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Developmental and reproductive toxicity testing of vaccines

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ABSTRACT

The majority of new preventative and therapeutic vaccines are now assessed for developmental toxicity according to guidelines issued by the FDA in 2006. Despite the absence of confirmed effects in humans, vaccines are frequently suspected of having adverse side-effects on the development of children. Such suspicions are perhaps unavoidable considering the extremely widespread use of vaccines. The preclinical developmental toxicology studies are designed to assess possible influences of each component of the vaccine formulation—and the induced antibodies—on the development of the conceptus, neonate and suckling organism. Immune modulation by a vaccine or an adjuvant could, for instance, affect the outcome of pregnancy by interfering with the natural shift in immune balance of the mother during gestation. Maternal immunoglobulins are transferred from the mother to the offspring in order to confer passive immunity during early life. This maternal antibody transport is prenatal in humans and monkeys, but tends to be delayed until after birth in other species. Therefore, a suitable model species needs to be chosen for preclinical studies in order to ensure exposure of the foetus to the induced maternal antibodies following vaccination. Rabbits are the best laboratory model for prenatal immunoglobulin transfer, but rodents are more practical for the necessary postnatal investigations. Non-human primates are the only appropriate models for the testing of vaccines that are not immunogenic in lower species. It is advisable to test new adjuvants separately according to the ICH S5(R2) guidelines. Preclinical paediatric investigations are not currently required for vaccines, even though most vaccines are given to children. Other areas of regulatory concern include developmental immunotoxicity and effects on the preimplantation embryo. Because of the limitations of the available animal models for developmental toxicity testing, pharmacovigilance is essential.

1. Background

The first technical guidance on the preclinical testing of preventative and therapeutic vaccines for developmental toxicity was issued by the CBER Division of Vaccines and Related Products Applications of the FDA in 2006 (FDA, 2006a). Before this, developmental and reproductive toxicity studies were rarely performed for vaccines. This approach was not entirely justified, since vaccination programmes at that time often included pregnant women (Gruber, 2003). Influenza vaccines, for example, were already being recommended for use during pregnancy by public health policy makers in the absence of specific regulatory approval for use of the product during pregnancy (Centers for Disease Control and Prevention, 1999). This practice was brought to the forefront of public attention by the recent worldwide H1N1 vaccination programmes (Centers for Disease Control and Prevention, 2009).

The first draft of the FDA guidance document was issued for comments in 2000 (FDA, 2000). A Workshop was organised jointly by the Society of Toxicology and the FDA in 2002 to discuss advances and regulatory considerations in the non-clinical safety evaluation of preventive vaccines. During the course of this meeting the possible risks of adverse effects of vaccines on human development were reviewed and recommendations were made concerning the need to develop new animal models and methods (unpublished). Many of these recommendations were subsequently incorporated into the final guidance document (FDA, 2006a). The European guidelines for the non-clinical testing of vaccines issued in 1997 (EMEA, 1997) state that embryo-foetal and/or perinatal studies may be necessary for vaccines that will be given to women of child bearing age or during pregnancy, but give no guidance on study designs. In the absence of guidance from the regulatory authorities of other regions, the FDA developmental toxicity study designs have become de facto the international standard for the testing of new vaccines.

2. Possible risks of vaccines to development

To date, there is no documented causal evidence of developmental or reproductive toxic effects in humans following the use of an approved vaccine. Live vaccines are contraindicated during pregnancy because of the risk of infection of the conceptus. The inadvertent use of smallpox vaccine during pregnancy, for instance, carries a risk of foetal vaccinia, but does not appear to result in birth defects

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or preterm delivery (Ryan et al., 2008). Despite the lack of causal evidence, vaccines are frequently suspected by the general population of having adverse side-effects on the development of children; the most recent point in case being the suspected implication of a swine flu vaccine in childhood narcolepsy (Daily Telegraph, 2010), even though the epidemiology data are reassuring (Schaefer, Fritzsche, Karbaum, Meister, & Weber-Schoendorfer, 2010). Such suspicions are unavoidable considering the extremely widespread use of vaccines in children, a small proportion of whom will inevitably fall ill following vaccination. Some longstanding concerns, such as the possible implication of vaccine products in the increasing incidence of childhood allergy, are more plausible than others (Offit & Hackett, 2003). Even in these cases, however, the minor unproven risk of increased sensitivity to allergy is far outweighed by the health benefits of vaccines in conferring protection against life-threatening disease (Forster, this issue).

The extremely long-lasting pharmacological efficacy of vaccines—i.e. lifelong or long-term immunity—presents unique considerations that are not applicable to other classes of medicinal agent. Following vaccination, the induced antibodies and/or sensitised T-cells or memory cells typically persist for decades. Also, unlike most other pharmaceutical agents, an administered vaccine often exerts its pharmacological action at the site of administration (e.g. an intramuscular injection site) and does not necessarily need to enter the general circulation in order to be effective. Thus, conventional pharmacokinetic measures of dose and exposure are not applicable. Antibody titres are measured for vaccines as a substitute for the pharmacokinetic and pharmacodynamic parameters evaluated for conventional drugs. Each component of a vaccine formulation—antigen, adjuvant, excipients, vehicle, etc.—needs to be evaluated for potential adverse effects on development. This is usually accomplished by evaluating the final vaccine formulation, though in most circumstances it is advisable to also test an adjuvant separately (see below).

