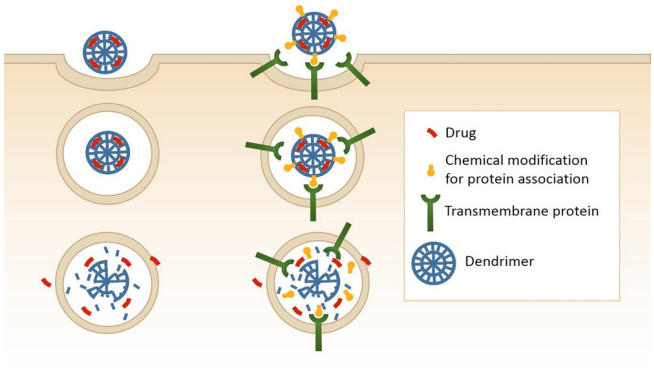

Figure 2 Models that explain the intracellular delivery of drugs mediated by polyamidoamine (PAMAM) dendrimers.
Dendrimers could be internalized into the cytoplasm by non specific endocytosis. As an alternative, dendrimers could be functionalized to allow the specific interaction with transmembrane proteins inducing their endocytosis. The intracellular delivery of drugs could be mediated by diffusion from the internal spaces of the dendrimers, or by their disorganization in the intracellular vesicles.

Main characteristic
- Organized as expansive growing layers
- Intramolecular spaces for drug storage/transport
- Great capacity for drug encapsulation
- Easy surface modification and functionalization

Citotoxicity
- Positive charges in the surface are associated with citotoxicity
- Neutralization of charges reduces toxicity
- Common modifications: PEG, pyrrolidone, carboxilation

Cell internalization
- Clathrin associated
- Caveoline associated
- Association to early and late endocytic markers

Neurobiological applications
- Targeting with T7 peptide and Angiopep-2 to brain blood barrier
- Demonstrated efficiency in mice brain glioma
- Applicability in inhibition of prion and Aβ peptide aggregation

Open questions
- Degradation and excretion pathway
- Intracellular trafficking and release of the cargo drug
- Detailed aspects of internalization
- Neuronal interactions and synaptic modulation

Box 1 Polyamidoamine (PAMAM) dendrimers: actual knowledge and future perspectives.

important global health problems are neurological disorders which are a continuous challenge for pharmacological research and companies. Over 1.5 billion people worldwide are suffering from the central nervous system (CNS) diseases. They currently represent 11% of the global burden of disease, which is expected to rise to 14% by 2020, becoming one of the largest and fastest growing areas of unmet medical need. A crucial aspect to consider when directing drug therapy specifically to the CNS is the ability to cross the blood brain barrier (BBB). About 98% of small molecule drugs fail to cross this barrier, whereas no large molecule drugs cross the BBB except for a few natural peptides and proteins, such as insulin (del Burgo et al., 2014; Xu et al., 2014). It is possible to functionalize PAMAM dendrimers with specific BBB transmembrane protein ligands that target the nanocarrier to the endothelial cells and help the drug internalization through specific endocytic processes. Even when a more detailed description about the mechanism of drug delivery is needed, it is probable that cargo molecules would be released from the dendrimer into the endothelial cells to reach the neuronal target. The main advances that have been reported are in relation with the use of LRP-1 receptor, transferrin receptor, EGF receptor and integrin receptors (del Burgo et al., 2014; Xu et al., 2014). For example, PAMAM functionalized with peptides derived from Kunitz domains (Angiopep-2, specific for LRP-1 receptor) are capable of targeting the brain tissue in mice models (Ke et al., 2009). Moreover, T7 peptide functionalized dendrimers loaded with the antitumor gene agent pORF-hTRAIL and doxorubicin target the transferrin receptor and induce a three times survival increase in a mice brain glioma model (Liu et al., 2012), which is a demonstration for the effectiveness of these new nanocarrier systems. For optimal therapeutic application, it is important to employ dendrimeric polymer systems which do not generate significant changes in the physiology of cells, and in particular in plasma membrane physiology and function. In this case, it is not enough that these compounds are not cytotoxic, is also important that properties such as the integrity of the plasma membrane and permeability to ions are not altered. Indeed, there is experimental evidence showing

that PAMAM having five generations (G5) did not effect the conductance of K^+ and Cl^- ions in hippocampal neurons, but produced an increased influx of Na^+ (Nyitrai et al., 2013b). While the authors attributed this phenomenon to the formation of pores in the plasma membrane as a result of the interaction between the positive charges of the dendrimers and the cell surface, the specificity that this pore shows for Na^+ ions is not fully explained. Moreover, it has been shown that PAMAM G5 are capable of inducing a significant increase in intracellular Ca^{2+} leading to mitochondrial depolarization in pyramidal neurons and astroglial cells, indicating that the focus must be not only in neuronal cells, but also in glial cells. These studies also show that astrocytes are more resistant than neurons to the cytotoxic effects of dendrimers (Nyitrai et al., 2013a). Another important application of dendrimeric polymer systems is the intervention of cells or tissues with genes or interfering nucleic acids. This has the advantage of eliminating the use of viruses as carriers for these kinds of molecules and confers specificity to the tissue or cells to be transfected. For example, a recent study by Brunner et al. (2015) demonstrated a high efficiency in neuronal gene silencing with a direct application to rabies infection, a relevant biomedical problem. In this case, a siRNA was linked to the surface of a dendrimeric polymer, and a covalently associated cannabinoid receptor ligand conferred specificity to the neurons (Brunner et al., 2015). Interestingly, beyond the nanocarreir properties of PAMAM dendrimers, it has been described to have neuropharmacological activity by itself. In this way, studies demonstrate that these polymers are able to prevent the Alzheimer's peptide $A\beta_{1–28}$ and prion peptide PrP 185–208 aggregation, processes associated to neurodegenerative diseases. The probable mechanism for this PAMAM effect is that the dendrimers interact with peptide monomers, and therefore inhibit their capability of growing into fibrils (Klajnert et al., 2007).

