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Polymeric nanoparticles - targeted drug delivery systems for treatment of CNS disorders and their possible endocrine disrupting activities

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Abstract. Drug delivery to the central nervous system (CNS) represents one of the most priority challenges in research and development of pharmaceutical nanotechnology products. Among the various non-invasive approaches for CNS delivery, nanoparticle carriers and particularly polymeric nanoparticles (PNs) seem to be one of the most interesting. This review deals with PNs as CNS drug delivery systems and their potential endocrine disrupting properties. Possible interference with the development of neuroendocrine-reproductive system is considered. Special regard is being paid to potential mechanisms of PNs toxicity. Necessity to investigate the toxicity of nanomaterials and their impact on human health are discussed.

Key words: endocrine disruption, nanomaterials, polymeric nanoparticles, nanotoxicology, reproduction, KiSS-1 system, polyethylene glycol, review

It is estimated that as much as 1.5 billion people worldwide suffer from some type of central nervous system (CNS) disorders representing 11 % of the global burden which is growing together with the aging of population. In order to find therapies for so-called "difficult-to-treat" brain disorders a large number of studies have been performed. However, in general, the failure of several CNS diseases treatment is not mainly attributed to the potency of the drugs itself, but to various barriers that inhibit the drug delivery to the brain (Tiwari and Amiji 2006; Patel et al. 2009).

During the past two decades, nanoparticle based targeted drug delivery, especially for cancer therapy, has attracted increasing attention (Wang et al. 2010; Kateb et al. 2010). Nowadays, among the various non-invasive approaches for CNS delivery, nanoparticle carriers and particularly polymeric nanoparticles (PNs) seem to be one of the most interesting. The use of ligands for blood-brain barrier (BBB) crossing and also the surfactant coverage of PNs facilitate an encapsulation of the drugs, their protection from excretion and metabolism, and delivering active agents across without infliction of any damage to the barrier (Olivier 2005; Tosi et al. 2007). In this respect the question arises whether PNs as drug delivery systems could have a potential to interact with the processes of brain development and neurotransmission which belong to the factors of a considerable importance for endocrine, reproductive and immune functions.

Notwithstanding, some nanomedical products are already used in therapy mainly for treatment of cancer (Wang et al. 2010), a little is known about the toxicity of non-drug loaded, empty nanoparticles. It is assumed that the identification of possible endocrine disrupting activity of drug carriers/nanoparticles, especially CNS

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drug delivery systems, and the development and validation of standard guidelines for toxicity testing strategies of nanoparticles should be in the centre of interest of several collaborative scientists to avoid unwanted side effects caused by targeted or accidental exposures to nanomaterials used in nanotherapy (Oberdörster 2010).

Endocrine disruptors

Although it has been recognized for decades that hormonally active or endocrine disrupting substances (EDSs), such as estrogen like pharmaceuticals, industrial chemicals, pesticides, fungicides, plasticizers and phytoestrogens, exert potentially deleterious effects upon the biological systems, the idea of endocrine disruption as a scientific discipline has emerged only recently (Gore 2006; Hotckiss et al. 2008). In response to emerging concerns that chemicals may have adverse effects on human health by altering the function of endocrine system (Colborn et al. 1993), Food Quality Protection Act mandated the US Environmental Protection Agency (US EPA) to develop and implement an Endocrine Disruptor Screening Program (EDSP). The aim of this program is to evaluate a number of *in vivo* and *in vitro* assays designed to detect such chemicals that can interact with the estrogen, androgen and thyroid systems in human, fish, and wildlife. Current research in this area deals with the problems how developmental exposures to EDSs may cause both immediate as well as latent effects on endocrine, reproductive and immune systems (Gore 2007; Gore 2008).

EDSs are natural or synthetic compounds that may alter hormonal functions by numerous mechanisms, including: 1. direct stimulation or inhibition of the endocrine system; 2. modulation or blocking the responses to endogenous steroid hormones; 3. alteration in biosynthesis or degradation of endogenous hormones; 4. actions involved in the regulation of various neural centers or the adenopituitary (Dickerson and Gore 2007).

Since the endocrine system is a complex involving the hormone, its receptor, co-regulatory factors, transcription elements, target genes and enzymes involved in the biosynthesis and metabolism as well as other molecular endpoints, the ability to assess the effects of EDSs is very complicated (Buck Louis et al. 2006). The timing of exposure to EDSs is critical, the early lifetime (fetal or early postnatal) and puberty being particularly vulnerable because of persisting EDSs effects. EDSs often act at environmentally relevant doses, show complex dose-response curves, and their actions involve cellular mechanisms that often occur via multiple signaling pathways. EDSs do not show only the impact on exposed individual, but may also be transmitted to subsequent generations through the germ line, probably via epigenetically conditioned mechanisms (Gore 2008; Schoeters et al. 2008).

