Exploration of biomedical dendrimer space based on *in-vivo* physicochemical parameters: Key factor analysis (Part 2)

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In nanomedicine, the widespread concern of nanoparticles in general, and dendrimers, in particular, is the analysis of key *in-vivo* physicochemical parameters to ensure the preclinical and clinical development of 'safe' bioactive nanomaterials. It is clear that for biomedical applications, biocompatible dendrimers, used as nanocarriers or active *per se*, should be devoid of toxicity and immunogenicity, and have adequate PK/PD behaviors (adequate exposure) in order to diffuse in different tissues. Functionalization of dendrimers has a dramatic effect on *in-vivo* physicochemical parameters. In this review, we highlighted key *in-vivo* physicochemical properties, based on data from biochemical, cellular and animal models, to provide biocompatible dendrimers. Up-to-date, only scarce studies have been described on this topic.

Introduction

Nanomedicine is defined as the medical application of nanotechnology and is the application of nanotechnology (the engineering of tiny machines) to the prevention and treatment of disease in the human body. This evolving discipline has the potential to change medical science dramatically. Nanomedicine can include a wide range of applications, including biosensors, tissue engineering, diagnostic devices, and many others. Nanomedicine will lead to many more exciting medical breakthroughs, for instance, in the domain of oncology. Nanoparticles (NPs) are key components of nanomedicine, and currently, a large variety of nanoparticle types exists [1]. Currently, the biomedical development of drug-delivery systems is an expanding therapeutic approach with great potential in nanomedicine. Thus, nanocarriers have been used not only for drug delivery but also for the delivery of genes and imaging agents and tissue-targeting, tumor therapy, and diagnostics, etc. [2–8].

Recently, we published a review of the analysis and discussion of simple guideline information, based onseveral translational requirements, for scientists of dendrimers moving towards Investigational New Drug (IND) application (evaluation of the safety profile before initiating clinical trials), the essential first step in entering clinical phase [9]. Continuing our effort to understand the properties of dendrimers required for success in the clinical

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phase, the aim of this original review is to analyze the different Absorption, Distribution, Metabolism, and Excretion and Toxicological (ADMET) parameters of dendrimers and to evaluate the key factors among them. In this second installment of our two-part series (see precedent article), we intend to define a non-equivocal piece of dendrimer parameter space based on realistic *in vivo* physicochemical properties Also we analyzed data from biochemical, cellular and animal models, to provide biocompatible dendrimers, improved physicochemical properties, toxicity profiles, *in vivo* behavior (PK/PD) for adequate exposure *versus* the clinical objectives, and to decrease risk assessments and consequently increase therapeutic value. Finally, we are convinced that developing and expanding the regulatory framework centered on the dendrimers physicochemical parameters of dendrimers will help to translate the technology successfully into the clinic.

Physicochemical properties of polymeric nanoparticles in nanomedicine

Nanoparticle therapeutics, with particle sizes ranging between 1 and 100 nm, are emerging as a new class of curative agents; for instance, against cancers. Thus, the combination of nanoparticle size and surface characteristics are the main key properties of anticancer nanoparticles to be developed. The diameter of nanoparticles for cancer therapeutics should be in the range of 10–100 nm. This range corresponds to the threshold for first-pass elimination by the kidney (10 nm) and the vasculature in tumors, which is leaky to macromolecules. Thus, nanoparticles show a strong ability to escape uptake by the nonspecific Reticulo Endothelial System (RES) from the lymphatic system. Importantly, the nanometric size of dendrimers (nanometer range) induces passive targeting effects, reducing, for instance, the nonspecific toxicity of the drugs carried. This effect is called the Enhanced Permeability and Retention (EPR) effect and is observed in inflamed tissues. Consequently, nanoparticles can leak out of the blood vessels and accumulate within tumors [10,11]. Nanoparticles with a surface charge either slightly positive or slightly negative in the 10–100 nm size range should have accessibility to tumors, show minimal self-self (aggregation) and self-non-self-interactions (*e.g.*, protein binding) and will also be able to access the liver. These attributes tend to avoid the interactions between nanoparticles and the negatively charged components of both the surface of cells and the inside surface of blood vessels. In addition, stabilization of nanoparticles by coated polyethylene glycol (PEG) polymers on their surface improve their protection from the immune system, reduce the charge-based contact typical of protein interactions, prevent rapid renal clearance due to the increased size of conjugates and prolong plasma half-life [12,13].

A very interesting analysis of the opportunities and challenges of multifunctional dendritic polymers in nanomedicine has been emphasized by Khandare and Haag *et al.* [14]. The 'Holy Grail' in nanomedicine is to design and synthesize new advanced macromolecular nanocarriers and to translate them from the laboratory to the clinic.

