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Nanomaterial interference with early human placenta: Sophisticated matter meets sophisticated tissues

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ABSTRACT

Next to nothing is known about nanoparticle and nanofiber trafficking at the feto-maternal interface in early human pregnancy. As the first trimester is thought to be crucial for the further placental and fetal development, it will be important to assess the possible risks of nanomaterial exposures during this period. There are some intriguing observations in nanotoxicology, however, indicating certain differences between classical toxicology and nanotoxicology. To understand nanomaterial-biokinetics and placental toxicity in early gestation, the special architecture, the hypoxic condition, the bilayer of villous trophoblast, the plugging of spiral arteries and the contribution of intrauterine glands to nutrition, as well as the delicate immunologic situation at the implantation site, will have to be considered. Unless nano-specific biokinetics are properly understood, it will be difficult to ensure identification of potential "nano-thalidomides" among all the newly engineered nanoparticles and fibers, based on the models available in reproductive toxicology.

1. Introduction

The rapidly growing field of nanotechnology has – not surprisingly and with a typical minor delay – raised concerns about a possible *reproductive* toxicity of these nanomaterials. Nanomaterials are natural, incidental or engineered particles or fibers with one or more external dimensions in the size range between 1 nm and 100 nm [1] They already surround us in daily life and are being increasingly introduced as therapeutics in medicine. The research field of reproductive nano-toxicology, however, is still in its infancy. While a recent database search (February 2013) in the Thomson Reuters "Web of Knowledge" [2] using the terms "nanoparticle" AND "toxicology" revealed more than 5500 entries, the terms "nanoparticle" AND "pregnancy", as well as "nanoparticle" AND "placenta" revealed less than 50, and even no hits at all for "nanoparticle" and "teratology". Besides the direct interference of nanomaterials with developmental processes in the embryo, interaction with the human placenta is one of the main issues in human developmental toxicology. Located at the materno-fetal interface, the placenta determines and regulates embryonic or fetal exposures. Moreover, placental toxicity can result in pathologies of pregnancy, such as abortion, IUGR, preterm birth or preeclampsia and thereby indirectly harm the offspring and the mother [3]. As for other organs in embryology, the aspect of development has to be considered for the placenta. From this point of view, dramatic changes in placental anatomy and function can be observed during the gestational period. Although our understanding of human term placenta is already quite comprehensive, early placentation is far from being completely understood. Our knowledge is only rudimentary concerning the very early placenta between weeks 4 and 6 (gestational age, based on last menstrual period) and mid second trimester placenta. Voluntary elective terminations of (healthy) pregnancies have offered good opportunities to analyze placental structure and metabolism in more detail at least between weeks 7 and 15 [4]. As the first trimester of pregnancy is thought to be crucial for the further placental and fetal development, it will be particularly important to assess the risks of nanomaterial exposures during this gestational period. It should also be kept in mind that the placenta is one of the most species-specific organs, showing remarkable differences in the morphology of the placental barrier between humans and, *e.g.* lab animals. For classical drugs, usually small lipophilic molecules, these morphologic differences may be of little relevance for their distribution and uptake into the fetal compartment. Species differences however, are expected to be important for large particles up to the 100 nm scale and beyond. Thus, conclusions from animal experiments must be drawn with

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caution, since only the great apes show a placentation similar to humans.

The growing concerns about nanomaterials in human pregnancy are based on very limited experimental evidence and some epidemiological data, altogether still insufficient for proper teratologic risk assessment and counseling. Concerns may also arise as a kind of side effect from the expectations raised by praise of the amazing nano features that are described, and by the promising novel therapeutic applications. According to act 1 in Wolfgang Goethe's Götz von Behrlichingen, we might be expecting "strong shadow, where there is much light".

2. Epidemiological and experimental nano developmental toxicity data

Little is known about nanoparticle and fiber trafficking at the feto-maternal interface in general and next to nothing about nanoparticle exposures in human pregnancy. Various routes of nanomaterial exposure are currently under investigation [5]. Most data exist on nanomaterial uptake by the respiratory system. In this context there is some epidemiologic evidence for an association between airborne particle exposure and adverse pregnancy outcome such as low birthweight [6]. The reviewed studies mainly investigated exposures to fine particulate matter of varying chemical composition up to a size of 2.5 μm, a major constituent of ambient air pollution. While the biologic plausibility remains to be fully confirmed, some experimental animal data support these observations. Nanoparticle inhalation in pregnancy was shown to cause adverse effects on the offspring's liver cells in mice and it was hypothesized that maternal inflammatory mediators induced by the nanoparticle exposure were indirectly responsible for this teratogenic effect [7]. However, the potential mechanisms involved in damaging DNA (strand breaks) in offspring liver certainly need to be further elucidated. Interestingly, and somehow in contradiction to the human epidemiologic data mentioned above, pregnancy and pregnancy-related parameters seemed unaffected in these mice. On the other hand, certain nanoparticles injected intravenously into mice did cause placental abnormalities and pregnancy complications such as fetal growth restriction, depending on particle size and surface chemistry. Yamashita et al. observed an increase in apoptosis, a reduction in the volume of spongiotrophoblast up to 50% and even a failure to form spiral artery channels. The authors correctly conclude that directly extrapolating their findings about nanoparticle-induced placental dysfunction to humans is not feasible, because of the differences in placental anatomy [8]. Nonetheless, these observations imply that special attention will have to be paid to possible detrimental effects of nanomaterials on the human placenta, and this will involve use of appropriate *in vitro* models.