Exponential increase in the number of scientific papers over the last years reflects the importance of endocrine disruption problems. Testing the endocrine disruption effects of chemicals is widely used, but there are also strong recommendations of several international institutions and regulatory agencies such as the US EPA, OECD and WHO to investigate the toxicity of new chemicals (Baker 2001) including nanomaterials (US EPA 2007) and their impact on human health. Numbers of scientists pay an emerging concern to the potential role of EDSs in increasing trends in early puberty in girls, in the pathogenesis of obesity and type 2 diabetes in human population. New concerns include some complex endocrine alterations induced by mixtures of chemicals including EDSs present in personal care products, nutraceuticals and phytosterols, and the potent human and veterinary pharmaceutical products (Hotchkiss et al. 2008).

Blood brain barrier and CNS drug delivery systems

The brain is the most protected organ in the human body. Specific interfaces with the systemic circulation presented by chorioid plexus, arachnoid epithelium and blood brain barrier (BBB) tightly regulate the exchanges between peripheral circulation and cerebrospinal fluid circulatory system (Fernandez et al. 2010). Notwithstanding, the blood flow to brain is very high and perfusion rate should be sufficient to deliver the drugs into the brain, where the cells are joined by tight junctions in the brain capillaries and nothing can be exchanged across the wall by free diffusion (Tiwari and Amiji 2006). Active targeting of BBB represents a promising non-invasive strategy for improving drug/gene delivery to CNS using various influx transport systems (Beduneau et al. 2007). The shuttle nutrients into the brain is conveyed by carrier-mediated transport (over 20 transporters e.g. glucose transporter proteins, essential amino acids transporters), adsorptive-mediated endocytosis systems (cationized albumins and immunoglobulins) and receptor mediated transport (insulin and insulin-like growth factor receptor, transferrin receptor, leptin receptor, low-density lipoprotein receptor related proteins 1 and

2 receptor, folic acid receptor and others) (Halmos et al. 1997; Partridge 2007). In contrast, expulsion of harmful molecules into peripheral circulation is represented mainly by the active efflux transport (e.g. P-glycoprotein and its two human isoforms: multi-drug resistance protein MDR1 and MDR2) (Tosi et al. 2008) (Fig. 1). In this respect, the BBB as important regulator of constant internal environment of the brain, makes difficult to treat brain diseases (Beduneau et al. 2007).

Several strategies were explored to increase the delivery of drugs to the brain. Among them were chemical delivery systems (lipid-mediated transport, the prodrug approach and the lock-in system), biological delivery systems, disruption of BBB, use of molecular "Trojan horses", and particulate drug carrier systems, as reviewed by Patel et al. (2009). To treat the brain pathologies such as brain cancers, strokes, Alzheimer and/or Parkinson's diseases, ischemia and HIV-dementia, the use of polimeric nanoparticles (PNs) represents one of the most promising approaches for drug delivery, and local sustained release of the new large molecules of therapeutics (Borm et al. 2006; Olivier 2005; Tosi et al. 2008). Moreover, the development of nanoparticles with a variety of unique targeting, imaging, and therapeutic components is very perspective to the coming era of personalized medicine potentially including the use of gene and siRNA (small interfering RNA) therapy (Kateb et al. 2010).

Polymeric nanoparticles and their properties

Recent application of nanoscience includes also the use of nanomaterials in biomedical research. There is increasing expectation in significant advances that nanomaterials will bring in improved treatment of diseases and the more sophisticated diagnosis (Suh et al., 2009). Nanoparticles can be engineered from components that recognize diseases at the cellular level, are visible on imaging studies, and deliver therapeutic compounds (Kateb et al. 2010). Especially, the research of nanoparticles as drug delivery systems focuses on more specific drug targeting and delivery, reduction in toxicity along with maintaining therapeutic effects, greater safety and biocompatibility and faster development of new safe medicines (De Jong and Borm 2008).

Nanoparticles, attractive for medical applications, have unique chemical and physical features, such as their surface to mass ratio that is much larger than that of other particles, their quantum properties, their ability to absorb and carry other compounds, their solubility, crystallinity, agglomeration/aggregation, surface chemistry and many others (Avgoustakis 2004; Dreher 2004; Powers et al. 2006; Drobne 2007; De Jong and Borm 2008; Oberdoster et al. 2010).