The physicochemical properties of therapeutic nanoparticles in general and dendrimers in particular play significant role in the modulation of PK/PD profile, affording specific pharmacological responses. A global view on the modulation of several physicochemical properties has been described by Choi and named 'Choi criteria' [15]. The nanoparticles' composition manages their biodegradation and toxicity, surface properties and control their targeting and biodistribution properties, whereas size and shape govern their excretion and clearance profiles. The main factors influencing the PK of nanoparticles (NPs) can be summed up as:

1) *Surface modification* such as a) charges. The cellular uptake increases with positive charges (electrostatic interactions) more than negatively or neutrally charged NPs. Indeed, positive charge can affect membrane integrity by interactions with the negative charges of membrane, b) Polymer coating. The PEG chain is the most common polymer coating used in nanomedicine. The introduction of PEG chains increases the plasma residence time and half-life of NPs, and reduces opsonisation effect. In addition, the layer plays an important role in the attraction of NPs to the cell membrane, and c) NP types. Liposomes, micelles, dendrimers, linear polymers, metals are the main NPs used in nanomedicine. For instance, in the oncology domain (chemotherapy), as nanocarriers of drugs, to improve the PK/PD of drugs.
2) *Route of administration*. The PK of the drug from NPs is related to the route of administration.
3) *Shape*. The Shape is related to the NPs types and sizes. Better cellular uptake was observed with rod shape over spherical NPs.
4) *Composition*. The NPs are composed of different elements influencing geometry/conformation and consequently their absorption, distribution, and elimination, and targeting ability. The composition Influence also the toxicity profile of NPs, and the endocytosis rates are related to the composition of NPs.
5) *Size*. NPs with a size of >10 nm can cross the cell membrane through passive targeting pathway, whereas <6 nm through active targeting pathway. The size influences plasma residence time, half-life and clearance.

Another interesting analysis was performed by Guo and O'Driscoll *et al.* concerning into the influence of nanoparticle formulations, careering a drug, on the PK, PD and biodistribution profiles following oral administration [16]. Several nanoparticles are included in this study as well as various peptide- and protein-like drugs as nanocarriers. The physicochemical properties of NPs on ADMET influence their profiles as follows: size, charge, surface polarity and bioadhesive properties. The PK/PD profiles are related to the nature of the nanoparticles such as biodegradable materials, and inorganic materials such as silica and gold. The biodistribution profiles are related to intestinal lymphatic system and whole-body distribution.

In vivo toxicity of dendrimers

It is evident that 'safe', non-toxic dendrimers are mandatory for clinical developments as both drug-delivery systems and as nanodrugs. So far, *in vitro* and *in vivo* studies are crucial to evaluate cell viability, hematological toxicity, immunogenicity, biocompatibility and biodistribution, and to establish risks/benefit ratios [17]. It is generally accepted that acceptable biocompatibility of dendrimers is related to rapid renal elimination rate or biodegradation followed by excretion [18]. Importantly, modification of the surface functionalities may induce tuning and improvement of

relevant properties, such as toxicity, encapsulation efficiency, biodistribution and pharmacokinetics, solubility, stability profiles, and drug release efficiency, etc. [19–22]. The pioneering work concerns the biological evaluation of G3, G5 and G7 PAMAM dendrimers against V79 cells, and in Swiss-Webster mice for *in-vitro*, *in-vivo* toxicity, immunotoxicity and biodistribution [23]. PAMAM dendrimers exhibited concentration-and generation-dependent toxicity against V79 cells (G3 < G5 << G7), 4- and. 24-h exposure times. 7- and 30-day *in-vivo* toxicity experiments, G3 and G5 PAMAM dendrimers do not show any major problem *in- vivo* toxicity *versus* G7 PAMAM dendrimer. No immunogenicity of any of the generations tested was observed. G3 PAMAM dendrimer showed the highest accumulation in kidney tissue, whereas G5 and G7 PAMAM dendrimers are mostly localized in pancreas. G7 PAMAM dendrimers showed high urinary excretion.

Very insightful analyses have been performed by Duncan and Izzo about the biocompatibility and toxicity of dendrimers for potential clinical applications [24]. We are in full agreement with their comments that biocompatibility statements of dendrimers, based only on their non-toxic and non-immunogenic effects, creates unhelpful dogma. Indeed, for *in-vivo* applications, there is a need for carefully considering toxicology and toxicokinetic studies for each dendrimer type, the protocols being tailored toward the proposed clinical uses. These studies can be based on learning lessons from past clinical experience, both with other different macromolecular therapeutics, such as antibodies and polymer therapeutics and with drug-specific delivery systems, such as liposomes and polymeric micelles. The final goals are able to predict the potential side effects of dendrimers, to understand their ADMET properties, and then to optimize the design of their chemical architecture.

Only a very few toxicological studies involving the *in vivo* administration of dendrimers have been reported so far. The *in vivo* biodisponibility is related to the core scaffold and the generation number, as well as to the nature of terminal groups.

Winnicka *et al.* showed that PAMAM dendrimers bearing carboxylate groups on their surface are less toxic than the corresponding cationic nitrogen-containing derivatives. Three daily doses of anionic G3.5 PAMAM administered by an *ip* route to mice at a total daily dose of 95 mg/kg caused no adverse weight change in C57 mice bearing B16F10 melanoma tumors [25].

In another study performed by Neerman and Simanek *et al.*, acute toxicity determinations in mice have been performed by the administration of 2.5, 10, 40 and 160 mg/kg of melamine-based dendrimers *via ip* injections. At 160 mg/kg, 100% mortality was observed after 6–12 h. At lower doses, liver damage but no renal damage was shown after 48 h. Hepatotoxicity was noticed at 40 mg/kg. No mortality was observed after three *ip* injections of 2.5–40 mg/kg of dendrimers at 3-week intervals. Subchronic doses of 40 mg/kg led to extensive liver necrosis. These studies suggest that the toxicity of these melamine-based dendrimers is comparable to that of cationic PAMAM dendrimers [26].

