Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

European Journal of Medicinal Chemistry 209 (2021) 112905

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Review article

Non-invasive intranasal administration route directly to the brain using dendrimer nanoplatforms: An opportunity to develop new CNS drugs

Serge Mignani [a, b, *], Xiangyang Shi [c, **], Andrii Karpus [d, e], Jean-Pierre Majoral [d, e, ***]

[a] Université Paris Descartes, PRES Sorbonne Paris Cité, CNRS UMR 860, Laboratoire de Chimie et de Biochimie Pharmacologiques et Toxicologique, 45, Rue des Saints Peres, 75006, Paris, France
[b] CQM - Centro de Química da Madeira, MMRG, Universidade da Madeira, Campus da Penteada, 9020-105, Funchal, Portugal
[c] State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai, 201620, PR China
[d] Laboratoire de Chimie de Coordination Du CNRS, 205 Route de Narbonne, 31077, Toulouse, Cedex 4, France
[e] Université Toulouse 118 Route de Narbonne, 31077, Toulouse, Cedex 4, France

ARTICLE INFO

Article history:
Received 4 September 2020
Received in revised form
28 September 2020
Accepted 30 September 2020
Available online 11 October 2020

Keywords:
Intranasal administration
Dendrimer nanoplatforms
Nose to brain transport pathways
In situ gel formation

ABSTRACT

There are several routes of administration to the brain, including intraparenchymal, intraventricular, and subarachnoid injections. The blood-brain barrier (BBB) impedes the permeation and access of most drugs to the central nervous system (CNS), and consequently, many neurological diseases remain undertreated. For past decades, to circumvent this effect, several nanocarriers have been developed to deliver drugs to the brain. Importantly, intranasal (IN) administration can allow direct delivery of drugs into the brain through the anatomical connection between the nasal cavity and brain without crossing the BBB. In this regard, dendrimers may possess great potential to deliver drugs to the brain by IN administration, bypassing the BBB and reducing systemic exposure and side effects, to treat diseases of the CNS. In this original concise review, we highlighted the few examples advocated regarding the use of dendrimers to deliver CNS drugs directly *via* IN. This review highlighted the few examples of the association of dendrimer encapsulating drugs (*e.g.*, small compounds: haloperidol and paeonol; macromolecular compounds: dextran, insulin and calcitonin; and siRNA) using IN administration. Good efficiencies were observed. In addition, we will present the *in vivo* effects of PAMAM dendrimers after IN administration, globally, showing no general toxicity.

Contents

* Corresponding author. Université Paris Descartes, PRES Sorbonne Paris Cité, CNRS UMR 860, Laboratoire de Chimie et de Biochimie Pharmacologiques et Toxicologique, 45, rue des Saints Peres, 75006, Paris, France.
** Corresponding author.
*** Corresponding author. Laboratoire de Chimie de Coordination du CNRS, 205 route de Narbonne, 31077, Toulouse, Cedex 4, France.
E-mail addresses: serge.mignani@parisdescartes.fr, serge.mignani@staff.uma.pt (S. Mignani), xshi@dhu.edu.cn (X. Shi), majoral@lcc-toulouse.fr, jean-pierre.majoral@lcc-toulouse.fr (J.-P. Majoral).

https://doi.org/10.1016/j.ejmech.2020.112905
0223-5234/

1. Introduction

Within the drug development field, drug delivery systems can be considered as engineered technologies for the controlled delivery of targeted or non-therapeutic agents in the past few decades [1–3]. Drug delivery systems represent a prominent thrust of pharmaceutical research. This therapeutic strategy has dramatically changed clinical practice and our knowledge of the physiological barriers, including membrane transporters, the transport of drugs in the circulatory system and then through cells and tissues. Importantly, the aim of this strategy is to keep the biologically active properties of the drugs, while decreasing the unacceptable side effects, limiting the optimal design of medications for many diseases (such as cancer), due to the interaction of the drug with healthy tissues that are not the target of the drug. Adequate drug delivery systems control the rate of drug release and placement of release of the drug, with the final goal of enhancing the use of existing medications more effectively and precisely [4].

Nanotechnology is revolutionizing pharmaceutical research and development (R&D) to develop smarter therapeutics and diagnostics, as well as delving into important changes, for example, in nanoformulations and nanocarriers on the delivery of therapeutic agents through the development of nanodelivery platforms, encompassing include liposomes, micelles, nanocrystals, nanoparticles and dendrimers. These nanoparticle carriers help to improve solubility and bioavailability and reduce off-target effects of loaded drugs. The ideal drug delivery system can be attained by nanomaterials with adequate biodegradability and biocompatibility profiles [5].

The main advantage of nanoparticulate drug delivery systems include the following points: 1) tunable nanoparticles for an active or passive drug targeting process; 2) sustained controlled release of the drug in targeted tissues or cells; 3) conjugated targeting ligands on the surface of particles or use of magnetic or ultrasonic guidance for site-specific targeting strategy; and 4) provide drugs with greater water solubility for adequate bioavailability and biocompatibility [6–8].

Compared to linear polymers, dendrimers, which are artificial discrete macromolecules, are highly tunable branched spherical macromaterials [9] constructed with a well-defined and controlled size, shape, molecular weight, and highly monodispersed properties, which provide a high degree of surface functionality [10]. As shown in Fig. 1 (G4 PAMAM dendrimer), the four main architectural components representing PAMAM dendrimers are as follows: a central functional core, interior branches, interior layers (generations), and terminal functionality groups. Generation (named Gn) can be defined as the number of points of convergence, counting from the core towards the surface of the dendrimer. The therapeutic agents may either be encapsulated into the three-dimensional void spaces of the dendrimers or conjugated or physically adsorbed (electrostatic interactions) onto the surface of the dendrimer [11,12]. Another strategy is to develop dendrimers, such as drugs, active *per* se in several therapeutic realms [13–19]. Over the last several years, we have embarked on this direction in order to develop new drugs based on phosphorus dendrimers, for instance, as anticancer [20,21] and anti-inflammatory agents [22].

There are several routes of administration to deliver dendrimers to the systemic circulation, such as oral and intravenous, as well other routes, which include transdermal and ocular [23,24]. Herein, we reviewed the recent research progress on alternate routes of the delivery of dendrimers using non-invasive intranasal (IN) administration. Generally speaking, the administration of drugs in the systemic circulation is governed by the acidic or enzymatic degradation of the drugs, as well the excessive first-pass effect [25] due to hepatic metabolism of the considered drugs, with the result of inducing ineffective treatment with this drug [26,27]. According to our knowledge, no data about the first-pass effects of dendrimers has been highlighted.

The general aspects of the delivery of dendrimers to the brain was analyzed by Zu et al. in an excellent review [28], and the use of dendrimers to tackle brain tumors (*in vitro* and *in vivo*) was highlighted by S. M. and J-P. M [29]. Note that, Albertazzi et al. observed that G4-C_{12} functionalized PAMAM dendrimers were able to diffuse into the central nervous system (CNS) and cross the cell membranes of primary neurons after intraparenchymal or intraventricular injection in animals, unlike G4 PAMAM dendrimers. Also, apoptotic cell death *in vitro* was observed using G4-C_{12} PAMAM dendrimers at a dose higher than μM and not with G4 PAMAM dendrimers [30]. Also, in an *in vitro* blood-brain barrier (BBB) model, G4 PAMAM dendrimers were able to cross the BBB and induced Mac-1 (CD11b) and chemokine receptor type 2 (CCR2) overexpression in murine microglia. Interestingly, Serramía et al. avocaded the *in vivo* delivery in mice of siRNA-NEF to the brain by 2G cationic dendrimers against HIV-infected human primary astrocytes and achieved gene silencing without causing cytotoxicity [31].

2. Nose to brain transport pathways: A concise overview

The delivery of therapeutics to the brain remain one of the most challenging assignments due to 1) the anatomical and physiological aspects of the BBB, which limit the transfer of most drugs (mainly hydrophilic compounds) from the vascular compartment to the brain tissue, ergo treating CNS disorders and 2) inadequate drug profile, such as solubility limiting their bioavailability [32,33].

Nose to brain delivery has been displayed in both preclinical and clinical studies with a variety of formulations, which include nasal sprays, powders, gels, and nanoemulsions [34,35]. The olfactory nerve pathway should be the portal of entry of NPs into the human brain *via* the olfactory bulbs [36–38]. Several invasive approaches have been developed to bypass the BBB and deliver drugs directly into cerebral lesions, including incorporating intracerebroventricular (ICV) [39] and intracerebral/intraparenchymal [40] administration, convection-enhanced delivery (CED) [41], and intrathecal [42] and intratympanic administration [43]. In contrast, non-invasive approaches, such as IN delivery of CNS drugs [44,45], can be considered similar to systemic administration but without enzyme degradation and the first-pass effect that is usually observed after oral administration. IN administration can allow direct delivery of drugs into the brain through the direct anatomical connection between the nasal cavity and brain, large surface area available for drugs, and without crossing the BBB [46–49]. The volume and the surface area of the nasal cavity are 15–20 ml and 150–200 cm^2, respectively [50]. Consequently, the IN administration route circumvents drug elimination by the liver and gastrointestinal tract, as well as filtration