Physical stability of nanoparticles for clinical application appears a very important issue. The lack of stability leads to the formation of secondary aggregates

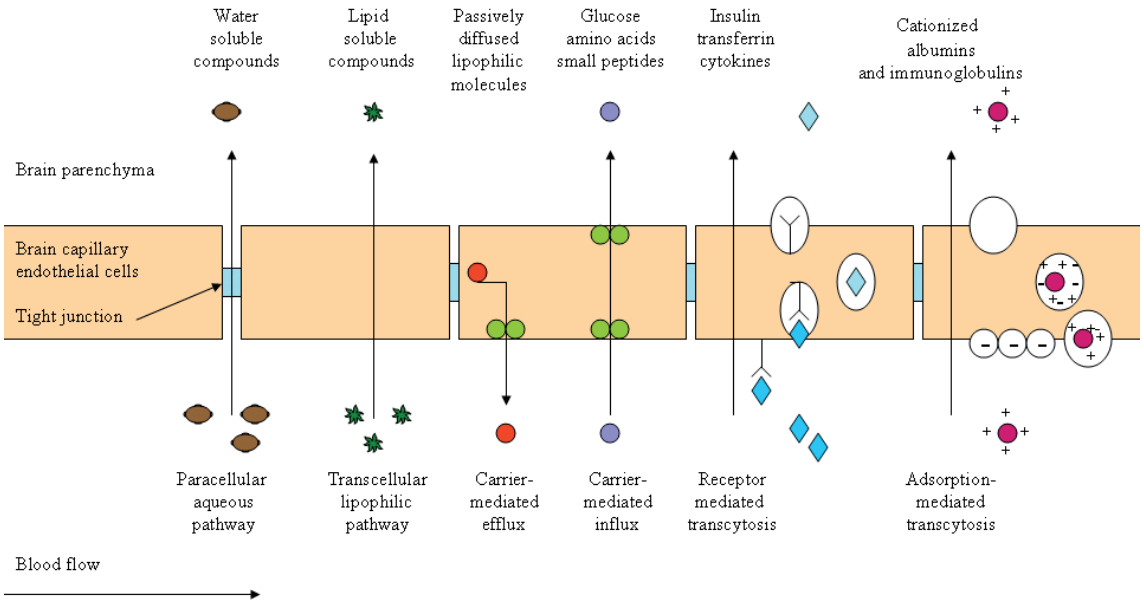

Fig. 1. Transport mechanisms across brain capillary endothelial cells.
Modified from Fernandez et al. (2010), Patel et al. (2009), Tiwari and Amiji (2006).

of particles that may cause blood vessel occlusion and make such aggregates more susceptible to clearance by the mononuclear phagocytic system (MPS). On the other hand, the dissociation of polymeric micelles/nanoparticles into individual polymers after application may lead to an unwanted rapid release of the enclosed drug, resulting in side effects *in vivo* (Plard and Bazile 1999; Wang et al., 2010).

Nanoparticles used in medicine represent a broad scale of materials, including liposomes, polymeric micelles, dendrimers, superparamagnetic iron oxide particles, colloidal gold, quantum dots and others (Wang et al. 2010). They can be either of biological origin like lipids, phospholipids, lactic acid, dextran and chitosan (organic nanoparticles: liposomes, micelles, dendrimeres) or their source material can be chemical like various polymers, carbon, silica, and metals (inorganic nanoparicles) (De Jong and Borm 2008). The most employed materials in engineering of nanoparticles are polypeptides and proteins, polysaccharides, polyethylene glycol and the vinyl polymers such as *N*-(2-hydroxypropyl)methacrylamide (HPMA). Especially, for drug delivery, biodegradable nanoparticle formulas are needed to ensure the effective transport and release of the drug. Inorganic nanoparticles are less biodegradable and show more applications for diagnosis rather than therapies (Ricci et al. 2006).

The term nanoparticle is used for a well-defined drug carrier system, generally of polymeric nature. Polymeric nanoparticles made from natural and artificial polymers are characterized by sizes ranging from 10 to 1000 nm, in which the drug can be loaded either in liquid or in the solid state, or adsorbed or chemically linked to the surface (Patel et al. 2009; Ricci et al. 2006). Generally, the size range of 30-200 nm is preferable, sufficient to avoid leakage into capillaries and small enough to avoid the mononuclear phagocytic system clearance (Wang et al. 2010).

