AGAH WORKSHOP
Critical Aspects of Integrated Drug Development - Expect the Unexpected!

Session 3: Reproductive and juvenile toxicity and paediatric drug development – “Everything you always wanted to know but were too afraid to ask”

Introduction
S. Plassmann

Outline

- Testing paradigm in reproductive toxicology
  - ICH S5 (R2)
  - FDA guidance (2011)
  - FDA pregnancy categories
  - EMA guidance (2009)
Outline

- Testing paradigm in reproductive toxicology
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Aim of reproductive toxicity programme (1)

- The combination of studies
  - Allow exposure of mature adults and all stages of development from conception to sexual maturity

- To allow detection of immediate and latent effects of exposure, observations should be continued through one complete life cycle, i.e. from conception in one generation through conception in the following generation

- For convenience of testing this integrated sequence can be subdivided into [...] stages [A-F]
Aim of reproductive toxicity programme (2)

- [...] this guideline does not fully cover exposures from weaning through puberty, nor does it deal with the possibility of reduced reproductive life span.
- To detect adverse effects for medicinal products that may be used in infants and juveniles, special studies (case by case designs) involving direct treatment of offspring, at ages to be specified, should be considered.

Treatment of offspring

Timing conventions (1)

Timing of pregnancy (rats, mice and rabbits)
- Day sperm positive vaginal smear and/or plug = day 0 of pregnancy
- Implantation = day 6-7 of pregnancy
- Closure of the hard palate = day 15-18 of pregnancy
- Period of organogenesis = DG 6/7 – 15-18

Duration of pregnancy
- Rat: 21 – 23 days
- Rabbit: 29 – 31 days
- Human: 40 weeks
### Reminder: Relative duration of organogenesis

**Proportion of total duration of pregnancy**

- **Rat**: >50%
- **Rabbit**: >40%
- **Human**: ca. 30%

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### Remaining days of pregnancy at the end of organogenesis [ca. days]

- **Rat**: ca. 4 - 6
- **Rabbit**: 10 – 12
- **Human**: 196 (28 weeks)
The accuracy of the time of mating should be specified since this will affect the variability of fetal and neonatal parameters.

[...] consistent in different studies to assure that no gaps in treatment occur

[...] to provide an overlap of at least one day in the exposure period of related studies.

[...] particularly with regard to delays in, or prolongation of parturition, reference to a postcoital time frame may be useful.

Timing conventions (2)

Stages of development (ICH) (1)

A. Premating to conception (adult male and female reproductive functions, development and maturation of gametes, mating behavior, fertilisation)

B. Conception to implantation (adult female reproductive functions, preimplantation development, implantation)
The reproductive cycle (A-B)

A

Male gametogenesis

pre-mating

Direct treatment

B

Mating

Fertilisation

Conception

Implantation (F1)

Stages of development (ICH) (2)

C. Implantation to closure of the hard palate (adult female reproductive functions, embryonic development, major organ formation)

D. Closure of the hard palate to the end of pregnancy (adult female reproductive functions, fetal development and growth, organ development and growth)

E. Birth to weaning (adult female reproductive functions, neonate adaptation to extrauterine life, preweaning development and growth)

F. Weaning to sexual maturity (postweaning development and growth, adaptation to independent life, attainment of full sexual function)
The reproductive cycle (C-F)

Male gametogenesis
Mating
Fertilisation
Implantation (F2)
Embryo and Foetal Development (Pregnancy)
Female gametogenesis
Embryo and Foetal Development (Pregnancy)

Direct treatment

F1-generation

C-section (F0)
F1 Males
Day 17 of gestation
F1 Females
Day 17 of gestation
F0 Females
Day 6 of gestation
Mating
Day 0
Birth
F0 Female treatment period

E: Birth
D: F0 Females
B: Puberty
Weaning PND 21
Lactation

Example: PPND/EFD study design

F0 necropsy
Selection of F1 generation
F1 necropsy on Day 14 of gestation
F1 Males
Mating
Behaviour studies
F1 Females
F0 Females