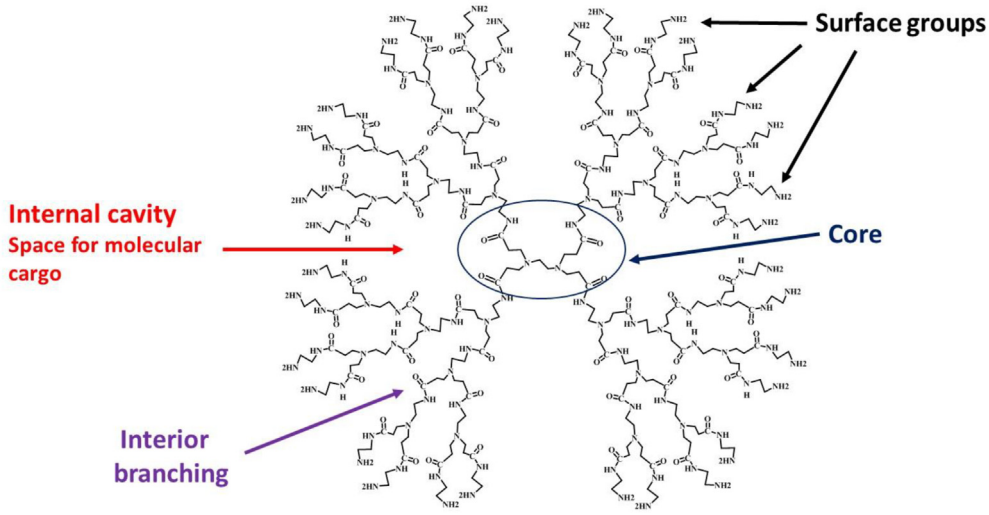


Fig. 1. 2D chemical structure of G4 PAMAM dendrimers with the different characteristics: core, surface, void space and interior branching.

through the kidney and degradation in the serum, increasing the pharmacokinetic/pharmacodynamic (PK/PD) profile and reducing systemic exposure, and thus the unwanted systemic side effects of CNS drugs [51]. It is hypothesized that drugs deposited on the nasal mucosa mainly travel to the brain *via* the olfactory neurons and via the trigeminal nerves [52], allowing the delivery of a high concentration of drug in different regions of the brain. This effect is based on the access to highly vascularized nasal mucosa for specific local brain delivery of CNS drugs. Diagram showing the three different pathways (DP-1, DP-2 and IDP-3) was depicted in Fig. 2. Importantly, the efficacy of the drug directly transported (DP-1 pathway) was strongly challenged. The amount of drug transported is less than 0.1% [53].

Drugs that are highly lipophilic with a low molecular weight showed high absorption (better nasal bioavailability) in the brain *via* the nasal mucosa. Importantly, about 98% of small molecules and almost all large proteins and genes are unable to cross the BBB [46,57]. Using IN administration, the bioavailability of larger drug molecules is improved. Useful methods for the administration of regular doses of IN drugs in awake non-anesthetized mice to target the brain without the use of anesthesia are highlighted [58]. Indeed, it is not trivial to administer IN drugs in mice. The technique can be learned and requires practice.

A notable review was published by Ul Islam and colleagues concerning the IN delivery of diverse brain targeting nanoformulations for the treatment of chronic neurological disorders such as Parkinson's Disease (PD), Alzheimer's Disease (AD), glioblastoma (GBM), epilepsy, multiple sclerosis, and cerebral palsy (CP) [59]. The most relevant and safe nanoformulations used are polymeric nanogels, polymeric nanoliposomes, niosomes, nanospheres and nanocapsules, polymeric nanomicelles, and dendrimers. For an example, a very interesting analysis was presented by Musumeci et al. concerning the use of polymeric nanoparticles, such as polylactic acid (PLA), poly (L-lactic-co-glycolide) (PLGA), polycaprolactone (PCL), gallen and xanthan gums, for the delivery of loaded antiepileptic drugs (small molecules), which include clonazepam, lorazepam, diazepam, lamotrigine, oxcarbazepine, clonazepam, and lamotrigine, as well thyrotropin-releasing

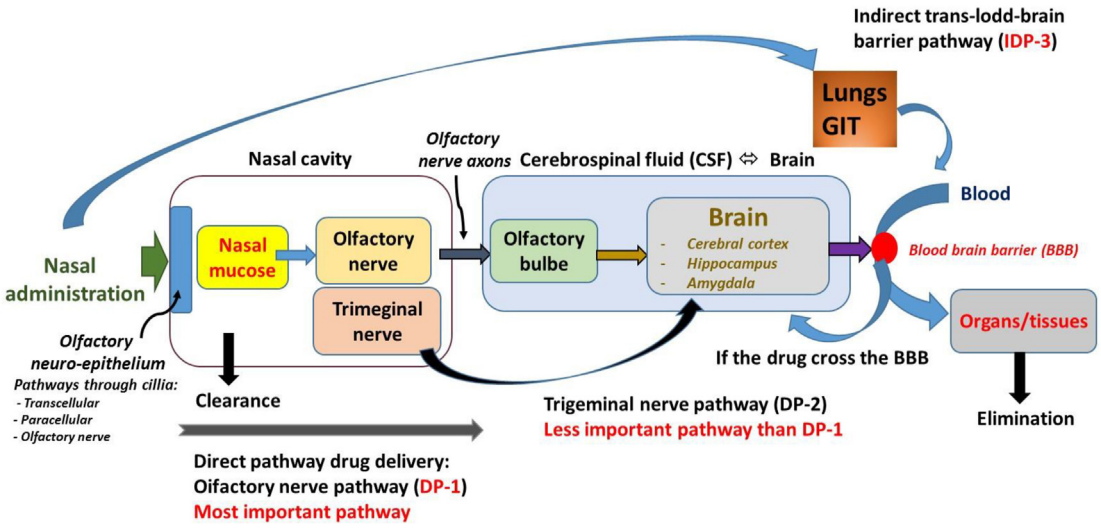


Fig. 2. Simplified pathways for target brain regions after intranasal administration. Adapted from Ref. [54–56] Three major nose-to-brain drug delivery pathways were highlighted 1) DP-1: major direct pathway trough olfactory nerve and olfactory bulb and then to brain; 2) DP-2: trigeminal nerve pathway (less important pathway); and 3) IDP-3: If the drugs can cross the BBB, they can reach the brain through indirect pathway: Lungs/Gastroinstinal tract (GIT) ⇨ blood ⇨ brain.

hormone (TRH) via IN administration [60]. *Ex vivo* and *in vivo* studies in several animals (*e.g.*, rabbits, rats, and mice) were performed. Interestingly, the authors listed the advantages and limitations of the use of IN administration for drug delivery. Fig. 3 summarized the global advantages and limitations of IN administration.

In order to overcome the very low drug transfer level using nasal solution showing low value of C_{max} (*e.g.* insulin, interferon [61]), nanoparticulate formulations, including nanoemulsions, lipids, or polymer particles, were developed to enhance the penetration of the drug as well the residence time within the nasal cavity [59,62]. Table 1 lists the major advantages and limitations of specific nanoformulations, except dendrimers (see conclusion), such as lipid-based nanoparticles, microemulsions and nanoemulsions, chitosan nanoparticles, PLGA nanoparticles and polymeric micelles for IN administration in drug delivery strategy.

3. Nose to brain transport pathways of drugs in association with PAMAM dendrimers using intranasal administration route

Notably, the effects of G0-G3 PAMAM dendrimers for the delivery of poorly absorbable drugs, such as insulin, using nasal absorption in rats were emphasized by Dong et al. [74] The nasal absorption of isothiocyanate-labeled dextran (average MW = 4400, FD4) was estimated by measuring the plasma insulin levels and glucose levels in rats. Five concentrations were used: 0.1% (w/v), 0.5% (w/v), 1% (w/v), 2% (w/v) and 5% (w/v).

Fig. 4 presents the PK parameters (*i.e.*, maximum (or peak) serum concentration (C_{max}), time to peak drug concentration (T_{max}), area under the curve (AUC) and absorption enhancement ratio) after IN administration of FD4 with various generations and concentrations of PAMAM dendrimers. The absorption enhancement ratios were calculated as the ratio of AUC values in the presence or absence of PAMAM dendrimers. For instance, these ratios were 1.0, 1.7, 2.6 and 3.4 for 5% (w/v) G0, 5% (w/v) G1, 5% (w/v) G2, and 5% (w/v) G3,

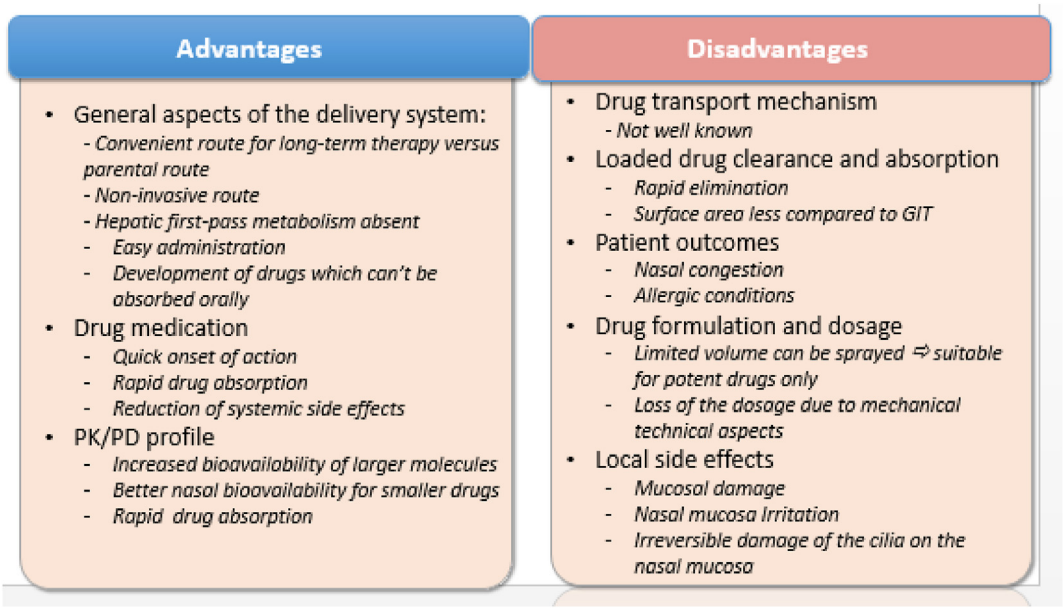


Fig. 3. General aspect of the major advantages and limitations of IN administration.