Polymeric nanoparticles or polymeric micelles typically have a core-shell structure. The core is either the hydrophobic part or the ionic part of the nanoparticles that contain therapeutic drugs. The shell provides the interactions with the solvent and makes the nanoparticle stable in the liquid (Yang et al. 2008). Selection of polymeric materials for the preparation of drug carriers is subjected to: 1. availability of suitable functional groups for covalent coupling with drugs; 2. biocompatibility (nontoxic, nonimmunogenic, nontrombogenic); 3. either to biodegradability or molecular weight below the renal excretion limit (Ricci et al. 2006). Various biodegradable polymers such poly(lactide acid) (PLA), poly(lactic–co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), chitosan, poly(alkylcyanoacrylate) (PACA), poly(lysine) and poly(aspartic acid) PAsp are used for preparation the core matrix. They show high drug-loading capacity and provide protection to the embedded drugs against chemical and enzymatic degradation (Tosi et al. 2008).

As late as the mid 1990s, long-circulating PLA and PLGA polymer nanoparticles have been made available that opened great opportunities for drug targeting. Nowadays, great number of nanoparticles is made with the use of US Food and Drug Administration approved PLA and PLGA polymers because of their biodistribution, biodegradability and biocompatibility properties. Degradation of these polymers realizes by an autocatalytic cleavage of ester bonds through spontaneous hydrolysis to oligomers and D, L-lactic, and glycolic acid monomers, substrates of the Krebs cycle (Avgoustakis 2004; Beduneau et al. 2007).

Time of elimination from the body is depending on their molecular weight and their conjugation with other surface material such as e.g. polymer polyethylene glycol (PEG) (Tosi et al. 2008). Exact surface characteristics of nanoparticles contribute to their stability, solubility, and aggregation tendency, the ability to transverse biological barriers, biocompatibility, and targeting ability (Randall 2007). It is generally accepted, that surface heterogenity may explain the rapid clearance of significant fraction of intravenously injected long-circulating nanoparticles by the mononuclear phagocyte system, mainly the Kupffer cells in the liver and the spleen macrophages (Avgoustakis 2004; Olivier 2005). Hydrophobic surfaces promote the adsorption of protein component and negative surfaces are activators of the complement system. In contrast, hydrophilic coating with PEG sterically stabilizes PLA and PLGA nanoparticles and reduces opsonization and phagocytosis *in vitro* or *ex vivo* (Olivier 2005). Generally, nanoparticles should end in endosomes or lysosomes of the cells, but their fate may be determined by surface charge as its change from negative to positive could influence the escape of the endosomes. Moreover, long time accumulation after administration, observed in case of quantum dots during 4 months, seems likely (De Jong and Borm 2009).

Modification of PNs surface properties changes considerably their biodistribution and facilitates drug delivery (Bondioli et al. 2010; Tosi et al. 2008; Vergoni et al. 2009). There have been developed polyethylene

glycol-coated (PEG-coated) nanoparticles with a great potential as long circulating systems after intravenous administration along with anti-aggregation properties (Gao et al. 2006). Prolonged presence in the circulation is provided by inhibiting recognition and phagocytosis by the mononuclear phagocytic system as present in the liver, spleen and lymph nodes (Niidome et al. 2006). Blood half-life for unmodified PLA and PLGA nanoparticles is generally around the 2-3 min (Olivier 2005), however due to modification by PEG, nanoparticles were characterized by long-circulating properties with a half life of 18 h after intravenous administration (Yamamoto et al. 2001).

PEG, linear polyether diol, is a relatively inert hydrophilic polymer approved by US Food and Drug Administration (FDA) for clinical use (Wang et al. 2010). Depending on increasing molecular weight it can occur as viscous liquid or waxy solid due to crystallization. PEGs are eliminated by renal and hepatic pathways. They have the lowest level of protein or cellular adsorption of any polymers (Rian et al. 2008), exerting low toxicity and low immunogenic potential (Bondioli et al. 2010).

The chain length, shape, total molecular weight, and density of PEG on the particle surface are the main parameters affecting nanoparticle surface hydrophilicity and phagocytosis (Wang et al. 2010). PEGylation of NPs improves their cytoplasmic transport rates possibly by reducing non-specific adhesion to cytoskeletal elements (Suh et al. 2007). At present, PEGylation of nanoparticles is still the most commonly used approach, although new materials have been developed to mimic the effect of PEG (Owens and Peppas 2006) and to compete with PEGylation in terms of better efficacy, reduced number of injections, adequate biocompatibility and versatility in polymer design (Rian et al. 2008).

