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Drug delivery to the central nervous system by polymeric nanoparticles: What do we know?☆

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ABSTRACT

Nanoparticles enable the delivery of a great variety of drugs including anticancer drugs, analgesics, anti-Alzheimer's drugs, cardiovascular drugs, protease inhibitors, and several macromolecules into the brain after intravenous injection of animals. The mechanism of the nanoparticle-mediated drug transport across the BBB appears to be receptor-mediated endocytosis followed by transcytosis into the brain or by drug release within the endothelial cells. Modification of the nanoparticle surface with covalently attached targeting ligands or by coating with certain surfactants that lead to the adsorption of specific plasma proteins after injection is necessary for this receptor-mediated uptake. A very critical and important requirement for nanoparticulate brain delivery is that the employed nanoparticles are biocompatible and, moreover, rapidly biodegradable, i.e. over a time frame of a few days. In addition to enabling drug delivery to the brain, nanoparticles, as with doxorubicin, may importantly reduce the drug's toxicity and adverse effects due to an alteration of the body distribution. Because of the possibility to treat severe CNS diseases such as brain tumours and to even transport proteins and other macromolecules across the blood–brain barrier, this technology holds great promise for a non-invasive therapy of these diseases.

Contents

1. Introduction

The blood–brain barrier (BBB) represents an insurmountable obstacle for most drugs, including neurological drugs, cytostatics, antibiotics, etc. One efficient possibility to deliver drugs including peptides [1–3] and even macromolecules [4] across this barrier is the employment of polymeric nanoparticles. This possibility was recently summarised in a short review in this journal [5]. Unfortunately, previous reviews frequently cite similar references and highlight similar points, often for studies that are repetitive or incremental over time. Focus on increasingly important pharmacological effects achieved with nanoparticle-based delivery as well as studies regarding mechanisms of nanoparticle-

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mediated drug transport are not often analysed. This review seeks to achieve this goal.

The above mentioned reports [1–3] demonstrate that overcoating of drug-loaded biodegradable poly(butyl cyanoacrylate) nanoparticles with certain surfactants such as polysorbate 80 (Tween® 80) or poloxamer 188 (Pluronic® F68) yields significant dose- and time-dependent [6] pharmacological effects in the CNS after intravenous injection into mice and also rats, whereas all the controls, including drug solution, empty nanoparticles, polysorbate 80 solution, simple mixtures of the components, i.e. nanoparticles, drug, and polysorbate 80, or uncoated drug-loaded nanoparticles did not achieve such effects [7]. These results clearly showed that the drugs indeed were transported across the BBB by the polysorbate-coated particles [1,8]. Similar results were obtained by overcoating of the poly(butyl cyanoacrylate) nanoparticles with polysorbate 20, 40, or 60 whereas a large number of other surfactants were not able to achieve a delivery across the BBB [9]. Alternative to poly(butyl cyanoacrylate), polylactic acid and polylactic acid-polyglycolic acid copolymers [10,11] as well as albumin [12] and chitosan [13,14] also can be used as nanoparticle materials.

Given these *in vivo* results demonstrating efficiency with particle-based penetration of the BBB using translatable drug delivery methods, new important questions remain to be addressed. These include: 1) the mechanism of nanoparticle-mediated drug transport across the BBB, and, 2) closely related to this, the influence of the surface properties and of targeting ligands, 3) the amount of drug that can be transported by this pathway in order to achieve a pharmacological effect, and 4) the aspect of toxicity. This review attempts to critically evaluate past *in vivo* results with a deliberate effort to identify mechanisms that might lead to new delivery breakthroughs, as well as to highlight key features of particle-based systems and approaches that seem to penetrate the BBB.

2. Definition of nanoparticles and particle size influence

This review follows the classical definition of nanoparticles in the Encyclopedia of Pharmaceutical Technology [15] and in the Encyclopedia of Nanoscience and Nanotechnology [16] which was formulated already 40 years ago [17]:

> *Nanoparticles for pharmaceutical purposes are solid colloidal particles ranging in size from 1 to 1000 nm (1 μm) consisting of macromolecular materials in which the active principle (drug or biologically active material) is dissolved, entrapped, or encapsulated, or to which the active principle is adsorbed or attached.*