Table 1
Major advantages and limitations of specific nanoformulations such as lipid-based nanoparticles, microemulsions and nanoemulsions, chitosan nanoparticles, PLGA nanoparticles and polymeric micelles for IN administration.

Nanoformulation	Advantages	Limitations	Selected example of drug loaded	References
Lipid-based nanoparticles	Improved bioavaibility of loaded drug High biocompatibility and biodegradability of the nanoformulation	Low drug loaded for hydrophilic drugs Lack of wide clinical studies	Valproic acid [63]	[64]
Microemulsions and nanoemulsions	Strong thermodynamic and kinetic stabilities of the nanoformulation Improved bioavaibility of the loaded drug	Require large concentration of co-surfactants	Olanzapine [65]	[66]
Chitosan nanoparticles	Improved absorption of the loaded drug Low toxicity and high biocompatibility of the nanoformulation Improved bioavaibility of loaded drug	Synthesis issues	- Pramipexole [67] - siRNA [68]	[69]
PLGA nanoparticles	Low toxicity and high biodegrability of the nanoformulation Strong loading capacity of the drugs Extended controlled drug release of the drugs	Toxicity issues for several of them	Olanzapine [70]	[71]
Polymeric nanomicelles	Low toxicity of the nanoformulation Improved bioavaibility of the loaded drug	Low capacity to incorporated drugs versus liposomes Low *in vivo* stability	Lurasidone [72]	[73]

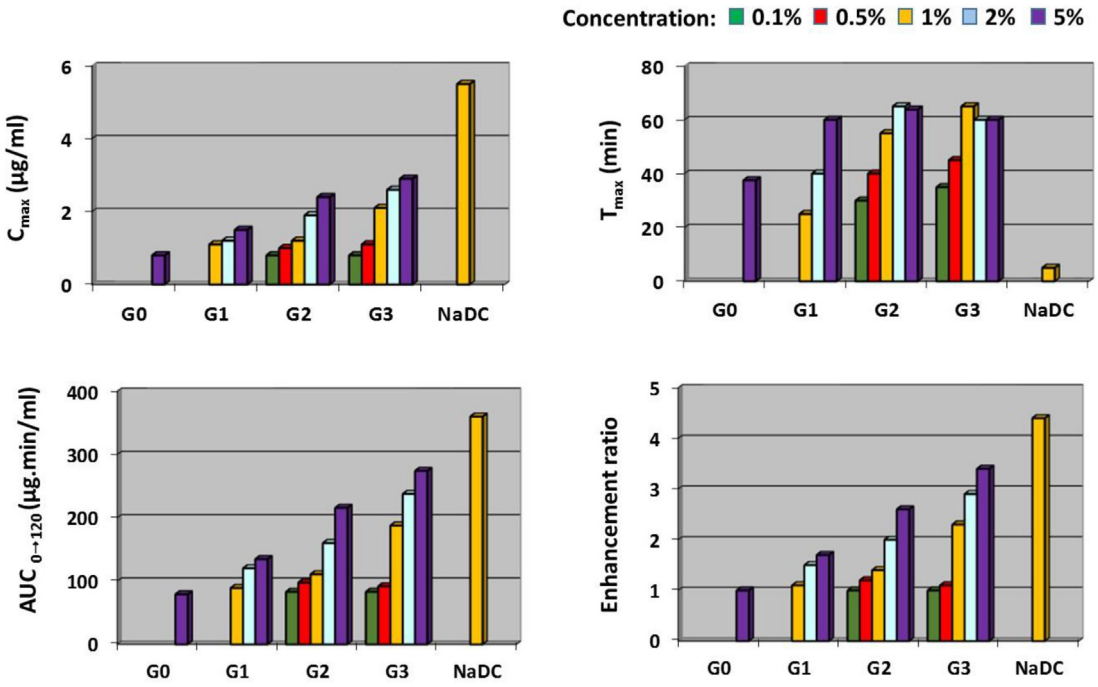


Fig. 4. PK profile parameters of FD4 with G0-G3 PAMAM dendrimers after nasal in rats.

respectively. For the same generation of PAMAM dendrimers, the absorption-enhancing effects were shown to be concentration- and generation-dependent: G3 > G2 > G1 > G0. After nasal absorption, all generations and concentrations of PAMAM dendrimers could increase the T_{max} of FD4, which was not always the case for C_{max} and AUC values of FD4. Consequently, PAMAM dendrimers prolonged the absorption time of FD4 after IN administration in rats. Interestingly, the G3 PAMAM dendrimer also improved the nasal absorption of macromolecules, such as fluorescein isothiocyanate-labeled dextran FD10 (MW = 9100), fluorescein isothiocyanate-labeled dextran D70 (MW = 71 600), insulin and calcitonin.

Based on the evaluation of total protein and lactate dehydrogenase (LDH) levels in nasal cavity lavage fluid, the toxicities of PAMAM dendrimers were determined. The damage to nasal tissue increased with the generation and concentration, but, very interestingly, it was lower than the standard positive control sodium deoxycholate (NaDC). As depicted in Figs. 4 and 2% and 5% (w/v) G2 and G3 PAMAM dendrimers significantly increased the amounts of total protein. Importantly, these increases were less than that caused by 1% (w/v) NaDC as a positive control.

As shown in Fig. 5, as for FD4, the C_{max}, T_{max} and AUC of FD10 and FD70 increased with the addition of 1% (w/v) G3 dendrimer,

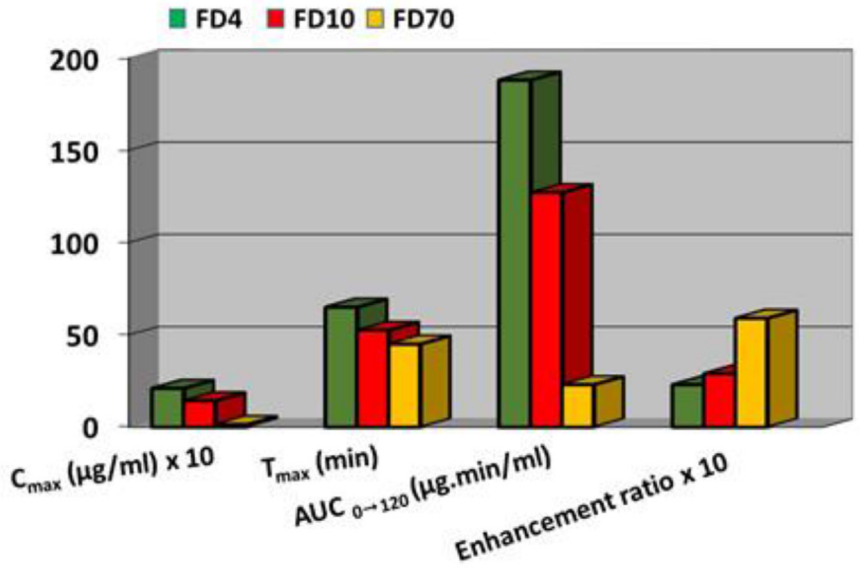


Fig. 5. PK parameters of FD4, FD10 and FD70 in rats.

suggesting that this dendrimer could increase the absorption of these drugs and prolong their absorption time after their IN administration.

Taken together, the studies showed that 1% (w/v) G3 PAMAM dendrimer displayed the strongest activity and lowest toxicity to deliver FD4.

As shown in Fig. 6, the nasal absorption of calcitonin, FD4, insulin, FD10 and FD70 with 1% (w/v) G3 dendrimer increased with the increase of their molecular weights. The evaluation of the positive zeta potential of the PAMAM dendrimers might trigger the absorption-enhancing effects of PAMAM dendrimers on the IN absorption of these macromolecules without any membrane damage to the nasal tissues.

Arguably, Katare and colleagues explored the effectiveness of dendrimers for the delivery, in the brain, of the antipsychotic drug haloperidol, which is a drug with poor water solubility, using G5 PAMAM dendrimers as nanocarriers [75]. Approximatively 40% of drug candidates showed poor water solubility, and consequently poor bioavailability, to be selected for clinical trials. In addition, haloperidol showed important side effects, such as catalepsy and motor suppression, in adult rats using acute administration [76]. Due to its poor water solubility, haloperidol can't be administered via the IN or intraperitoneal route without a soluble formulation. Dendrimers increased the water solubility of haloperidol by about 100 times, using a combination of 1% G5 PAMAM dendrimers, 20% ethanol and 20% Tween 20 to increase the haloperidol concentration up to 1223 μg/ml *versus* 11.5 μg/ml alone. The number of haloperidol molecules complexed with each dendrimer molecule is 9.4. No significant modifications of the zeta potential (~11–13 mV) or size (10–20 nm) were observed by the incorporation of haloperidol, allowing nose to brain transport as well colloid instabilities. As the size of G5 PAMAM dendrimers is ~5.4 nm, the formation of soluble aggregates can be envisaged due to the presence of Tween 20 as a surfactant [77].