Actually, several different approaches have been developed in order to cross BBB and to target PNs to the CNS including: 1. magnetic-NP approach, 2. nanogel approach, 3. emulsifying wax and Brij 72 approach, 4. surface charge-based approach, 5. surfactant-based approach, 6. PEG approach, 7. ligand-based approach (Fig. 2) (Tosi et al. 2008). For example, peptide-modified PNs and polysialylation of the nanoparticle surface are actively studied by the pharmaceutical nanotechnology to improve the ability of PNs to cross the BBB and to enhance their half-life (Bondioli et al. 2010; Constantino et al. 2005; Tosi et al. 2007; Tosi et al. 2010; Vergoni et al. 2009) after intravenous administration. To enhance intranasal administration of peptides, proteins and DNA (biotech drugs) or nanoparticles, the surface modification of nanoparticles with biorecognitive ligands such as

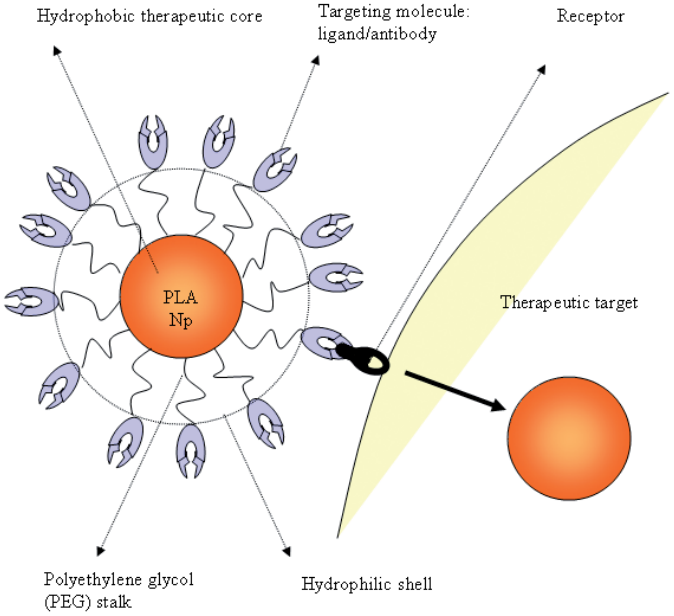

Fig. 2. Polymeric functionalized nanoparticle – ligand based approach. Interaction of targeting molecules (peptides, proteins, antibodies, etc.) with the receptors at the target site facilitates to increase movement across the brain blood barrier and deliver therapeutic molecules to the target. PLA – polylactide acid, Np – nanoparticle. Modified from Tiwari and Amiji (2006); Tosi et al. (2008), Wang et al. (2010).

lectins (e.g. wheat germ agglutinin), proteins of nonimmunological origin, are widely used (Gao et al. 2006).

For more useful control of drug release, stimuli-sensitive polymers that are actively responding to environmental signals, such as surrounding temperature, pH, electricity, light, ionic strength, and others are used. Controlled release of loaded drugs guarantees the maintenance of therapeutic dose for an extended time period and the avoidance of adverse effects induced by high drug concentration in systemic circulation (Wang et al. 2010).

KiSS-1/GPR54 system - a key factor in central neuronal regulation of hypothalamic-adenopituitary-gonadal (HPG) axis

Normal reproductive function depends on hypothalamic-adenopituitary regulation of gonadal function through the secretion of gonadotropin-releasing hormone (GnRH) and the adenopituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulation hormone (FSH), which then act on the ovaries and testes to stimulate gonadal maturation, gametogenesis, and steroidogenesis. These processes are controlled in turn by the feedback effects of gonadal steroids acting on the brain and adenopituitary (Moore and Price 1932; Smith 2008).

Hypothalamic GnRH neurons do not express estrogen receptors alpha (ERα) or androgen receptors (AR), which are though to mediate steroidal feedback effects, suggesting that other steroid-sensitive neurons receive and transmit steroid feedback signals to the reproductive axis (Kauffman 2009). Virtually, all GnRH neurons coexpress GPR54 (G-protein-coupled receptor 54), cognate receptor for kisspeptins, peptides encoded by the *KiSS1* gene (West et al. 1998), considered as indispensable players in the regulation neuroendocrine reproductive axis (Tena-Sempere 2010).