Heiden and Peterson *et al.* presented the development of low-generation PAMAM dendrimers in zebrafish [27]. Polycationic G4 PAMAM dendrimers were toxic and attenuated growth and development of zebrafish embryos at sublethal concentrations, whereas G3.5 PAMAM dendrimers showed no sublethal signs of toxicity or increased mortality. In addition, RGD (Arg-Gly-Asp) conjugation of G4 PAMAM dendrimers eliminated their toxicity and mortality compared to unconjugated G4 dendrimers. Generally speaking, the zebrafish model can be used in the early stage as a screening tool to study the systemic circulation of nanoparticulate drug-delivery systems *in vivo* [28].

Moreover, Polyanionic phosphorus dendrimers [29,30] were found to be extremely useful in the treatment of rheumatoid arthritis *via* intravenous or oral administration. One of these dendrimers reduced levels of inflammatory cytokines with an absence of cartilage destruction and bone erosion and exhibited anti-osteoclastic activity on mouse and human cells [31]. No toxicity was observed for these polyanionic phosphorus dendrimers.

In general, the *in vivo* toxicity of dendrimers depends on the chemical structure, size, generation, duration of exposure and the nature of the terminal groups. The increase in generation leads to an increase in toxicity. The effects of nanomaterials, including dendrimers, based on their physicochemical properties, on *in vivo* toxicity have been nicely highlighted by Aillon and Laird Forrest *et al.* [32].

Pharmacokinetic (PK) and pharmacodynamics (PD) behaviors of dendrimers

Figure 1 highlights the relationship between the pharmacokinetic parameters such as area under the curve (AUC), hepatic and renal clearance, and the physicochemical properties (size and charge), of dendrimers after the intravenous administration in mice of two types of dendrimer: PAMAM dendrimers and lysine dendrimers, of different generations. Neutral, amphiphilic, weak anionic and weak hydrophobic dendrimers showed high plasma residence time and half-life, corresponding to low total clearance [33].

Relative accumulation of PAMAM dendrimers of different generations in specific organs after intravenous administration in mice has been studied [34]. ^{153}Gd-radiolabeling PAMAM dendrimers with an overall negative surface charge were used: The dendrimers were cleared from the blood circulation and were mainly distributed in the kidney and liver, depending on their size (generation). The increase of the accumulation of G5-G7 PAMAM dendrimers in the blood *versus* the decrease in kidney was observed, whereas the G8-G9 PAMAM dendrimers showed rapid elimination from the blood, with accumulation in the liver *versus* kidney.

Based on the *in-vivo* data of around 130 different nanoparticle types (fullerenes, metal oxides, polymers, liposomes, dendrimers, quantum dots and gold colloids), Nel *et al.* [35] and Khandare and Haag *et al.* [14] analyzed the *in-vivo* biocompatibility of nanoparticles based on physical characteristics such as their zeta potential, rigid core size and hydrophobicity. Figure 2 shows the main physical characteristics of nanoparticles for their *in-vivo* biocompatibility. Low *in-vivo* biocompatibility of NPs was observed with 1) cationic NPs (high positive zeta potential), 2) low hydrophilicity, and 3) high rigid core size, while high *in-vivo* biocompatibility was observed with 1) anionic, 2) high hydrophilicity and 3) low rigid core. NPs with low hydrophobicity and high rigid core size are recognized by the reticuloendothelial system (RES), and NPs with strong enhanced permeation and retention (EPR), for instance as anticancer drug delivery systems are average sized and have a