Interestingly, from the G5 PAMAM dendrimer-haloperidol formulation (PAMAM-loaded haloperidol), haloperidol was released rapidly in 0.1 N HCl *versus* PBS. About 70% and 30% of haloperidol was released within the first hour in 0.1 N HCl and PBS, respectively. There were no significant differences in the binding efficacy on the D2 dopamine receptor with the different formulations, blank dendrimers (control) and dendrimer-haloperidol formulations. *In vivo* behavioral effects in adult rats showed that the dendrimer-haloperidol formulation displayed suppression of catalepsy after IN administration, which was similar to that obtained by IP administration with the same formulation at 30 min and 60 min after administration. No *in vivo* effect, based on catalepsy score, was observed by oral administration (gavage) of the dendrimer-haloperidol formulation, as well IP administration of haloperidol alone until 60 min after administration of the same dose of haloperidol. No *in vivo* effect, based on motor suppression effects, was observed 2 h after administration, IN and IP routes, contrary to the oral route with the dendrimer-haloperidol formulation or IP administration of haloperidol alone, showing a similar level of activity.

The concentration of haloperidol in the plasma and brain was evaluated, showing that the highest concentrations of haloperidol were found following IP administration of the dendrimer-haloperidol formulation compared to IN administration. The highest concentrations of haloperidol were obtained in olfactory and cerebellum tissues, which was better than the striatum and plasma, using IP administration of the dendrimer-haloperidol formulation versus IN administration. The ratio of percent of dose administered *per* g of tissue Intraperitoneal (IP) administration *versus* IN administration (IP/IN) for the cerebellum, olfactory bulb and striatum was ~1.8, ~1.6 and ~0.75, respectively, demonstrating the strong accumulation of haloperidol in these areas by the IP or IN route. The ratio of dose administered *per* ml of plasma was ~3 for the dendrimer-haloperidol formulation *versus* haloperidol alone by IP administration. In more detail, the ratio of percent dose per g of striatum to percent dose per ml of plasma was ~6, ~35 and ~15 for dendrimer-haloperidol formulation (IP), dendrimer-haloperidol formulation (IN) and haloperidol (IP), respectively. Clearly, these data suggested a higher percentage of haloperidol dose reached the striatum following IN administration compared to IP administration, subsequently inducing the behavior effects. PAMAM-loaded haloperidol improved brain concentrations after IN administration, with 6.7-fold lower doses of dendrimer-haloperidol formulation versus IP administration.

Another interesting approach to deliver drugs to the brain via nose was emphasized by Xie et al., using dendrimers *via in-situ* gel (Scheme 1) [78]. This approach used an *in situ* stable gel formation for IN administration [79]. For instance, polymers, almotriptan and opiorphin [80,81] were delivered to the brain via IN administration. The authors presented the physicochemical properties and

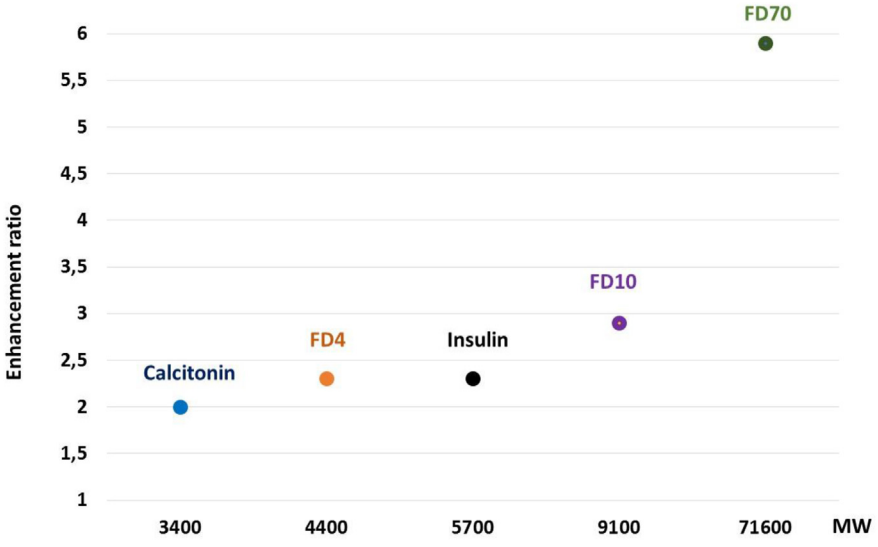
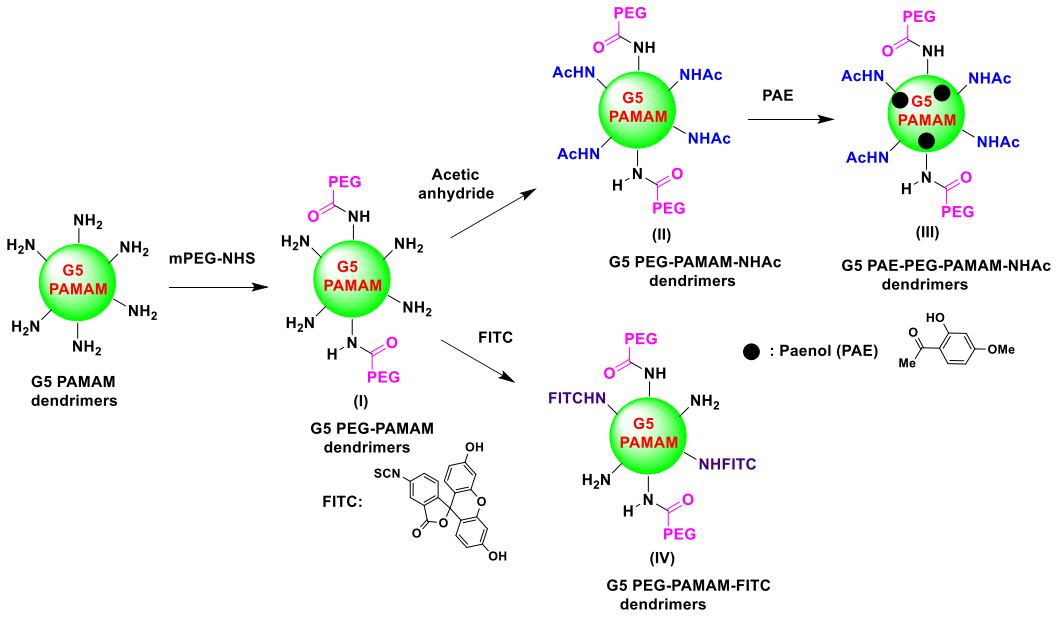


Fig. 6. Intranasal absorption of calcitonin, FD4, insulin, FD10 and FD70 with 1% (w/v) G3 dendrimer.



Scheme 1. Synthesis of G5 PEG-PAMAM-NHAc dendrimers (**II**), G5 PAE-PEG-PAMAM-NHAc (**III**) and G5 PEG-PAMAM-FITC dendrimers (**IV**).

biological effects of the nasal brain transport system using functionalized G5 PEG-PAMAM dendrimers prepared from G5 PAMAM dendrimers, bearing 128 primary amino groups, and encapsulating the poorly water soluble natural product paeonol (PAE, log P = 2.054, solubility = ~560 mg/ml in pH 7.4 PBS) as a central neuroprotective agent [82] and protection in rat hippocampal neurons [83] with *in situ* gel (Scheme 1). PAE has low bioavailability profile due to its rapid metabolism [84]. Two types of dendrimers were prepared: G5 PAE-polyethylene glycol (PEG)-PAMAM-NHAc (III) physically encapsulated with PAE in the void space of the dendrimer and G5 PEG-PAMAM-FITC (II) also for imaging purpose.

After PEGylation of the G5-PAMAM dendrimer, the G5 PEG-PAMAM dendrimers (I) had a size of 11.55 nm and zeta potential of +4.81 mV *versus* 5.41 nm and +8.23 mV for G5 PAMAM dendrimers. The introduction of a PEG chain on the surface of PAMAM dendrimers increased the shielding effect of PEG. The N-acetylation reaction of (I) affords (II) with significantly increased size (~71 nm) and decreased positive charge (+2.60 mV) due to changes in the conjugation of the PEG chains. No significant difference in size was observed by the encapsulation of PAE, corresponding to dendrimer (III) [72.4 nm *versus* (II)], indicating that the encapsulation of PAE occurred in internal cavities rather than complexed to the surface. The PAMAM dendrimers (III) and (IV) showed a zeta potential of +0.57 mV and +9.60, respectively. Both the morphology and size of lyophilized PAMAM dendrimers were evaluated by transmission electron microscopy (TEM) and scanning electron microscopy (SEM) techniques. Drug loading efficiency (amount of PAE in complexes/amount of complexes) and encapsulation efficiency (amount of PAE in complexes/amount of complexes of PAE added) of (III) were determined by HPLC and were ~54% and ~14%, respectively.

Interestingly, based on characterization on optimization of the *in-situ* gel (Table 1), stable gel was formed from (III)/deacetylated gellan gum (DGG) within 30 s based on Design Expert software (Stat-case Inc., USA). The formulation composition (%w/v) of *in situ* gel was: (III) = 1, DGG = 0.45, HMPC = 0.3, mannitol = 1, chlorine acetate = 0.01, vitamin E = 0.01 and distilled water = 100. The main characteristics of *in situ* gel are indicated in Table 2, indicating a high level of mucosal adhesion, indicating that the gel could remain in contact with the mucosa for a longer period of time, consequently increasing drug absorption. Rheology analysis demonstrated that the gel had a stable 3-D network space structure.

In vitro release studies of PAE from free PAE, (**III**) and (**III**)/DGG showed that 80% of the release rate of free PAE was observed at 2, 4 and 6 h, respectively, whereas ~100% of the release was observed at 4, 8 and 11 h, respectively. Clearly these data confirmed the sustained release of PEA from the internal cavities of PAMAM dendrimers based two consecutive processes: release of (**III**) from the *in-situ* gel and then PEA from the PAMAM dendrimers. These two processes were validated by two different kinetic models.