Kisspeptins, members of a group of peptide hormones known as RF-amides with common Arg-Phe-NH_2 motif at the C-terminus have essential role in GnRH neuron firing, GnRH pulsatile secretion, steroid feedback, the onset of puberty, and the ovulatory LH surge (Millar et al. 2010).

Consistent population of kisspeptin neurons identified across mammalian species is located in the arcuate nucleus (ARC). In addition to the arcuate population and distinct population outside the hypothalamus recently described in the mice, kisspeptin neurons have also been identified in the preoptic region in the anteroventral periventricular nucleus (AVPV) (Lehman et al. 2010). High degree of colocalization of ERα, AR, and progesterone receptor (PR) in ARC and AVPV kisspeptin cells implicates these neurons and their signals through GPR54 as primary mediators of gonadal steroid feedback control of GnRH release in mammals (Colledge et al. 2010; Roseweir and Millar 2009). In general, gonadal steroid estradiol has an opposite effect on kisspeptin populations, stimulating *KiSS1* transcripts and peptides in preoptic region (AVPV) and inhibiting it in the ARC. It was suggested that anatomical differences might underlie functional differences in phenotypically different kisspeptin cells (Lehman et al. 2010). In this sense, the ARC population so called KNDy cells (kisspeptin-neurokinin B-dynorphin), that colocalize also other neuropeptides as neurokinin B and dynorphin, is critical for negative feedback influence of estradiol and progesterone on GnRH neurons. On the other hand, kisspeptin cells in AVPV expressing galanin and/or tyrosine hydroxylase mediate positive feedback, resulting in the ovulatory LH surge (Lehman et al. 2010; Millar et al. 2010).

The hypothalamic-adenopituitary-gonadal axis is regulated by a plethora of excitatory and inhibitory hypothalamic factors and is highly sensitive to the organizing and activating effects of endogenous steroids during fetal and perinatal life (Bellingham et al. 2009). Although genetic factors may play a role in development of sexually differentiated features, most identified sex differences in the brain and behavior result from the influence of perinatal sex steroid signaling. Sex differences in the brain range from these in synapse morphology to neuron size or number to specific gene expression or protein levels. In this connection, hypothalamic expression of the *KiSS-1* gene as well as the expression of genes for estrogen receptors *ERα* and *ERβ* are very sensitive to neonatal imprinting by estrogen (Navarro et al. 2004).

One important sexually differentiated trait is the ability of adult females, but not adult males, to display an estrogen-induced, circadian-dependent GnRH/LH surge i.e. positive feedback. KiSS1 system is considered a critical component in this process. Kisspeptin neurons in hypothalamic AVPV are sexually differentiated with adult females possessing many more KiSS1 cells than males. Similar sex differences in kisspeptin protein levels in AVPV have also been reported in adult mice and rats (Navarro et al. 2004; Kauffman 2009). In contrast to the AVPV population, kisspeptin neurons in the ARC of rodents exert no sex difference in their number (Lehman et al. 2010).

KiSS-1 system is required for central activation of the hypothalamic-adenopituitary-ovarian axis at puberty. Persistent expression of hypothalamic *KiSS-1* and *GPR54* mRNA is detected in mice, rats and primates throughout postnatal development, with maximum expression levels at puberty in both male and female rats (Roseweir and Millar 2009). Eventually, hypothalamic expression of *KiSS-1* and *GPR54* mRNA changes throughout the estrous cycle and it is significantly increased after gonadectomy (Navarro et al. 2004).

Recently, the mutations of gene function encoding *GPR54* have been linked to hypogonadotropic hypogonadism, both in rodents and humans. Reproductive phenotypes in men with hypogonadotropic hypogonadism characterized by the failure of pubertal development, decreased levels of sex steroid hormone with inappropriately low levels of gonadotropins, absent spermatogenesis and ovulation, and impaired menstrual cyclicity demonstrate, that kisspeptin/GPR54 function is required at all phases of the life cycle when the secretion of GnRH is robust (Chan et al. 2009; Kauffman 2009).

Taken together, hypothalamic KiSS-1/GPR54 system appears a pivotal factor in central regulation of the gonadotropic axis at puberty and in adulthood. The alterations in reproductive function that manifest later in life, such as earlier or delayed timing of puberty, reduced fertility, reduced sexual behavior, no maturation of follicles in the ovary could result from the exposure to sex steroid mimetics or hormone active chemicals during development of the regulatory reproductive axis (Bellingham et al. 2009).