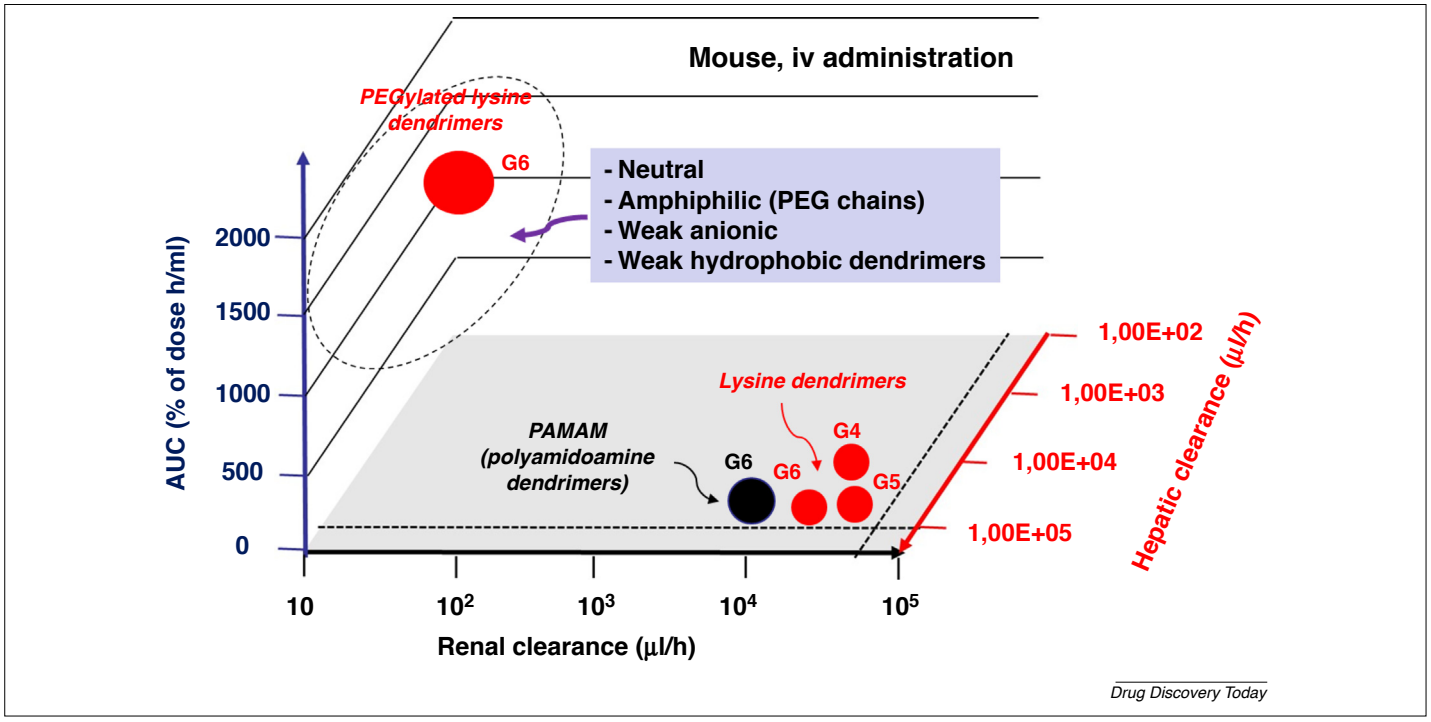

FIGURE 1

Relationship between the pharmacokinetic parameter AUC and hepatic and renal clearance, and the physicochemical properties (size and charge) of PAMAM and lysine dendrimers (intravenous administration in mice). Neutral, amphiphilic, weak anionic and weak hydrophobic dendrimers showed high plasma residence time and half-life, corresponding to low total clearance. Adapted from Ref. [33].

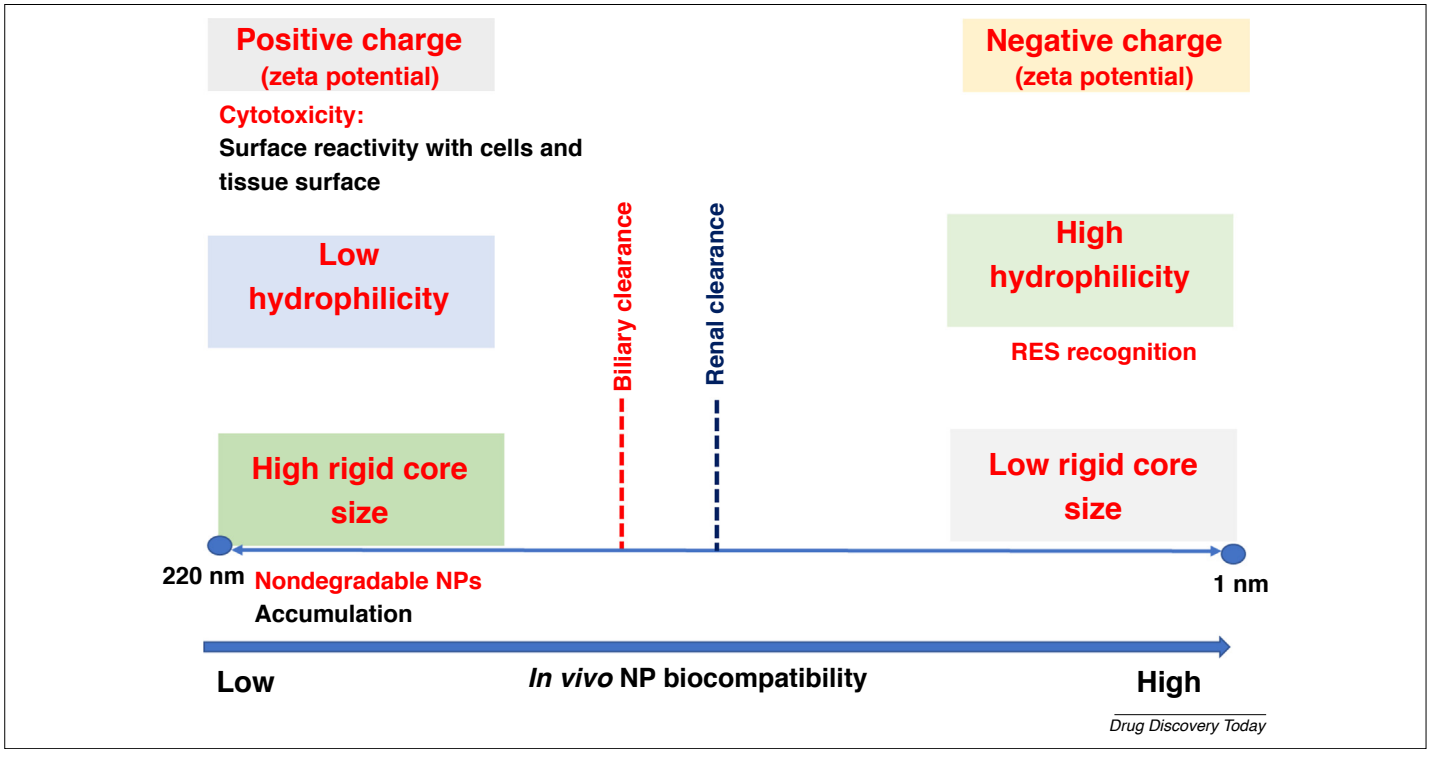

FIGURE 2

Physical characteristics of nanoparticles for their *in-vivo* biocompatibility. Low *in-vivo* biocompatibility of NPs was observed with 1) cationic, 2) low hydrophilicity, and 3) high rigid core size, while high in-vivo biocompatibility was observed with 1) anionic, 2) high hydrophilicity and 3) low rigid core. Adapted from Ref. [14].

neutral surface charge. From a broader perspective, EPR-based therapeutics improve the PK/PD profile of a nano-encapsulated drug (nanoformulation) [14]. Globally, nanoparticles are cleared by the kidneys or biliary tract [36].