An additional complication is the need to assess the possibility that the induced antibodies may have the potential to cross-react with endogenous tissues due to molecular mimicry between the infectious organism and the human host. Developmental toxicity could arise when the affected host cells play an active role in a developmental process. Furthermore, the endogenous antigenic molecules may only be present during restricted periods of development, leading to phase-specific effects. For this reason, it is necessary to expose the model organism throughout its entire development (i.e. up to maturity) in developmental toxicity testing to be sure of covering all possible periods of vulnerability. An example of molecular mimicry occurs between polysaccharide Group B Neisseria meningitides (GBM) vaccines and mammalian neural cell adhesion glycoproteins (NCAMs) (Verdier, Barrow, & Bruge, 2003). Polysialiated NCAMS play an important role in the remodelling of various tissues at specific times during development. Once development of the tissue is complete, the NCAMS are deactivated by removal of the sialic acid polymer. Mouse antibodies raised against GBM polysaccharide have been shown to cross react with activated human NCAMS, but no adverse effects on development have been demonstrated in-vivo. Exposure of the embryo to maternal antibodies is prevented by the placental barrier (see below), so adverse effects of cross-reacting antibodies during organogenesis are unlikely. However, NCAMS control some critical aspects of development that occur later in development, e.g. brain maturation or secondary sexual development of the testes, when maternal and/or endogenous antibodies are present.

Most vaccines are designed to provoke a humoral (adaptive) immune response. Adjuvants are designed to enhance this response, either through immune-modulating effects or by improving the presentation of the antigen. Immune stimulation by either the vaccine antigen or a co-administered adjuvant may alter the natural balance between the innate and adaptive arms of the immune system. The resting balance of the maternal immune system shows a natural shift during pregnancy in order to reduce the risk of rejection of the embryo and to accommodate the normal immune interactions between the maternal tissues and the developing conceptus. During pregnancy, cellular immune activity, modulated by TH1 cells, is depressed while humoral immune activity, modulated by TH2 cells, is increased (Thellin & Heinen, 2003). Specific immune stimulation by a vaccine antigen or non-specific stimulation by an adjuvant could conceivably interfere with the shifted balance during pregnancy and thus adversely influence the outcome of gestation. Cytokines of macrophage origin, for example, have been shown to cause pregnancy loss and abortion (Raghupathy, 1997).

On the other hand, non-specific immune stimulation of the mother with adjuvants or cytokines, such as GM CSF or IFNγ, has been shown to have a protective effect in laboratory animals against the dysmorphogenic action of many known teratogens (Holladay et al., 2002). The mode of action of this protective effect remains to be elucidated.

It is reasonable to assume, therefore, that vaccines—or adjuvants—may have the capacity to affect the outcome of gestation, owing to their inherent pharmacological activity on the immune system. The end result of these influences may be beneficial or detrimental for the developing conceptus or child.

3. Animal models

Passive immunity conferred from the mother serves to protect the newborn from infectious disease during early life, until the infant's own immune system becomes fully functional. The conferred immunity involves the transfer of maternal immunoglobulins (mainly IgGs) from the mother to the offspring, which takes place at different times relative to birth in various mammalian species. This transfer is essentially prenatal in the human. Old World primate species also show prenatal maternal antibody transfer, as do lagomorphs (rabbits and guinea pigs). Rodents, however, have very limited prenatal maternal immunoglobulin (Ig) transfer, while the transfer is entirely postnatal in most other species (e.g. dogs, cats and pigs) (Pentsuk & van der Laan, 2009).

In early gestation, the placental barrier prevents exposure of the developing embryo to maternal immunoglobulins and the embryo does not have the capacity to produce its own antibodies. Therefore, the induced antibodies following vaccination are unlikely to cause dysmorphogenesis via a direct interaction with the embryonic tissues during organogenesis. The human chorioallantoic placenta develops active transport mechanisms for IgG—via the FcRn receptor—starting from about mid gestation, after which an interaction with the developing foetal tissues becomes a possibility (although the period of vulnerability to most teratogenic agents is already over). Non-human primate species show a similar placental function as humans. In rodents and lagomorphs, the FcRn receptors reside in the inverted yolk sac (or vitalline) placenta. Rabbits have the unique characteristic of transporting small, but significant quantities of IgM to the foetus in addition to IgG (Baintner, 2007). In humans, other primates and rabbits, the foetal IgG levels generally reach, or exceed, the maternal titres by the time of birth. Prenatal IgG titres are much lower in rodents, but reach maternal titres within a few days after birth (Halliday, 1955).

On the basis of the above considerations, primate species are the most appropriate models to study of the effects of vaccines on intrauterine development. There is increasing ethical pressure, however, to avoid the use of monkeys in pharmaceutical safety testing whenever possible. Rabbits or rodents are generally preferred for this purpose and are also much more practical than primates for reproductive toxicity testing (Barrow, 2009). Rats and mice are the most practical species when postnatal examinations are required, although more expertise in the area of postnatal studies in rabbits has been acquired over recent years (see below). Rabbits have a higher foetal exposure