For biocompatibility purposes [86], the *in vitro* cytotoxicity of PAMAM dendrimers were evaluated using the cell viability of HepG2 cells and has the following order: G5 PAMAM dendrimers > (**I**) > (**II**) > PAE > (**III**). A concentration-dependent effect was observed with regard to the cytotoxicity, and the viability increased when the concentration of dendrimers decreased. No cytotoxicity was observed with a concentration <0.1 μM. The introduction of a PEG chain in G5 PAMAM dendrimers decreased its cytotoxicity due to the reduction of the exposure of amino groups on the surface, decreasing the interactions with the negatively charged cell membranes to lead to the rupture and

Table 2
Major characteristics of *in situ* gel formulation.

Viscosity	112 ± 3.2 mPa	Increased after gel phase transformation ⇨increased adhesion time of the mucosa ⇨ sustained release of the drug at specific absorption site [85] ⇨ decrease of the clearance of nasal mucus and cilia
Gel strength	28 ± 3 s	⇨ suitable for nasal absorption by decreasing the clearance of nasal mucus and cilia
Water-holding capacity	95.21% ± 1.58%	Good gel stability
pH	6.17 ± 0.17	No irritation of the nasal mucosa (nasal mucus pH: 5.5–7)

dissolution of cell membranes. For G5 PAMAM dendrimers and (**I**), about 50% of viability was obtained at ~10 μM, whereas for (**II**) and (**III**), 90–100% of viability occurred up to 100 μM. *In vitro* cellular uptake and localization of (**IV**) in HepG2 cells using confocal microscopy showed that (**IV**) exhibited strong fluorescence in the cytoplasm and nucleus of cells. Importantly, there was significant accumulation of (**III**)/DGG in the rat brain after IN administration and *in situ* gel formation, after phase transformation in the nasal mucus and then adhering to nasal mucosa, with a maximum accumulation after 12 h, which was also validated by fluorescent imaging technique using (**IV**). A small amount of accumulation of PAE was observed in solution with a maximum at 2 h. The accumulation of PEA with (**III**)/DGG was about 2.5 times higher than PAE in solution after 12 h (and similar after 2 h). Taken together, these *in vivo* experiments showed that, after IN administration, the combination within situ gel strongly increased the nasal absorption and nasal brain transport of PAE.

The delivery of siRNA to the brain remains a challenge due to the BBB preventing the passage of hydrophilic and relatively large molecules, such as siRNAs. Kim and colleagues investigated the specific complexation of high mobility group box-1 (HMGB1) siRNA with PAMAM dendrimers, as a gene vector, and the delivery of this complex in the post-ischemic rat brain through the IN route. HMGB1 acts as a pro-inflammatory cytokine, is released by necrotic cells, and secreted by macrophages and monocytes [87]. Localization of siRNA in the rat brain after IN delivery using fluorescent-labeled siRNA demonstrated that the maximum siRNA delivery efficiency occurred at a weight ratio of 5 (PAMAM/siRNA), versus 1.2, 2.5 and 10, in both primary cortical neuronal cultures and post-ischemic rat brain. The zeta potential at the weight ratio of 5 was +22.3 mV, and the particle size was ~189 nm. IN administration of 2 μg HMGB1 siRNA/PAMAM complexes at a weight ratio of 5, at 1 h after IN delivery, was significantly depleted in several regions of the brain, such as the striatum and pre-frontal cortex (pre-motor cortex). More interestingly, IN delivery of HMGB1 siRNA, 2 or 5 μg, strongly suppressed the cerebral infarct volume in rats after cerebral ischemia by a maximum of approximately 48% and 53%, respectively, at 48 h after 60 min of middle cerebral artery occlusion (MCAO), which is a focal cerebral ischemia model, and recovered from neurological and behavioral deficits (3 h pre- or 1 h post-MCAO) in the rotarod performance score test.

4. *In vivo* neurological effects of nasal exposure of PAMAM dendrimers

In vivo neurological effects of nasal exposure to G4 PAMAM dendrimers were highlighted by Win-Shwe et al. [54] A single dose of PAMAM dendrimers (3 or 15 μg/mouse) administered to old male BALB/c mice. Then, the animals were sacrificed, and the olfactory bulb, hippocampus, and cerebral cortex were collected. Neurological biomarkers in the blood and in the brain were analyzed. No significant change was observed in standard serum biochemical biomarkers versus the control. Consequently, no general toxicity was observed in the study. Microarray analyses demonstrated alterations (1.5-fold up-regulation or down-regulation) in several gene expression levels (up- or down-regulation), within a pluripotent network, such as the serotonin-anxiety pathway, TGF-beta receptor signaling, prostaglandin synthesis regulation, complement-coagulation cascades, chemokine signaling pathway and non-odorant GPCR signaling pathways in brain tissues, including the olfactory bulb, cerebral cortex, and hippocampus. In addition, a significant increase of the expression levels of neurotrophins, which target neurotoxicants and play a key role in neuroimmune responses [88], including *Ngf* and *Bdnf* observed (*via* mRNA expression evaluation) in the hippocampus and cerebral cortex at the dose of 15 μg PAMAM dendrimer in mice. Consequently, G4 PAMAM dendrimers appeared to be non-toxic, but the expression of some neurological-related genes was induced by high dose treatment.

5. Nose to brain transport pathways of drugs in association with hyperbranched polyglycerol dendritic nanoparticles using intranasal administration route

Interestingly, Zhang et al. described the preparation of a novel nanocarrier type based on the grafting of β-cyclodextrin (β-CD) with hyperbranched polyglycerols (HPGs) for nasal insulin delivery in diabetic rats [89]. This strategy avoided the proteolysis of insulin by reducing its bioavailability. Peptide and protein drugs showed low bioavailability through nasal administration due to their high molecular weight and hydrophilicity, despite the main advantage of the nasal administration which avoids the first-pass liver metabolism process (*vide supra*). HPGs are well-defined dendritic branched polymers with free hydroxyl groups. Controlled condensation of β-CD with HPG furnished the HPG-g-CD copolymers. It has been reported that cyclodextrins open the tight junctions so increasing the paracellular transport [90]. Three types of HPG-g-CD copolymers were prepared, HPG-*g*-CD1, HPG-*g*-CD2, and HPG-*g*-CD3 with different mass ratios of HPG/β-CD at 1:1, 1:2.5, and 1:5, respectively. Five ratios of polymer/insulin were developed at 0.2:1, 0.4:1, 1:1, 1.5:1, and 2:1. Interestingly, these NPs exhibited a strong capacity to encapsulate insulin with an efficiency of 88%. The size of the prepared NPs ranged between 200 and 340 nm, with a positive charge-based zeta potential evaluation of NPs with loaded insulin ranging between +10 and + 13 mV, and between +25 and + 43 mv without insulin, respectively. These results are favorable to get good biopharmaceutical properties of these NPs using IN. In vitro release studies of insulin with HPG-g-CD1, HPG-g-CD2 and HPG-g-CD3 indicated that the release rate of insulin was much faster under acidic pH (4.0, acetate buffer) than physiological pH in PBS. Insulin release is 75–90% after 10 h at pH = 4 versus 40% after 10 h at pH = 7.4. The cell viability assay in Caco2-2 cell line (MTT assay) demonstrated no toxic effects of HPG-g-CD1, HPG-g-CD2 and HPG-g-CD3 with a concentration up to 200 μg/mL. Importantly, significant *in vivo* efficiencies of insulin-loaded HPG-g-CD NPs in diabetic rats were obtained with HPG-g-CD1, HPG-g-CD2 and HPG-g-CD3 versus the control, due to the strong decrease of the blood glucose concentrations. A strong hypoglycemic effect was observed with a maximum glucose decrease of 80% at 2 h after dosing, and a low blood glucose level was maintained for about 4 h. Using the confocal laser scanning microscopy technique, it could be seen that HPG-g-CD1, HPG-g-CD2 and HPG-g-CD3 crossed the nasal mucosal epithelium.

6. Conclusion and perspectives

IN administration of drug delivery represents an attractive route which quickly and accurately accesses the brain, and it can enhance patient compliance regarding the administration of CNS drugs. The nose to brain delivery strategy, as a non-invasive technique, is a cost-efficient choice for the direct transport of drugs to the brain. The main advantages of IN administration are as follows: 1) non-invasive route; 2) absence of hepatic first-pass metabolism; 3) quick onset of action; 4) improvement of drug availability; and 5) convenient route, whereas the limitations are as follows: 1) histological toxicity; 2) nasal irritation; and 3) local side effects [91]. Nethertheless, clinical application of IN formulations remains a long way to develop. In addition, limitations of the IN drug delivery systems remains challenging such as poor drug permeability from nasal mucosa, mucocillary clearance low drug retention time, and

nasomucosal toxicity. Several strategies using auxiliary agents were proposed to solve these issues such as the use of permeation enhancers, mucolytic agents, mucoadhesive agents, *in situ* gelling agents, and enzyme inhibitors [92].

This review presented and analyzed the few examples of the association of dendrimer encapsulating drugs (*e.g.*, small compounds: haloperidol and PAE; macromolecular compounds: dextran, insulin and calcitonin; and siRNA) using IN administration. Good efficiencies were observed. In addition, we presented and discussed the *in vivo* effects of PAMAM dendrimers after IN administration.

The major advantages of dendrimers are as follows: 1) strong thermodynamic and kinetic stabilities; 2) low toxicity for several of them; 3) strong loading capacity of the drugs (small and large molecules); 4) improved bioavaibility of loaded drugs; and 5) site specific and controlled drug release, whereas the limitations are: 1) few clinical studies; 2) few *in vivo* examples using IN administration to set rules for drug development until clinical trials with dendrimers [93]; 3) few data about the role of the generation on the effectiveness. In this direction, in a recent tutorial review, Tomalia et al. analyzed the architectural components of branches of dendrimers in the development of drug delivery systems based on dendrimer encapsulation properties [94,95]. Several 'nano-periodic' property patterns were analyzed related to the generation of dendrimers such as congestion, surface chemistry, size, shape, flexibility and container properties. Similar studies will be important to be performed to understand in depth the role of dendritic generation in the effectiveness of IN administration.