Buerki-Turnherr et al. recently reviewed 14 studies focusing directly on placental transfer of nanoparticles [9]. Only three of these studies were performed in human systems, employing the quite sophisticated *in vitro* perfusion of term placenta cotyledons and a rather artificial human choriocarcinoma cell line (BeWo)/fibroblast co-culture system. These studies could correspond to the situation in late pregnancy, but it is certainly not possible to draw substantial conclusions from them about nanoparticle interactions with the early human placenta. The data published so far indicate that placental transfer of some nanosized materials, *e.g.* dendrimers designed for drug delivery, occurs at least in trace amounts in third trimester placenta [10]. The extent of transfer varied, also size dependently [11] and sometimes there was a high degree of retention of nanomaterials in the placental tissues, *e.g.* of 70–300 nm liposomes or 10–30 nm PEGylated gold particles. The latter were shown not to cross human term placenta in measurable amounts [12], similar to albumin coated gold particles, while 5 nm IgG coated gold particles could easily cross the barrier and were detected on the fetal side of the *in vitro* systems [13]. In an elaborate review on reproductive toxicity of engineered nanoparticles, Sørig Hougaard and Campagnolo summarize that variable degrees of developmental toxicity and adverse effects on fertility have been observed *in vitro* and in animals, predominantly mice and zebra fish. They conclude, however, that a more systematic approach toward developmental nanotoxicity will be essential in the future, to advance our understanding of the underlying pathophysiology and to direct research toward risk assessment rather than hypothesis generation [14].

3. Nanotoxicology nano-biokinetics and reproductive toxicology

On the strength of past experience, hydrophilic molecules and particles >1 kDa are usually effectively prevented from crossing the placental barrier. This applies not only to proteins, such as immunoglobulins (150 kDa) and modified therapeutic Fab-fragments of ~48 kDa like Abciximab [15], but also to heparin (4–6 kDa) and insulin (~6 kDa). Their distribution appears to be restricted to the maternal circulation unless they are specifically transported by the syncytiotrophoblast, such as IgG from week 13 onwards [16] or vitamin B12. Notably, the main pharmacokinetic principle responsible for accumulation of nanoparticle based diagnostics and therapeutics in tumor tissue which has been taken advantage of so far is the so called enhanced permeability and retention (EPR) effect, which is based on the high permeability of tortuous and leaky tumor vessels for large molecules and particles [17]. Enhanced permeability is however not a key feature of early placenta, nor of placenta in general, hence one might assume that the growing embryo should in general be quite well protected from most nanoparticles.

Nevertheless, some intriguing observations in nanotoxicology point to differences between classical toxicology and nanotoxicology. As Maynard et al. discuss in their comprehensive review on "the new toxicology of sophisticated materials", nanoparticles and nanofibers have been shown to cause harm which cannot be well assessed by use of current methods of risk assessment, a phenomenon Maynard et al. referred to as *emergent risk* [18]. Such emergent risks are described to arise from unanticipated penetration of particles into normally inaccessible tissues, and also from poorly understood abrupt size-dependent changes in interactions with biological systems. Especially novel, increasingly sophisticated nanomaterials, *e.g.* active materials that specifically respond to local environments or signals by changing their properties, or smart self assembling materials, should be expected to carry emergent risks. Specific targeted drug delivery, achieved by linking monoclonal antibodies to nanoparticles, has for example been shown to significantly enhance drug delivery to the trophoblast in placental explants *in vitro* and in choriocarcioma xenotransplants *in vivo* [19]. Anyway, emergent risks are not novel experiences for teratologists. They resemble the thalidomide tragedy, where a relatively harmless drug for adults was unexpectedly able to cause severe damage to the embryo. Thus, teratogenicity as such is probably one of the emergent risks to consider particularly in nanotoxicology.

Intentional tailoring of nano materials to have distinct novel nanofeatures by manipulation of the physicochemical properties at the nanoscale is one of the major aims of nanotechnology. Changes in biokinetics and toxicity as compared to bulk material can therefore be expected. Experts in the field, *e.g.* the MINChar initiative, have proposed lists of parameters relevant for understanding nano-features [20]. Not only does the size matter, according to