Perspectives: In recent years, nanoparticles of several kinds have been developed in clinical trials or patents directed to relevant biomedical problems (Cheng et al., 2015), which is a demonstration that neurological applications of PAMAM dendrimers are a real and feasible alternative. One of the issues to be addressed regarding the use of nanocarriers in central nervous system is related to the possibility to increase the tissue specific drugs delivery. In this regard, the studies of dendrimers passing through the BBB and the understanding of specific association with tissues or cell groups are highly relevant. Therefore, the generation of nanocarriers with activity in brain nuclei or specific nerve centers to increase the effectiveness and reduce secondary effects in treatments against neurodegenerative disorders, psychiatric pathology and addiction is an area for future possibilities. Another interesting aspect that neuropharmacology should address is the contribution that nanocarriers could have in behavioral pharmacological studies or higher neural functions, as a first step towards a possible application to human neuropathology. To reach this goal, it is necessary to focus on studies that describe aspects of nanocarrier activity at the subcellular level. Of particular interest is to know the possible applications of this kind of drug delivery system in the regulation of synaptic function (**Box 1**). It is also necessary to further analyze the effects that dendrimers might have on the basal activity of different components in neurons such as ion channels, neurotransmitter transporters, vesicle mobilization systems, among other relevant aspects for neurological applications.

Felipe Vidal, Leonardo Guzman*

Laboratory of Molecular Neurobiology, Department of Physiology, Faculty of Biological Sciences, University of Concepcion, Chile

***Correspondence to:** *Leonardo Guzman, Ph.D., joseguzman@udec.cl.*
***Accepted:** 2015-05-09*
***doi:**10.4103/1673-5374.160063* ***http**://www.nrronline.org/*
Vidal F, Guzman L (2015) Dendrimer nanocarriers drug action: perspective for neuronal pharmacology. Neural Regen Res 10(7):1029-1031.

References

Albertazzi L, Serresi M, Albanese A, Beltram F (2010) Dendrimer internalization and intracellular trafficking in living cells. Mol Pharm 7:680-688.

Brunner K, Harder J, Halbach T, Willibald J, Spada F, Gnerlich F, Sparrer K, Beil A, Mockl L, Brauchle C, Conzelmann KK, Carell T (2015) Cell-penetrating and neurotargeting dendritic siRNA nanostructures. Angew Chem Int Ed Engl 54:1946-1949.

Cheng CJ, Tietjen GT, Saucier-Sawyer JK, Saltzman WM (2015) A holistic approach to targeting disease with polymeric nanoparticles. Nat Rev Drug Discov 14:239-247.

del Burgo LS, Hernandez RM, Orive G, Pedraz JL (2014) Nanotherapeutic approaches for brain cancer management. Nanomedicine 10:905-919.

Janaszewska A, Ciolkowski M, Wrobel D, Petersen JF, Ficker M, Christensen JB, Bryszewska M, Klajnert B (2013) Modified PAMAM dendrimer with 4-carbomethoxypyrrolidone surface groups reveals negligible toxicity against three rodent cell-lines. Nanomedicine 9:461-464.

Ke W, Shao K, Huang R, Han L, Liu Y, Li J, Kuang Y, Ye L, Lou J, Jiang C (2009) Gene delivery targeted to the brain using an Angiopep-conjugated polyethyleneglycol-modified polyamidoamine dendrimer. Biomaterials 30:6976-6985.

Klajnert B, Cangiotti M, Calici S, Majoral JP, Caminade AM, Cladera J, Bryszewska M, Ottaviani MF (2007) EPR study of the interactions between dendrimers and peptides involved in Alzheimer's and prion diseases. Macromol Biosci 7:1065-1074.

Lee CC, MacKay JA, Frechet JM, Szoka FC (2005) Designing dendrimers for biological applications. Nat Biotechnol 23:1517-1526.

Liu S, Guo Y, Huang R, Li J, Huang S, Kuang Y, Han L, Jiang C (2012) Gene and doxorubicin co-delivery system for targeting therapy of glioma. Biomaterials 33:4907-4916.

Nyitrai G, Heja L, Jablonkai I, Pal I, Visy J, Kardos J (2013a) Polyamidoamine dendrimer impairs mitochondrial oxidation in brain tissue. J Nanobiotechnology 11:9.

Nyitrai G, Keszthelyi T, Bota A, Simon A, Toke O, Horvath G, Pal I, Kardos J, Heja L (2013b) Sodium selective ion channel formation in living cell membranes by polyamidoamine dendrimer. Biochim Biophys Acta 1828:1873-1880.

Perumal OP, Inapagolla R, Kannan S, Kannan RM (2008) The effect of surface functionality on cellular trafficking of dendrimers. Biomaterials 29:3469-3476.

Svenson S (2009) Dendrimers as versatile platform in drug delivery applications. Eur J Pharm Biopharm 71:445-462.

Wang S, Li Y, Fan J, Wang Z, Zeng X, Sun Y, Song P, Ju D (2014) The role of autophagy in the neurotoxicity of cationic PAMAM dendrimers. Biomaterials 35:7588-7597.

Xu L, Zhang H, Wu Y (2014) Dendrimer advances for the central nervous system delivery of therapeutics. ACS Chem Neurosci 5:2-13.

Zhu S, Hong M, Zhang L, Tang G, Jiang Y, Pei Y (2010) PEGylated PAMAM dendrimer-doxorubicin conjugates: in vitro evaluation and in vivo tumor accumulation. Pharm Res 27:161-174.