As mentioned by Mintzer and Grinstaff [21], circulation time, organ uptake and tumor accumulation are the critical factors for the *in-vivo* efficiency of dendrimers as anticancer delivery agents. For instance, modified PAMAM dendrimers with folic acid targeting of anticancer drugs such as methotrexate improved their therapeutic response in nude female mice with human epithelial cancer, as highlighted by Kukowska-Latallo and Baker [37].

A very interesting and instructive analysis has been performed by Fréchet *et al.* concerning the PK properties of asymmetric bow-tie dendrimers related to their molecular weights and chain numbers (architecture effects) [19,38]. Bow-tie dendrimers bearing both a G3-hydroxyl-terminated branching and a G1-G3 branching group with three types of PEG chains (5, 10, and 20 kDa, *vide supra*) were found most active. Importantly, all these bow-tie dendrimers showed very low cytotoxicity effects against MDA-MB231 breast cancer cell lines. Significant degradation was observed over 15 days in buffer solutions at pH 5.0 or pH 7.4 due to the hydrolysis of both ester and carbamate groups. Biodistribution studies (mice, *iv* route) disclosed several important points: a) long circulation times with elimination half-lives of 31–50 h were observed with bow-tie dendrimers [G3]-(PEGx)8-(G3)-(OH)8 with x = 5000, 10,000 and 20,000 molecular weights, whereas b) dendrimers [G2]-(PEGx)4-(G3)-(OH)8 with x = 45,000 and 85,000 molecular weights displayed and elimination half-live of ~25 h *versus* [G2]-(PEGx)4-(G3)-(OH)8 with x = 5000 showing a half-life of 11 h. These results are in full agreement with the molecular weight cutoff for renal filtration of linear PEGs, which is 30,000–40,000 Da [39]. Less than 4% and 7–16% of these dendrimers were excreted in the urine (48 h) and in the feces (48 h), respectively. Interestingly, the circulation half-life depended on the architecture of the bow-tie dendrimers, related to the number of PEG chains. The half-lives of [G1]-(PEGx)2-(G3)-(OH)8 with x = 20,000 molecular weight (44,000 Da) and [G3]-(PEGx)8-(G3)-(OH)8 with x = 5000 molecular weight (~44,000 Da) were 1.5 h and 31 h, respectively. In addition, the *iv* administration into tumoured C57BL6 mice with B16F10 melanoma cells, of the largest dendrimers [G3]-(PEGx)8-(G3)-(OH)8 with x = 10,000 molecular weight and [G3]-(PEGx)8-(G3)-(OH)8 with x = 20,000 molecular weight showed similar biodistribution behaviors, with high levels of dendrimers in both tumors (10–15%) and blood (18–20%) at 48 h. Interestingly, a single dose of Doxorubicin-functionalized bow-tie dendrimer (named SPN-L6Dox), with hydrazone cleavage principally in the tumor cured mice bearing C-26 colon carcinomas, as has been highlighted by Lee and Fréchet *et al.* [40]. An interesting analysis of several parameters about the design of dendrimers for drug delivery has been recently published by Sebestik *et al.* [41].

Another very interesting study has been highlighted by Kaminskas and Porter *et al.* regarding the characterization and tumor targeting of SPN-L-Dox (*vide supra*) [42]. The PK profiles of SPN-L-Dox, as well as the corresponding fragments SPN-L and SPN-NH2, corresponding to the dendrimer without Dox and without Dox and cleavable linker, respectively, were performed to evaluate the effect of the surface nature of the dendrimers and their distribution in different tissues. These PK studies were performed after *iv* administration in rats. The data clearly demonstrated that the full dendrimer (SNP-L-Dox) exhibited lower clearance (Cl), higher AUC correlated with higher exposure, lower volume of distribution (VD) correlated with higher concentration in the blood, and higher half-life (T1/2) correlated with higher circulation time, than SPN-NH2 and SPN-L.

In another study by the Okuda group [43], the effect of PEGylation of G4-G6 dendrimer based on poly(L-lysine) or poly(L-ornithine) on the biodistribution characteristics has been described (mice, *iv* route). The following events were observed: a) non-PEGylated G4-G6 dendrimers were fully eliminated from circulation within minutes of injection (liver and kidney accumulation) and b) the increase of the generation number of cationic dendrimers induced strong hepatic accumulation, low renal accumulation, and high retention time (from minutes to over 24 h).

Importantly, the same group showed that G6-PEGylated cationic lysine dendrimers are selectively accumulated in malignant cells through EPR effect in mice (*iv* route) [44]. The two PEGylated dendrimers with low and high numbers of PEG 5000 Da chains (10 and ~76) attached to the surface of dendrimers were used. No accumulation in the kidney was observed with PEGylated dendrimers. The dendrimers with the highest number of PEGylated chains displayed strong tumor accumulation correlated with good plasma level. The opposite effect was obtained with non-PEGylated dendrimers, which showed negligible tumor accumulation and rapid clearance.