We are convinced and strongly encouraged that this friendly route of administration for the treatment of acute and chronic conditions, for instance for elderly patients, requiring high drug exposure, such as cancers, that require life-long treatment or rapid action. In addition, within the dendrimer space [96], the use of dendrimers as nanocarriers and as active drugs per se in the oncology domain should be extended based on the following points: 1) to treat brain cancers, which are very difficult to tackle; 2) combination therapy [97] based on the release of several anticancer drug types, including natural products [98], siRNAs [99], mRNAs [100], antisense [101] and aptamers [102]; and 3) dendrimers as anticancer agents [103]. Importantly, this convenient non-invasive route easily allowed translation from the successes obtained in preclinical studies into human clinical studies as a smart system for all people suffering brain cancers.

Finally, as of May 30, 2020, over 6 million confirmed cases of COVID-19 and 370 000 deaths worldwide affording severe acute respiratory syndromes were listed. Currently no drugs are available for a fully effective treatment of COVID-19 which rapidly become a global pandemic [104]. Importantly, very recently, Starpharma announced the complete antiviral testing for the dendritic system SPL7013 (active principle of Vivagel) against SARS-CoV-2 using IN administration. More than 99% effectivity against Sar-Cov-2 was observed with higher selectivity index compared to antiviral remdesivir and hydroxychloroquine [105,106]. .

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

X. Shi, S. Mignani and J-P. Majoral thank the PRC NSFC-CNRS 2019 (21911530230 for X.S. and 199675 for S.M. and J-P.M). S. Mignani and X. Shi acknowledge the support of FCT-Fundação para a Ciência e a Tecnologia (Base Fund UIDB/00674/2020 and Programmatic Fund UIDP/00674/2020, Portuguese Government Funds) and ARDITI-Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação through the project M1420-01-0145-FEDER-000005-CQM+ (Madeira 14–20 Program). S. Mignani, A. Karpus and J-P. Majoral acknowledge transnational Euro-NanoMed III funded projects including a proper acknowledgement of ERANET EuroNanoMed III and the respective funding partner organizations. J. P. Majoral thanks CNRS (France) for financial support.

References

[1] R. Mohammad, Z.A. Qazi, Tajuddin, trends in nanotechnology for practical applications. Applications of targeted nano drugs and delivery systems, Chapter 11 (2019) 297–325.
[2] O. Farokhzad, C.; robert langer, R., impact of nanotechnology on drug delivery, ACS Nano 3 (2009) 16–20.
[3] G. Tiwari, R. Tiwari, B. Sriwastawa, L. Bhati, S. Pandey, P. Pandey, S.K. Bannerjee, Drug delivery systems: an updated review, Int. J. Pharm. Investig. 2 (2012) 2–11.
[4] J.H. Lee, Y. Yeo, Controlled drug release from pharmaceutical nanocarriers, Chem. Eng. Sci. 125 (2015) 75–84.
[5] M. Athar, J.A. Das, Therapeutic nanoparticles: state of the art of the medicine, Adv. Mater. Rev. 1 (2014) 25e37.
[6] S.R. Mudshinge, A.B. Deore, S. Patil, C.M. Bhalgat, Nanoparticles: emerging carriers for drug delivery, Saudi Pharmaceut. J. 19 (2011) 129–141.
[7] S. Parveen, R. Misra, S.S.K. Sahoo, Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging, Nanomed. Nanotechnol. Biol. Med. 8 (2012) 147–166.
[8] J. Khandare, M. Calderon, N.M. Dagia, R. Haag, Multifunctional dendritic polymers in nanomedicine: opportunities and challenges, Chem. Soc. Rev. 41 (2012) 2824–2848.
[9] Kannan, R. M.; Nance, E.; Kannan, S.; Tomalia, D. A., Emerging concepts in dendrimer-based nanomedicine: from design principles to clinical applications. J. Intern. Med. 214, 276, 579–617..
[10] D.A. Tomalia, L.S. Nixon, D.M. Hedstrand, The role of branch cell symmetry and other critical nanoscale design parameters in the determination of dendrimer encapsulation properties, Biomolecules 10 (2020) 642.
[11] E. Abbasi, S.F. Fekri Aval, Akbarzadeh, M. Milani, H.T. Nasrabadi, S.W. Joo, Y. Hanifehpour, K. Nejati-Koshki, R. Pashaei-Asl, Dendrimers: synthesis, applications, and properties, Nanoscale Res. Lett. (2014) 9. Article number: 247.
[12] A. Santos, F. Veiga, A. Figueiras, Dendrimers as pharmaceutical excipients: synthesis, properties, toxicity and biomedical applications, Materials 13 (2020) 65.
[13] T.D. McCarthy, P. Karellas, S. Henderson, M. Giannis, D.F. O'Keefe, G. Heery, J.R.A. Paull, B.R. Matthews, G. Holan, Dendrimers as Drugs: discovery and preclinical and clinical development of dendrimer-based microbicides for HIV and STI prevention, Mol. Pharm. 2 (2005) 312–318.
[14] D. Maciel, C. Guerrero-Beltrán, R. Ceña-Diez, H. Tomás, M. Muñoz-Fernández, J.M. Rodrigues, New anionic poly(alkylideneamine) dendrimers as microbicide agents against HIV-1 infection, Nanoscale 11 (2019) 969–9690.
[15] H. Holota, J. Magiera, S. Michlewska, M. Kubczak, N. Sanz del Olmo, S. Garcia-Gallego, P. Ortega, F. Javier de la Mata, M. Ionov, M. Bryszewska, In vitro anticancer properties of copper metallodendrimers, Biomolecules (2019) 9.
[16] M. Gouveia, J. Figueira, M.G. Jardim, R. Castro, H. Tomas, K. Rissanen, J. Rodrigues, Poly(alkylidenimine) dendrimers functionalized with the organometallic moiety $[Ru(\eta^5\text{-}C_5H_5)(PPh_3)_2]^+$ as promising drugs against cisplatin-resistant cancer cells and human mesenchymal stem cells, Molecules 23 (2018) 1471.
[17] Y.M. Chabre, R. Roy, in: D. Horton (Ed.), Design and Creativity in Synthesis of Multivalent Neoglycoconjugates, vol. 63, 2010, pp. 165–393.
[18] R. Shukla, T.P. Thomas, J. Peters, A. Kotlyar, A. Myc, J.R. Baker, Tumor angiogenic vasculature targeting with PAMAM dendrimer–RGD conjugates, Chem. Commun. (2005) 5739.
[19] A.M. Holmes, J.R. Heylings, K.W. Wan, G.P. Moss, Antimicrobial efficacy and mechanism of action of poly(amidoamine) (PAMAM) dendrimers against opportunistic pathogens, Int. J. Antimicrob. Agents 53 (2019) 500–507.
[20] L. Chen, S. Mignani, A.M. Caminade, J.-P. Majoral, Metal-based phosphorus dendrimers as novel nanotherapeutic strategies to tackle cancers: a concise overview, Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol. (2019) 11.
[21] L. Chen, J. Li, Y. Fan, J. Qiu, L. Cao, R.;S.,M. Laurent, A.-M. Caminade, J.-P. Majoral, X. Shi, Revisiting cationic phosphorus dendrimers as a nonviral vector for optimized gene delivery towards cancer therapy applications, Biomacromolecules (2020), https://doi.org/10.1021/acs.biomac.0c00458.
[22] M. Hayder, M. Poupot, M. Baron, D. Nigon, C.O. Turrin, A.M. Caminade, J.-P. Majoral, R.A. Eisenberg, J.J. Fournié, A. Cantagrel, R. Poupot, D. J-L, A phosphorus-based dendrimer targets inflammation and osteoclastogenesis in experimental arthritis, Sci. Transl. Med. 81 (2011), 81ra35.
[23] S. Mignani, S. El Kazzouli, M. Bousmina, J.-P. Majoral, Expand classical drug administration ways by emerging routes using dendrimer drug delivery