C. Willoughby, HLS (2012), adapted
**Example: PPND/EFD study design**

- **F0 Females**
  - Day 0 of lactation
- **F1 Females**
  - Day 6 of gestation
- **F1 Males**
  - Day 17 of gestation
- **Mating**
- **Birth**
- **Day 17 of gestation**
  - F1 necropsy
  - Selection of F1 generation
- **C-section (F0)**
  - F0 necropsy
- **F0 Female treatment period**
  - F0 Females

**Outline**

- **Testing paradigm in reproductive toxicology**
  - ICH S5 (R2)
  - FDA guidance (2011)
  - FDA pregnancy categories
  - EMA guidance (2009)
Classes of reproductive toxicity include:
- male fertility
- female fertility
- parturition
- lactation

Classes of developmental toxicity include:
- mortality
- dysmorphogenesis (structural abnormalities)
- alterations to growth
- functional impairment

Reproductive or Developmental Toxicity Endpoints - Positive Signal

Overview of integration

Factors

Summary/integration of positive findings
**Overview of integration**

Analyse any positive nonclinical signal for reproductive or developmental toxicity

Level of concern for adverse effects in humans?

Multiple factors contribute!

Use scientific judgment!

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**Adequacy/predictivity of model?**

Test species pharmacologically relevant ("responder")?

Toxicity profile in test species overall consistent with the human toxicity profile?

DMPK profile in test species qualitatively similar to humans?
Relative impact of factors

Some can have greater impact than others

Some may not be relevant

Factors to be considered

- Maternal/paternal toxicity
- Cross-species concordance of DART effects
- Multiplicity of effects
- Concordance between test species and humans (DMPK/genealogical toxicity)
- Dose-response relationship
- Rare events
- Similarity between pharmacologic and toxicologic mechanisms
- Class alerts
- Relative exposure

AGAH Workshop Expect the Unexpected 2014
Rare events?

Developmental toxicity studies lack statistical power to detect subtle increases in rare events

Increased frequency = increased concern

But:
Absence of rare events does not decrease concern!

Note:
Of particular concern in reproductive toxicity studies
Sound background database of extreme importance!

Relative exposures?

Scientifically plausible link between the exposure metric and the adverse reproductive or developmental effect?

If so, then higher emphasis on exposures
Safety Ratio (SR)

Parameter to estimate relative safety

Comparison of systemic drug exposures
- In patients at therapeutic doses
- With those in animals at the no-observed-adverse-effect level (NO(A)EL)

Reminder

NOAEL and SRs refer to systemic maternal drug exposures!

Embryofoetal (tissue) exposures are mostly unknown!
Consideration of a class effect to be based on adverse reproductive or developmental effects previously demonstrated in humans by

- closely related chemical entities (parent or metabolites)
- compounds with related pharmacologic effects

If there is a "class alert" for the drug

- Then the concern is increased

Decreased concern only where a class of compounds

- Although demonstrating adverse effects in animals
- Was previously shown definitively to have no adverse effects on human reproduction or development

**Class Alerts?**

Reminder

Your signal for reproductive toxicology may have been identified in a general toxicity not reproductive toxicity study!
“A weight of evidence approach should then be applied to arrive at an overall conclusion for reproductive or developmental toxicity.

Summary/Integration of Positive Findings (2)

- “The following are examples of possible summary risk conclusions for the evaluation:
  - **Does Not Appear to Increase Risk:** The drug is not anticipated to increase the risk of adverse developmental (or reproductive) outcomes in humans when used in accordance with dosing information in the product label.
  - **May Increase Risk:** The drug may increase the risk of adverse developmental (or reproductive) outcomes in humans when used in accordance with the dosing information in the product label.
  - **Predicted to Increase Risk:** The drug is expected to increase the risk of adverse developmental (or reproductive) outcomes in humans when used in accordance with the dosing information in the product label.”
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How is the outcome of the assessment currently reflected in (new US) drug labels?
**Current Labelling (FDA)**