The biodistribution of cationic PAMAM dendrimers and neutral N-Ac PAMAM dendrimers in B16 melanoma and DU145 prostate cancer models have been investigated [45]. Nonspecific distributions and rapid clearance from the blood within 24 h were observed for both dendrimers, but greater tissue deposition was obtained with PAMAM dendrimers *versus* corresponding N-Ac dendrimers. Accumulation was greatest in the lungs, liver, and kidneys *versus* tumors tissues (~3% of the initial dendrimer loading) after 1 h. N-Ac dendrimers were excreted through urine more rapidly (three-fold more rapidly than cationic PAMAM). No deleterious effects were observed with both dendrimers with non-tumors-bearing mice, and low levels of dendrimers were present only in the kidney over 12 weeks.

Different generations of PAMAM dendrimers (G1–G3) were co-spray-dried with rifampicin, an antibiotic used to treat several types of bacterial infections, including tuberculosis, leprosy, and Legionnaires' disease, to produce inhalable microspheric particles (microsphere formulations) for pulmonary delivery [46]. This very interesting study was based on the evaluation of the pharmacokinetic profiles of rifampicin, such as Cmax, tmax, t1/2, mean residence time (MRT) and AUC, with different formulations (G1-G3 PAMAM dendrimers) following intrapulmonary administrations for 60 h. The G3 PAMAM microsphere formulation induced better PK profile of rifampicin and consequently better bioavailability *versus* G1 and G2 PAMAM dendrimers, due to better rifampicin absorption rate. The absorptions (1/h) were 0.14, 0.10 and 0.05 for G1, G2, and G3 PAMAM, respectively. G3 PAMAM dendrimer induced a good sustained drug release delivery system, maintaining rifampicin for a longer period of time in the RES. Importantly, these formulations-maintained rifampicin plasma concentration above its minimal inhibitory concentration

(MIC) with a sufficient time gap. The pharmacokinetic parameters of rifampicin with G1, G2 and G3 microsphere formulations can be summed up as: 1) T_{max} (h) (amount of time that a drug is present at the maximum concentration in plasma) for G1 PAMAM, G2 PAMAM and G3 PAMAM is 12 h, 12 h and 24 h, respectively, 2) $T_{1/2}$ (h) (Half-life) for G1 PAMAM, G2 PAMAM and G3 PAMAM is 16.27 h, 37.30 and 45.61, respectively, 3) C_{max} (μg/mL) (Maximum plasma concentration) for G1 PAMAM, G2 PAMAM and G3 PAMAM is 1.4, 0.9 and 0.8 μg/ml, respectively, 4) $AUC_{(0 \rightarrow 60\ h)}$ (μg/ml) (Area under the curve) for G1 PAMAM, G2 PAMAM and G3 PAMAM is 23.49, 53.79 and 65.81, respectively, and 5) F (%) (Abs. bioavaibility, the dose-corrected area under curve (AUC) non-intravenous (*e.g.* oral) divided by AUC intravenous) for G1 PAMAM, G2 PAMAM and G3 PAMAM is 0.79, 0.80 and 0.91, respectively.

Another important study about the PK and pulmonary lymphatic exposure of G4 poly-lysine dendrimers following *iv* and aerosol administration to rats and sheep was also highlighted by Ryan *et al.* [47]. Higher plasma concentrations were achieved when dendrimer was administered to the lungs of rats when compared to aerosol administration. Plasma PKs were similar between sheeps and rats.

A specific organ is the brain, and neurons are a difficult target for nanocarriers. In the nanomedicine to target neurons for chemotherapy, the objective is to design pH-sensitive drug-delivery nanoparticles that accumulate in intracellular acidic vesicles and then selectively release the drugs. An instructive example has been described by Patel *et al.* [48]. Sialic acid, glucosamine, and concanavalin A were anchored to poly(propyleneimine) (PPI) dendrimers and evaluated for the delivery of paclitaxel (PTX) to the brain. MTT assay on U373MG human astrocytoma cells showed the better antiproliferative activity of the PTX encapsulated with functionalised PPI dendrimers than PTX-PPI and plain PTX: PTX-Sialic acid-PPI > PTX- Glucosamine-PPI > PTX- concanavalin A-PPI > PTX-PPI > free PTX. The *in-vivo* pharmacokinetics and biodistribution studies in rats showed a significantly higher accumulation of PTX in brain *versus* free PTX. Blood circulation time increased in all ligand-conjugated PPI dendrimers as compared to PTX-PPI and free PTX, as well the $AUC(0 \rightarrow \infty)$ and the mean residence time (MRT. All the pharmacokinetic values of PTX-Sialic acid-PPI, PTX- Glucosamine-PPI and PTX- concanavalin are very similar. The pharmacokinetic parameters of PTX-Sialic acid-PPI, PTX- Glucosamine-PPI, PTX- concanavalin A-PPI, PTX-PPI and free PTX can be summed up as: 1) C_{max} (μg/ml) for PTX, PTX-PPI and PTX-functionlized PPI (average of the parameter values of PTX-Sialic acid-PPI, PTX- Glucosamine-PPI and PTX-concanavalin) is 548.4, 538.8 and 537.7 μg/ml respectively, 2) K_{el} (h^{-1}) (elimination rate) for PTX, PTX-PPI and PTX-functionlized PPI is 0.119, 0.092 and 0.080 h^{-1}, respectively, 3) $T_{1/2}$ (h) for PTX, PTX-PPI and PTX-functionlized PPI is 5.81, 7.53 and 8.62 h, respectively, 4) $AUC_{(0 \rightarrow \infty)}$ (μg/ml) for PTX, PTX-PPI and PTX-functionlized PPI is 4113.95, 5499.23 and 6212 μg/ml, respectively, and 5) MRT (Mean residence time) (h) for PTX, PTX-PPI and PTX-functionlized PPI is 7.69, 8.42 and 10.35 h, respectively.