systems: a Concise overview, Adv. Drug Deliv. Rev. 35 (2013) 1316–1330.
[24] Y. Cheng, Z. Xu, M. Ma, T. Xu, Dendrimers as drug carriers: applications in different routes of drug administration, J. Pharm. Sci. 97 (2008) 123–143.
[25] R. Saikat, What is meant by the First-Pass Effect of drugs?. https://socratic.org/questions/what-is-meant-by-the-first-pass-effect-of-drugs, 2016.
[26] Drug absorption, distribution and44 elimination, pharmacokinetics. http://www.columbia.edu/itc/gsas/g9600/2004/GrazianoReadings/Drugabs.pdf.
[27] Merck Manual Professional version. https://www.merckmanuals.com/professional/clinical-pharmacology/pharmacokinetics/drug-absorption.
[28] Y. Zhu, C. Liu, Z. Pang, Dendrimer-based drug delivery systems for brain targeting, Biomolecules 9 (2019) 790, https://doi.org/10.3390/biom9120790.
[29] S. Mignani, J.-P. Majoral, Dendrimers as macromolecular tools to tackle from colon to brain tumor types : a concise overview, New J. Chem. 37 (2013) 3337–3357.
[30] L. Albertazzi, L. Gherardini, M. Brondi, S.S. Sulis, A. Bifone, T. Pizzorusso, G.M. Ratto, G. Bardi, In vivo distribution and toxicity of PAMAM dendrimers inthe central nervous, Mol. Pharm. 10 (2013) 249–260.
[31] J. Serramía, S. Susana Álvarez, E. Elena Fuentes-Paniagua, M.I. Clemente, J. Sánchez-Nieves, R. Rafael Gómez, J. de la Mata, A. Muñoz-Fernández, In vivo delivery of siRNA to the brain by carbosilane dendrimer, J. Contr. Release 200 (2015) 60–70.
[32] S. Svenson, A.S. Chauhan, Dendrimers for enhanced drug solubilization, Nanomedicine 3 (2008) 679–702.
[33] H. Kulhari, D. Pooja, S.K. Prajapati, A.S. Chauhan, Performance evaluation of PAMAM dendrimer based simvastatin formulations, Int. J. Pharm. 405 (2011) 203–209.
[34] A. Patel, N. Surti, A. Mahajan, Intranasal drug delivery: novel delivery route for effective management of neurological disorders, J. Drug Deliv. Sci. Technol. 52 (2019) 130–137.
[35] M.C. Bonferoni, S. Rossi, G. Sandri, F. Ferrari, E. Gavini, G. Rassu, P. Giunchedi, Nanoemulsions for “nose-to-brain” drug delivery, Pharmaceutics 11 (2019) 84.
[36] G. Oberdörster, Z. Sharp, V. Atudorei, A. Elder, R. Gelein, W. Kreyling, C. Cox, Translocation of inhaled ultrafine particles to the brain, Inhal. Toxicol. 16 (2004) 437–445.
[37] A.J.D. De Lorenzo, The olfactory neuron and the blood–brain barrier, in: G.E.W. Wolstenholme, J. Knight (Eds.), Taste and Smell in Vertebrates, J. & A.Churchill, London, UK, 1970, pp. 151–176.
[38] A. Elder, R. Gelein, V. Silva, T. Feikert, L. Opanashuk, J. Carter, R. Potter, A. Maynard, Y. Ito, J. Finkelstein, G. Oberdorster, Translocation of inhaled ultra-fine manganese oxide particles to the central nervous system, Environ. Health Perspect. 114 (2006) 1172–1178.
[39] T. Siegal, R. Rubinstein, F. Bokstein, A. Schwartz, A. Lossos, E. Shalom, R. Chisin, J.M. Gomori, In vivo assessment of the window of barrier opening after osmotic blood-brain barrier disruption in humans, J. Neurosurg. 92 (2000) 599–605.
[40] M.F. Bennewitz, W.M. Saltzman, Nanotechnology for delivery of drugs to the brain for epilepsy, Neurotherapeutics 6 (2009) 323–336.
[41] B.S. Carson, Q. Wu, B. Tyler, L. Sukay, R. Raychaudhuri, F. DiMeco, R.E. Clatterbuck, A. Olivi, M. Guarnieri, New approach to tumor therapy for inoperable areas of the brain: chronic intraparenchymal drug delivery, J. Neuro Oncol. 60 (2002) 151–158.
[42] X. Yi, D.S. Manickam, A. Brynskikh, A.V. Kabanov, Agile delivery of protein therapeutics to CNS. J. Contr. Release 190 (2014) 637–663.
[43] X. Zhang, G. Chen, L. Wen, F. Yang, A.-L. Shao, X. Li, W. Long, L. Mu, Novel multiple agents loaded PLGA nanoparticles for brain delivery via inner ear administration: in vitro and in vivo evaluation, Eur. J. Pharmaceut. Sci. 48 (2013) 595–603.
[44] F. Erdőa, L.A. Borsa, D. Farkasa, A. Bajzaa, S. Gizurarsonb, Evaluation of intranasal delivery route of drug administration for brain targeting 143 (Brain Res. Bull.) (2018) 155–170.
[45] L.R. Hanson, W.H. Frey, Intranasal delivery bypasses the blood-brain barrier to target therapeutic agents to the central nervous system and treat neurodegenerative disease, BMC Neurosci. 9 (Suppl 3) (2008) S5, https://doi.org/10.1186/1471-2202-9-S3-S5, 2008, 9 (Suppl.3).
[46] S.V. Dhuria, L.R. Hanson, W.H. Frey, Intranasal delivery to the central nervous system: mechanisms and experimental considerations, J. Pharm. Sci. 99 (2010) 1654–1673.
[47] C. Marianecci, F. Rinaldi, P.N. Hanieh, L. Di Marzio, D. Paolino, M. Carafa, Drug delivery in overcoming the blood–brain barrier: role of nasal mucosal grafting, Drug Des. Dev. Ther. 11 (2017) 325–335.
[48] F.W.H.M. Merkus, M.P. van den Berg, Can nasal drug delivery bypass the blood-brain barrier? Drugs R 8 (2007) 133–144.
[49] K. Selvaraj, K. Gowthamarajan, V.V.S.R. Karri, Nose to brain transport pathways an overview: potential of nanostructured lipid carriers in nose to brain targeting, Artif. Cell Nanomed. B 46 (2018) 2088–2095.
[50] M. Kapoor, J.C. Cloyd, R.A. Siegel, A review of intranasal formulations for the treatment of seizure emergencies, J. Contr. Release 237 (2016) 147–159.
[51] T.P. Crowea, H.W. West Greenlee, A.G. Kanthasamy, W.H. Hsu, Mechanism of intranasal drug delivery directly to the brain, Life Sci. 195 (2018) 44–52.
[52] Z. Wang, G. Xiong, W.C. sang, A.G. Schätzlein, I.F. Uchegbu, Nose to brain delivery, J. Pharmacol. Exp. Therapeut. 370 (2019) 593–601.
[53] A. Mistry, S. Stolnik, L. Illum, Nanoparticles for direct nose-to-brain delivery of drugs, Int. J. Pharm. 379 (2009) 146–157.
[54] T.-T. Win-Shwe, H. Sone, Y. Kurokawa, Y. Zeng, Q. Zeng, H. Nitta, S. Hirano, Effects of PAMAM dendrimers in the mouse brain after a singleintranasal instillation, Toxicol. Lett. 228 (2014) 207–215.
[55] C.V. Pardeshi, V.S. Belgamwar, Direct nose to brain drug delivery via integrated nerve pathways bypassing the blood–brain barrier: an excellent platform for brain targeting, Expet Opin. Drug Deliv. 10 (2013) 957–972.
[56] Y. Feng, H. He, F. Li, Y. Lu, J. Qi, W. Wu, An update on the role of nanovehicles in nose-to-brain drug delivery, Drug Discov. Today 23 (2018) 1079–1088.
[57] W.M. Pardridge, The blood-brain barrier: bottleneck in brain drug development, NeuroRx 2 (2005) 3–14.
[58] L.R. Hanson, J.M. Fine, A.L. Svitak, K.A. Faltesek, Intranasal administration of CNS therapeutics to awake mice, JoVE 74 (2013) 4440.
[59] S. Ul Islam, A. Shehzad, M. Bilal Ahmed, Y.S. Lee, Intranasal delivery of nanoformulations: a potential way of treatment for neurological disorders, Molecules 25 (2020) 1929.
[60] T. Musumeci, A. Bonaccorso, G. Puglisi, Epilepsy disease and nose-to-brain delivery of polymeric nanoparticles: an overview, Pharmaceutics 11 (2019) 118.
[61] R. Thorne, L. Hanson, T. Ross, D. Tung, W. Frey Ii, Delivery of interferon- to the monkey nervous system following intranasal administration, Neuroscience 152 (2008), 785–79.
[62] Z. Patel, B. Patel, S. Patel, C. Pardeshi, Nose to brain targeted drug delivery bypassing the blood-brain barrier: an overview. Drug Invent. Today 4 (2012) 610–615.
[63] S. Eskandari, J. Varshosaz, M. Minaiyan, M. Tabbakhian, Brain delivery of valproic acid via intranasal administration of nanostructured lipid carriers: in vivo pharmacodynamic studies using rat electroshock model, Int. J. Nanomed. 6 (2011) 363–371.
[64] N. Yadav, S. Khatak, Solid lipid nanoparticles-a review, Int. J. Appl. Pharm. 5 (2013) 8–18.
[65] M. Kumar, A. Misra, A. Mishra, P. Mishra, K. Pathak, Mucoadhesive nanoemulsion-based intranasal drug delivery system of olanzapine for brain targeting, J. Drug Target. 16 (2008) 806–814.
[66] H.Y. Karasulu, Microemulsions as novel drug carriers: the formation, stability, applications and toxicity, Expet Opin. Drug Deliv. 5 (2008) 119–135.
[67] R. Raj, S. Wairkar, V. Sridhar, R. Gaud, Pramipexole dihydrochloride loaded chitosan nanoparticles for nose to brain delivery: development, characterization and in vivo anti-Parkinson activity, Int. J. Biol. Macromol. (2018) 27–35.
[68] M. Van Woensel, T. Mathivet, N. Wauthoz, R. Rosière, A.D. Garg, P. Agostinis, V. Mathieu, R. Kiss, F. Lefranc, L. Boon, Sensitization of glioblastoma tumor micro-environment to chemo-and immunotherapy by Galectin-1 intranasal knock-down strategy, Sci. Rep. 7 (2017) 1217.
[69] N. Csaba, M. Garcia-Fuentes, M.J. Alonso, The performance of nanocarriers for transmucosal drug delivery, Expet Opin. Drug Deliv. (2006) 463–478.
[70] U. Seju, A. Kumar, K. Sawant, Development and evaluation of olanzapine-loaded PLGA nanoparticles for nose-to-brain delivery: in vitro and in vivo studies, Acta Biomater. 7 (2011) 4169–4176.
[71] F. Danhier, E. Ansorena, J.M. Silva, R. Coco, A. Le Breton, V. Préat, PLGA-based nanoparticles: an overview of biomedical applications, J. Contr. Release 161 (2012) 505–522.
[72] V. Pokharkar, S. Suryawanshi, V. Dhapte-Pawar, Exploring micellar-based polymeric systems for effective nose-to-brain drug delivery as potential neurotherapeutics, Drug Deliv. and Transl. Res. 10 (2020) 1019–1031.
[73] R. Jayaraj, V. Chandramohan, E. Namasivayam, Nanomedicine for Parkinson disease: current status and future perspective, Int. J. Pharm. Bio. Sci. 4 (2013) 692–704.
[74] Z. Dong, H. Katsumi, T. Sakane, A. Yamamoto, Effects of polyamidoamine (PAMAM) dendrimers on the nasal absorption of poorly absorbable drugs in rats, Int. J. Pharm. 393 (2010) 244–252.
[75] Y.K. Katare, R.D. Daya, C.S. Gray, R.E. Luckham, J. Bhandari, A.S. Chauhan, R.K. Mishra, Brain targeting of a water insoluble antipsychotic drug haloperidol via the intranasal route using PAMAM dendrimer. Mol, Pharmaceutics 12 (2015) 3380–3388.
[76] J.L. Wiley, R.L. Evans, Evaluation of age and sex differences in locomotion and catalepsy during repeated administration of haloperidol and clozapine in adolescent and adult rats, Pharmacol. Res. 58 (2008) 240–246.
[77] Y.Y. Cheng, Q.L. Wu, Y.W. Li, J.J. Hu, T.W. Xu, New insights into the interactions between dendrimers and surfactants: 2. Design of new drug formulations based on dendrimer-surfactant aggregates, J. Phys. Chem. B 113 (2009) 8339–8346.
[78] H. Xie, L. Li, Y. Sun, Y. Wang, S. Gao, Y. Tian, X. Ma, C. Guo, F. Bo, L. Zhang, An available strategy for nasal brain transport of nanocomposite based on PAMAM dendrimers via in situ gel, Nanomaterials 9 (2019) 147.
[79] R.M. Singh, A. Kumar, K. Pathak, Mucoadhesivein situnasal gelling drug delivery systems for modulated drug delivery, Expet Opin. Drug Deliv. 10 (2012) 115–130.
[80] N. Youssef, A.A. Kassem, R.M. Farid, F.A. Ismail, M.A.E. El-Massik, N.A. Boraie, A novel nasal almotriptan loaded solid lipid nanoparticles in mucoadhesive in situ gelformulation for brain targeting: preparation, characterization and in vivo evaluation, Int. J. Pharm. 548 (2018) 609–624.
[81] P. Mura, N. Mennini, C. Nativi, B. Richichi, In situ mucoadhesive-thermosensitive liposomal gel as a novel vehicle for nasal extended delivery of opiorphin, Eur. J. Pharm. Biopharm. 122 (2018) 54–61.
[82] L. He, X. Tong, J. Zeng, Y. Tu, S. Wu, M. Li, H. Deng, M. Zhu, X. Li, H. Nie, H. Yang, F. Huang, Paeonol suppresses neuroinflammatory responses in LPS-