Could polymeric nanoparticles interfere with the development and function of reproductive system?

PNs as promising carriers for CNS drugs exert their action in the brain where reproductive neuroendocrine axis is regulated by gonadotropin-releasing hormone neurosecretory system (Dickerson and Gore 2007). Notwithstanding, they have favourable safety profiles such as providing sustained drug release and prolonged effects in the target organ, main questions on the mechanisms of toxic action of PNs inside the brain are still lacking (Drobne 2007).

There is a strong evidence that the exposure to EDSs at critical developmental points across the life cycle (prenatal, neonatal, pubertal, perimenopausal) can disturb the function of hypothalamic centers governing the hypothalamic-adenopituitary-gonadal axis. As a consequence, disrupted reproductive function and inappropriate sexual behaviour may be detected later in the life (Henley and Korach 2006; Kuoki et al. 2003; Tena-Sempere et al. 2004). If PNs interferes with sexually dimorphic action of estrogen in sensitive period for brain sexual differentiation, the identification of this potential will provide new insight into the mechanisms of toxicity of PNs and their contribution to some disturbances. Exposure to PNs during critical developmental window spanning the late embryonic and early postnatal period in rodents might induce morphological and neurochemical changes including the size, cell number and neurochemistry of hypothalamic brain regions. Alterations in the normal hormonal milieu during this critical period could result in the development of inappropriately masculinized, de-masculinized, feminized and de-feminized brain, which may manifest later in the life as deficits in reproductive function and behaviour (Dickerson and Gore 2007; Gore 2008).

One could hypothesized, that PNs exposure analogous to EDSs (Bellingham et al. 2009) might disturb estrogen feedback systems within hypothalamus, which may have consequences in the initiation of puberty in rodents (Navarro et al. 2001). The hypothalamic *KiSS-1* gene expression during early critical developmental periods in rodents is significantly and persistently reduced if rats are neonatally inappropriately exposed to xenosteroids (Navarro and Tena-Sempere 2008; Tena-Sempere 2010). The kisspeptin fibre density in discrete hypothalamic nuclei is also decreased along with altered gonadotropin secretion and gonadotropin-releasing hormone neuronal activation (Tena-Sempere 2010). Thus, KiSS-1 system provides the basis for potential endocrine disruption of reproductive maturation and function (Bellingham et al. 2009; Tena-Sempere 2009).

Especially, the delivery of drugs intentionally targeted into brain raise many questions. What is the mechanism of toxic action, how does the reactive surface of nanoparticles interact with internal environment inside the brain, how PNs interfere with sex hypothalamus neuroendocrine differentiation, and how do nanopartilces disrupt the regulation of neural network controlling of endocrine axis: hypothalamic gonadotropin-releasing hormone neurons – pituitary gonadotropes – gonads?

Because the immune system is closely related to endocrine system and many hormones can influence functions of various cell types of immune system (Utsujama et al. 2002), together with the reproduction and developmental processes, the immune system is also considered to be one of the major targets of PNs (Zolnik et al. 2010).

Toxicology of polymer nanoparticles

It is assumed that annual production of nanoparticles will increase from the present estimated 2300 tons to 58,000 tons by 2020. With increasing production and marketing of nanoparticles containing products along with continuing findings of their new applications it is very bewildering that our knowledge about their interactions with biological systems as well as our understanding of their potential toxicity still remains rudimentary (Lewinski et al. 2008). Moreover, because of unpredictable number of such interactions much higher attention should be paid to nanotoxicology of nanoparticles both from the material`s as well as from biological viewpoint than ever before (Suh et al. 2009). Dermal exposure, the entry through respiratory system and gastrointestinal tract suggest media for nanoparticle toxicity in dependence on their distinct physicochemical properties (Fernandez et al. 2010).

The main molecular mechanism of *in vivo* nanotoxicity is the induction of oxidative stress. Engineered nanomaterials may disturb the oxidative balance of the cell and it may result in abnormally large concentrations of intracellular reactive oxygen or nitrogen species that can react with proteins, lipids or nucleic acids, leading to abnormal cellular functions (Marquis et al. 2009). Destabilization of the balance between the production of reactive species results in disturbing biological system's ability to detoxify or to repair the system (Hagens et al. 2007). Because of slow clearance and tissue accumulation of nanomaterials in the organs of mononuclear phagocytic system, these ones represent main targets of oxidative stress (Wang et al. 2010).