A noticeable study by Albertazzi *et al.* regarding the *in-vivo* biodistribution and toxicity of G4 PAMAM dendrimers and G4-C12 modified PAMAM dendrimers in the Central Nervous System (CNS) has been performed [49]. As highlighted previously, biodistribution and toxicity also depend on the nature of the PAMAM's surface groups (*vide supra*). Based on imaging techniques, these studies described the cell internalization properties and diffusion of G4 PAMAM dendrimers as well G4-C12 functionalized PAMAM dendrimers in primary neuronal cultures and in the CNS of live mice by intraparenchymal or intraventricular injections. Confocal imaging studies on primary neurons clearly showed that all prepared dendrimers were able to cross the cell membrane and reached intracellular localization following endocytosis pathways. Interestingly, G4-C12 functionalized PAMAM dendrimers displayed good diffusion in the CNS tissue *in-vivo* and penetrated living neurons. G4-C12 functionalized PAMAM dendrimers induced strong apoptotic cell death of neurons *in vitro*. G4 PAMAM dendrimers diffused in the brain parenchyma, whereas G4-C12 functionalised PAMAM dendrimers did not.

Berberine (BBR) is a nitrogenous cyclic natural alkaloid with potential anticancer activity but has a poor pharmacokinetic profile. BBR was conjugated (~37.5%) as well as encapsulated (~30%) with G4 PAMAM dendrimers [50]. These two systems were safe and biocompatible, based on *ex vivo* hemolytic toxicity studies. *In vitro* release studies (water and PBS pH = 7.4) showed a sustained release pattern of BBR. In water, ~72–98% of BBR was released (24 h), whereas in PBS ~80%–98% of BBR was released (24 h). Good antiproliferative activities were observed, but the BBR conjugated with G4 PAMAM dendrimer showed the strongest activity against MCF-7 and MDA-MB-468 breast cancer cells. Importantly, *in-vivo* pharmacokinetic parameters, using *iv* administration in albino rat models were shown to be strongly improved. Both half-life (t1/2) and AUC of BBR were ameliorated *versus* plain BBR. The pharmacokinetic parameters of G4 BBR-PAMAM dendrimers can be summed up as: 1) co initial concentration (μg/ml) for BBR, encapsulated BBR-PAMAM dendrimer and conjugated BBR-PAMAM dendrimer is 24.37, 33.2 and 43.13 μg/ml, respectively, 2) $T_{1/2}$ (h) for BBR, encapsulated BBR-PAMAM dendrimer and conjugated BBR-PAMAM dendrimer is 6.70, 10.34 and 14.33 h, respectively, 3) $AUC_{(0 \rightarrow \infty)}$ (μg/mL) for BBR, encapsulated BBR-PAMAM dendrimer and conjugated BBR-PAMAM dendrimer is 1424.42, 2005.38 and 2471.17 μg/ml, respectively, and 4) elimination rtae (h^{-1}) for BBR, encapsulated BBR-PAMAM dendrimer and conjugated BBR-PAMAM dendrimer is 0.01034, 0.067 and 0.048 h^{-1}, respectively.

Another study has been published by Zhang *et al.* regarding the biodistribution and microglia-targeting of G3.5 PAMAM dendrimers related to the nature of their surface in a rabbit model of Cerebral Palsy (CP), a chronic childhood disorder [51]. In these studies, neutral and anionic PAMAM dendrimers were used, and their transport and neuroinflammation effects of the dendrimers were observed. Both neutral G3.5 PAMAM dendrimers (hydroxyl groups on the surface) and anionic PAMAM dendrimers (carboxyl groups on the surface) were absorbed by the fetus and demonstrated bi-directional transport between fetus and mother. Neutral hydroxyl PAMAM dendrimers were the most effective in crossing the fetal blood-brain barrier (BBB), targeting activated microglia and inhibiting fetal inflammation.

The same team highlighted that the neutral G6 hydroxyl PAMAM dendrimers improved CNS penetration and showed anti-inflammatory effect following intravenous administration in a canine brain injury model of hypothermic circulatory arrest (HCA) [52].