This definition deviates from the definition of physicists and material scientists who limit the upper size of nanoparticles to 100 nm. However, up to 1000 nm size appears to be of no important influence concerning uptake into cells of the reticuloendothelial system (RES), i.e. macrophages and endothelial cells, and also most other parts of the body. Schäfer et al. [18] demonstrated in vitro using electron microscopy that human macrophages endocytose nanoparticles independent of size, while Gao and Jiang [19] reported an only small, i.e. 20% increase in methotrexate delivery to the brain using 70 nm sized methotrexate-loaded poly(butyl cyanoacrylate) nanoparticles overcoated with polysorbate 80 after intravenous injection. In addition, this increase was not statistically significant in many parts of the brain. No differences in methotrexate brain delivery occurred between 170, 220, and 345 nm sized particles. This insignificant particle size influence can be attributed to the mechanism of nanoparticle uptake and of bound drugs into the brain (see next section).

It has to be kept in mind that the drug payload decreases with a reduced particles size. On the other hand, with sizes above 1000 nm in the micrometer range the danger of embolisation of the lung capillaries is increasing in size- and dose-dependent manner [20].

3. Mechanism of nanoparticle-mediated uptake of drugs into the brain

About eight possibilities exist for the mechanism of uptake of nanoparticles and of bound drugs into the brain that were proposed in an earlier review in this journal [2,21]:

1. An increased retention of the nanoparticles in the brain blood capillaries combined with an adsorption to the capillary walls. This could create a higher concentration gradient that would increase the transport across the endothelial cell layer and as a result enhance the delivery to the brain.
2. The polysorbate 80 used as the coating agent could inhibit the efflux system, especially P-glycoprotein (Pgp).
3. A general toxic effect on the brain vasculature.
4. A general surfactant effect characterised by the solubilisation of the endothelial cell membrane lipids that would lead to membrane fluidisation and to an enhanced drug permeability across the blood–brain barrier.
5. Opening of the tight junctions between the brain blood vessel endothelial cells. The drug could then permeate through the tight junctions in free form or together with the nanoparticles in bound form.
6. Endocytosis by the endothelial cells followed by the release of the drugs within these cells and delivery to the brain.
7. Transcytosis through the endothelial cell layer.
8. A combination of the above effects.

As discussed in detail in a recent review [22], the nanoparticle-mediated transport across the BBB seems to occur by endocytosis of the particles by the brain capillary endothelial cells after intravenous injection followed by nanoparticle transcytosis across these cells. Earlier reviews [2,3,21,22] already pointed out that mechanisms 1–6 appear to be of no major relevance: Creation of high drug concentration gradients by adherence of the nanoparticles to the inner surface of the blood capillary walls (mechanism 1) would not be sufficient for an effective and pharmacologically relevant drug transport across the endothelial cell layer since the diffusing drug still would have been subjected to the highly efficient efflux transporters such as Pgp in the luminal membranes of these cells. These efflux transporters also cannot be blocked by the presence of the 1% polysorbate 80 in the injected nanoparticle suspension because the pre-injection of polysorbate 80-coated empty nanoparticles 5 or 30 min before injection of a dalargin solution did not induce any pharmacological effects [23]. If efflux transporter inhibition would have been the underlying mechanism, these transporters would have been inactivated by the polysorbate bound to the empty nanoparticles which then would have enabled the drug flux across the endothelial cells. The fact that pre-injection of polysorbate-coated empty nanoparticles did not achieve such a transport of drug in solution into the brain also refutes mechanisms 3, 4. and 5, permeabilisation of the blood–brain barrier by toxic effects (mechanism 3) or by membrane solubilisation caused by the surfactant (mechanism 4) as suggested by Olivier et al. [24] and Calvo et al. [25] and also opening of the tight junctions (mechanism 5). This conclusion was further substantiated by electron microscopic studies [23,26,27], histological investigations [7,28], and toxicological experiments (see Section 10), which did not reveal any toxic effects at therapeutic levels. Additionally, a surfactant-induced permeability enhancement appears to be unlikely as no pharmacological responses were observed after injection of dalargin nanoparticles coated with other surfactants such as poloxamers 184, 338, 407, poloxamine 908, Cremophor® EZ, Cremophor® RH 40, and polyoxyethylene-(23)-laurylether (Brij® 35) [9]. The opinion that toxicity is not the mechanism for the nanoparticle-mediated drug transport across the BBB further was corroborated by the experiments of San et al. [29] and of Koziara et al. [30]. Moreover, the electron microscopic studies by Zensi at al. [26,27] and by Kreuter et al. [23] showed that the tight junctions (mechanism 5) did not open after intravenous administration of the nanoparticles. The latter result also is supported by the