Nanoparticles can trigger an inflammatory process resulting in the release of cytokines and chemokines such as IL-6, IL-1β, TNF-α, C-reactive protein and transcription factors, and in the activation of cascades. By such a way, several pro-inflamatory markers suggest that nanoparticles may promote low-level systemic inflammation at distant organs and tissues (Fernandez et al. 2010).

Nanoparticles comprised in intravenous preparations are formulated to allow the targeting to specific compartments within blood and accumulation within specific target organs or tissues (Borm et al. 2006). Nanoparticles used for drug delivery and imaging are intentionally engineered to interact with the cells. Therefore, it is really important to ensure that nanoparticles will undergo biodegradation in the cellular environment and to understand what cellular responses will degraded nanoparticles induce (Lewinski et al. 2008). Though most of the nanoparticles used as drug delivery systems were described non-toxic *in vivo*, there are several studies with reported toxicities of nanomaterials as reviewed by De Jong and Borm (2008).

Intrinsic properties of nanodrugs including brain-drug carriers raised some important questions regarding the potential for nanoparticles to exert toxic effects in the brain environment. Since the surface of nanoparticles as contact layer is crucial determinant of particle response, unique surface properties have to be investigated from toxicological point of view and no discrimination should be made between drug toxicity and empty non-drug loaded nanoparticle toxicity (De Jong and Borm 2006).

In the case of biodegradable nanoparticles, PEG chains are incorporated as copolymers throughout the particle and some surface PEG chains are always available even when the surface layers are already degraded (Wang et al. 2010). This fact again highlights that pharmacological characteristics of coating material should be also considered. Nowadays, PEGylation is still the most commonly used approach for solution of nanoparticle opsonization (Owens and Peppas 2006). PEG is not known to be metabolized in humans, it is minimally absorbed and rapidly excreted in feces (90-100 %) with no known confirmed toxicity resulting from the limited absorption (Pelham et al. 2008). On the other hand, Gajdova et al. (1993) reported that Tween 80 with PEG350 as an active ingredient has potential to behave as hormone/estrogen active agent. Neonatal exposure of female rats to Tween 80 significantly accelerated their maturation, prolonged the estrous cycle, and induced persistent vaginal estrus and squamous cell metaplasia of epithelial lining of the uterus. Moreover, ovaries were without corpora lutea, and had degenerative follicles (Gajdova et al. 1993). Although the components of PNs (PEG, PLA, and PLGA) are Food and Drug Administration (FDA)-approved, clinical trials testing their use as drug curriers are still lacking. Considering toxicity of PNs used for drug delivery in CNS from endocrine point of view should not be omitted since disruption of hypothalamic-adenopituitary-gonadal axis and kisspeptine/GPR45 neurosecretory system might be induced by therapeutically used PNs.

Like genetically modified organisms, the future of nanotechnology will depend on public acceptance of the risk versus benefits. Widespread application of nanomaterials provides enormous potential for human

exposure during the whole life cycle, and for environmental release, what poses difficult controllable issue (Tsuji et al. 2006).

There is a strong indication of WHO, US EPA (US EPA 2007) and OECD to investigate the toxicity of nanomaterials and their impact on human health (Drobne 2007). The existing *in vivo/in vitro* toxicological tests for evaluating risks of nano-scale substances should be used because of their familiarity and interpretability (Balbus et al. 2007) that have to be refined in future. Series of complementary *in vivo* and *in vitro* tests will contribute to determination of until unknown effects of PNs, to identification of target organs and possible mechanisms of toxicity. The observation of the number selected endpoints at the animals, organs, cells, proteins and nucleic acids levels will allow to record some changes/disturbances and will provide new knowledge for direct application in pharmacological nanotechnologies to guide the future development of nanodrugs and to avoid negative effects of nanoparticles during nanotherapy (Drobne 2007; Holsapple et al. 2005).

It is assumed that PNs as very attractive drug delivery systems will be broadly used in therapy because of their beneficial actions on the therapeutic potential of both established and new drugs. Still, no regulatory requirements to test nanoparticles for health, safety, and environmental impacts has been formalized (Oberdorster 2010). Understanding the physicochemical, molecular, biochemical and physiological processes of nanoparticles, and investigation their unique biological effects is imperative for nanomedicine to become a reliable and sustainable treatment modality (Fernandez et al. 2010). In this connection, multidisciplinary collaborative approach involving material scientists, physicians, and toxicologists will have crucial role for the successful present and future developments of diagnostic, therapeutic and preventive applications of nanomaterials (Oberdorster 2010).

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