Xu *et al.* presented an original nanocarrier that crossed the BBB based on G5 PAMAM dendrimers bearing both folic acid (FA) and borneol (BO) to deliver Doxorubicin (DOX) in glioma. The folate receptor (FR) alpha is selectively overexpressed in several epithelial malignant cells [53]. The bicyclic monoterpene alcohol BO is a well-known safe material derived from traditional Chinese medicine and facilitates BBB permeability as well as reducing the toxicity of PAMAM dendrimer. This construction reduced the cytotoxicity, *versus* PAMAM dendrimer, against both human brain microvascular endothelial cell line (HBMEC) and C6 rat glioma cells (IC50 s ~μM range). Interestingly, the introduction of BO onto the surface of dendrimers improved, two-fold, the brain penetration in comparison with PAMAM dendrimer without BO. The FA-BO-PAMAM/DOX nanoparticle displayed significant antiproliferative activity (IC50 = 2.48 μM) *versus* free DOX (IC50 = 0.73 μM), whereas BO-PAMAM/DOX showed an IC50 of 6.17 μM. The FA-BO-PAMAM/DOX nanoparticle showed a prolonged half-life, increased AUC and improved DOX accumulation in the brain tumor. Significant malignant cell growth inhibition (~57.5% *versus* ~17%) and improved median survival time (28 days *versus* 18 days) were observed when FA-BO-PAMAM/DOX was administrated by *iv* route to rats bearing xenograft, compared to plain DOX. The pharmacokinetic parameters of DOX, BO-PAMAM/DOX, and FA-BO-PAMAM/DOX can be summed up as: 1) $AUC_{(0 \rightarrow \infty)}$ (μg/mL) for DOX, BO-PAMAM/DOX, and FA-BO-PAMAM/DOX is 10.36, 124.01 and 129.46 μg/ml, respectively, 2) $T_{1/2\beta}$ (h) for DOX, BO-PAMAM/DOX, and FA-BO-PAMAM/DOX is 4.51, 11.66, and 12.60 h, respectively, 3) Vc (central volume of distribution, ml/kg) for DOX, BO-PAMAM/DOX, and FA-BO-PAMAM/DOX is 5.81, 7.53, and 8.62 respectively, 4) Cl (Total volume clearance, ml/h/kg) for DOX, BO-PAMAM/DOX, and FA-BO-PAMAM/DOX is 4113.95, 5499.23, and 6215 ml/h/kg, respectively, and 5) MRT (mean residence time, h) for DOX, BO-PAMAM/DOX, and FA-BO-PAMAM/DOX is 5.46, 16.31, and 16.58 h, respectively.

Systemic disposition, after no parenteral and parenteral administration, was strongly affected by dendrimer-based nanomedicine properties such as core, surface groups, charge, hydrophobicity, hydrodynamic radius (2.5–8 nm) which is correlated with molecular weight (~30–200 kDa), and also related with between the size of therapeutic proteins, except antibodies, and nanoparticles as delivery systems. [54] The hydrodynamic radius increases with the molecular weight and the dendrimer generation, and the decrease of membrane permeability. Table 1 highlights the effects of size, structure, surface characteristics (*e.g.* charge) on *in-vivo* disposition [54,55].

Conclusion and perspectives

In recent decades, a new class of polymeric materials such as dendrimers has attracted striking interest in nanomedicine. Biocompatibility of dendrimers, which is related to toxicity, represents an important factor for their biomedical applications. The analysis of the different Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) parameters of dendrimers as well as their PK/PD parameters represent key factors for their preclinical and clinical development. For instance, adequate exposure, based on PK/PD behaviors, *versus* the clinical objectives is a critical objective to be achieved. In this original review, we highlighted key *in-vivo* physicochemical properties, based on data from biochemical, cellular and animal models, to provide biocompatible

TABLE 1

Effects of size, structure, surface characteristics on *in-vivo* disposition of dendrimers

Parameters of dendrimers	Effects
Influence of dendrimer size and surface characteristics	a) Biodistribution and PK profile - *Cationic dendrimers* G > 8: tissues: pancreas, liver and spleen G < 4: tissue: kidney Rapid removal from the blood circulation after parenteral administration by urinary and intestinal excretion (<G5, <3.5 nm) - *Anionic and neutral dendrimers* Tissues: Liver, kidney, lungs, and blood ° After parenteral administration: low generation of neutral dendrimers (<3.5 nm): rapid clearance from blood and facile elimination into the urine, whereas larger uncharged dendrimers avoid renal filtration, and show extended blood residence times (avoid renal filtration) ° Weak anionic dendrimers induce good biodisponibility. In some cases, increase of the opsonized and RES uptake ° PEGylated dendrimers: increase the size, the hydrophilicity, the drainage and the circulation half-lives] low uptake *via* RES and enhance parenteral bioavailability In general after parenteral administration: ° Increase size or surface hydrophobicity [increase uptake *via* RES which is not major targets for dendrimer biodistribution ° Dendrimer absorption from parental injection sites: Increase the size] increase the injected dose in the local lymph capillaries. Opposite effect with small dendrimers b) Route of administration The different ways to administrate dendrimers were noted by several of us [56] - Parenteral administration: large impact due to small changes such as core, flexibility, and shape - Dendrimers and oral drug absorption: PAMAM dendrimers increase the Caco2 permeability of drugs: cationic > anionic > uncharged or PEGylated, and with the increase of the hydrophobicity of dendrimers - Dendrimers and transdermal drug absorption: cationic > uncharged > anionic and smaller > larger dendrimers c) Toxicity - *Cationic dendrimers* G >5: toxic and G≤ 5: non-toxic - *Anionic and neutral dendrimers* Non-toxic

dendrimers. Improvement of physicochemical properties, toxicity profiles, *in-vivo* behavior for adequate exposure will decrease risk assessments and consequently increase the therapeutic value of dendrimers for medical applications. Functionalization of dendrimers has a dramatic effect on their ability to diffuse in different tissues distributions and exposure *via* their PK/PD behaviors. Up-to-date, only scarce studies have been described on this topic, which makes this review very timely. The right design of tailored dendrimers in light of future clinical applications remains a critical objective to be achieved.

Finally, we are convinced that developing and expanding the regulatory framework based on the physicochemical parameters of dendrimers will help to translate the technology successfully into the clinic.

Conflict of interest

The authors declare no conflicts of interest.

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