activated microglia cells, Inflammation 39 (2016) 1904–1917.
[83] J. Wu, N. Song, X. Wei, H. Guan, X. Zhang, Protective effects of paeonol on cultured rat hippocampal neurons against oxygen-glucose deprivation-induced injury, J. Neurol. Sci. 264 (2008) 50–55.
[84] L.Q. Ding, T.Y. Qiu, Z.X. Liu, L.X. Chen, M.B. Oppong, D.Q. Zhang, B.L. Zhang, G. Bai, F. Qiu, Systematic characterization of the metabolites of paeonol in rats using ultra performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry with an integrative strategy, J. Chromatogr. Biomed. Appl. 1065–1066 (2017) 70–78.
[85] H.S. Mahajan, S. Gattani, In situ gels of Metoclopramide Hydrochloride for intranasal delivery: in vitro evaluation and in vivo pharmacokinetic study in rabbits, Drug Deliv. 17 (2010) 19–27.
[86] T. Lv, T. Yu, Y. Fang, S. Zhang, M. Jiang, H. Zhang, Y. Zhang, Z. Li, H. Chen, Y. Gao, Role of generation on folic acid-modified poly (amidoamine)dendrimers for targeted delivery of baicalin to cancer cells. Mater. Sci. Eng. C Mater. Biol. Appl. 75 (2017) 182–190.
[87] Wang, H.; O Bloom; Zhang, O.; Vishnubhakat, J. M.; Ombrellino, M.; Che, J.; Frazier, A.; Yang, H.; Ivanova, S.; Borovikova, L.; Manogue, K. R.; Faist, E.; Abraham, E.; Andersson, J.; Andersson, U.; Molina, P. E.; Abumrad, N. N.; Sama, A.; Tracey, K. J., HMG-1 as a late mediator of endotoxin lethality in mice. *Science* 199, 285, 248–251.
[88] L. Aloe, S.D. Skaper, A. Leon, R. Levi-Montalcini, Nerve growth factor andautoimmune diseases, Autoimmunity 19 (1994) 141–150.
[89] X. Zhang, X. Zhang, Z. Wu, Z. Gao, S. Shu, Z. Wang, C. Li, β-Cyclodextrin grafting hyperbranched polyglycerols as carriers for nasal insulin delivery, Carbohydr. Polym. 84 (2011) 1419–1425.
[90] M. Hinchcliffe, L. Illum, Intranasal insulin delivery and therapy, Adv. Drug Deliv. Rev. 35 (1999) 199–234.
[91] J. Rohrer, N. Lupo, A. Bernkop-Schnürch, Advanced formulations for intranasal delivery of biologics, Int. J. Pharm. 553 (2018) 8–20.
[92] A. Alexander, S. Dwivedi, T.K. Giri, S. Saraf, S. Saraf, D.K. Tripathi, Approaches for breaking the barriers of drug permeation through transdermal drug delivery, J. Contr. Release 164 (2012) 26–40.
[93] S. Mignani, J. Rodrigues, H. Tomas, R. Roy, X. Shi, J.-P. Majoral, Bench-to-bedside translation of dendrimers: reality or utopia? A concise analysis, Adv. Drug Deliv. Rev. 136–137 (2018) 73–81.
[94] D.A. Tomalia, Linda S. Nixon, D.M. Hedstrand, The role of branch cell symmetry and other critical nanoscale design parameters in the determination of dendrimer encapsulation properties, Biomolecules 10 (2020) 642.
[95] S. Mignani, X. Shi, J. Rodrigues, R. Roy, A. Muñoz-Fernández, V. Ceña, J.P. Majoral, Dendrimers toward translational nanotherapeutics: concise key step analysis, Bioconjugate Chem. 31 (2020) 2060–2071.
[96] S. Mignani, S. El Kazzouli, M.M. Bousmina, J.-P. Majoral, Dendrimer space concept for innovative nanomedicine: a futuristic vision for medicinal chemistry, Prog. Polym. Sci. 38 (2013) 993–1008.
[97] S. Mignani, M. Bryszewska, B. Klajnert-Maculewicz, M. Zablocka, J.-P. Majoral, Advances in combination t herapies based on nanoparticles for efficacious cancer treatment: an analytical report, Biomacromolecules 16 (2015) 1–27.
[98] S. Mignani, J. Rodrigues, H. Tomas, M. Zablocka, X. Shi, A.M. Caminade, J.-P. Majoral, Dendrimers in combination with natural products and analogues as anti-cancer agents, Chem. Soc. Rev. 47 (2018) 514–532.
[99] S. Biswas, V.P. Torchilin, Dendrimers for siRNA delivery, Pharmaceuticals 6 (2013) 161–183.
[100] C. Song, Y. Xiao, Z. Ouyang, M. Shen, X. Shi, Efficient co-delivery of microRNA 21 inhibitor and doxorubicin to cancer cells using core–shell tecto dendrimers formed via supramolecular host–guest assembly, J. Mater. Chem. B 8 (2020) 2768–2774.
[101] S. Mignani, X. Shi, M. Zablocka, J.-P. Majoral, Dendrimer-enabled therapeutic antisense delivery systems as innovation in medicine, Bioconjugate Chem. 30 (2019) 1938–1950.
[102] S. Mignani, X. Shi, V. Ceña, J.-P. Majoral, Dendrimer– and polymeric nanoparticle–aptamer bioconjugates as nonviral delivery systems: a new aapproach in medicine, Drug Discov. Today 25 (2020) 1065–1073.
[103] S. Mignani, N. El Brahmi, S. El Kazzouli, R. Laurent, S. Sonia Ladeira, A.-M. Caminade, E. Pedziwiatr-Werbicka, E.M. Szewczyk, M. Bryszewska, M.M. Bousmina, T. Cresteil, J.-P. Majoral, Original multivalent gold(III) and dual gold(III)–copper(II) conjugated phosphorus dendrimers as potent antitumoral and antimicrobial agents, Mol. Pharm. 14 (2017) 4087–4097.
[104] Y. Duan, Y. Yao, S.A. Kumar, H.-L. Zhu, J. Chang, Current and future therapeutical approaches for COVID-19, Drug Discov. Today 25 (2020) 1545–1552.
[105] R. Wilson, A. Chan, Starpharma holdings limited. https://www.afp.com/ar/news/1312/starpharmas-spl7013-covid-19-nasal-spray-virucidal-against-sars-cov-2-202009140052971.
[106] https://www.coronavirus-journal.fr/spl7013-covid-19-spray-nasal-star-pharma-virucide-sras-cov-2/.