- United States FDA Pharmaceutical Pregnancy Categories
  - Introduced in 1979
  - Under debate since years
  - Pregnancy categories are still in use
  - For exact definitions, see reference 43
- Assessment not reflected in label at present (at least not visibly)
- Other guidance refers back to pregnancy categories
  - Example: Nonclinical Safety Evaluation of Drug or Biologic Combinations (2006)

**Pregnancy categories (FDA)**

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Controlled studies show <strong>no risk</strong>. Adequate, well-controlled studies in pregnant women have failed to demonstrate a risk to the fetus in any trimester of pregnancy.</td>
</tr>
<tr>
<td>B</td>
<td><strong>No evidence of risk in humans.</strong> Either animal studies show risk but human findings do not, or if no adequate human studies have been done, animal findings are negative.</td>
</tr>
<tr>
<td>C</td>
<td><strong>Risk cannot be ruled out.</strong> Human studies are lacking, and animal studies are either positive for fetal risk or lacking. However, potential <strong>benefits</strong> may <strong>justify</strong> the potential risks.</td>
</tr>
<tr>
<td>D</td>
<td><strong>Positive evidence of risk.</strong> Investigational or post-marketing data show risk to the fetus. However, potential <strong>benefits</strong> may <strong>outweigh</strong> the potential risks. If needed in a life-threatening situation or serious disease, the drug may be acceptable if safer drugs cannot be used or are ineffective.</td>
</tr>
<tr>
<td>X</td>
<td><strong>Contraindicated in pregnancy.</strong> Studies in animals or humans, or investigational or post-marketing reports, have demonstrated positive evidence of fetal abnormalities or risk which clearly outweighs any possible benefit to the patient.</td>
</tr>
</tbody>
</table>
Consistency of pregnancy labelling across different therapeutic classes

- FDA poster publication (2010)
- Objective: To evaluate consistency of pregnancy labelling across different therapeutic classes from the top 20 therapeutic classes
- Top-selling 22 (24) drugs taken from Drugs.com listing of the top 200 drugs of 2010
- Focus on teratogenicity and whether the drug was reported to cross the placental barrier
- Out of the 20 therapeutic classes surveyed, 9 (45%) were consistently labelled across class
- The majority was classified Pregnancy category C (60%)

Pregnancy categories (FDA)

- **Category A**: Controlled studies show no risk. Adequate, well-controlled studies in pregnant women have failed to demonstrate a risk to the fetus in any trimester of pregnancy.

- **Category B**: No evidence of risk in humans. Either animal studies show risk but human findings do not, or if no adequate human studies have been done, animal findings are negative.

- **Category C**: Risk cannot be ruled out. Human studies are lacking, and animal studies are either positive for fetal risk or lacking. However, potential benefits may justify the potential risks.

- **Category D**: Positive evidence of risk. Investigational or post-marketing data show risk to the fetus. However, potential benefits may outweigh the potential risks. If needed in a life-threatening situation or serious disease, the drug may be acceptable if safer drugs cannot be used or are ineffective.

- **Category X**: Contraindicated in pregnancy. Studies in animals or humans, or investigational or post-marketing reports, have demonstrated positive evidence of fetal abnormalities or risk which clearly outweighs any possible benefit to the patient.
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EMA Guidance (2009)

- This guidance integrates non-clinical and clinical assessment
- It gives detailed information
  - On the assessment of effects both from a non-clinical and clinical perspective
  - And how the assessment will be reflected in the label
- Statistical basis for human data
  - Number of prospectively collected pregnancies to establish (absence of) signal
- However, not as detailed instructions as to how to discuss non-clinical data as in FDA guidance
  - Similar considerations as in FDA guidance
- Typical differences include
  - Level of maternal toxicity (minimal)
  - Reference is made to a NOAEL
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The Applicability of Animal Reproductive Data in Human Drug Safety
S. Plassmann

Outline

- Examples
  - ACE Inhibitors
  - Endothelin antagonists
  - Thalidomide
  - Triptanes (Sumatriptan)
  - Epigenetic modification

- Summary and conclusions
- Take home messages
Examples

Prospective interpretation of data for human risk assessment

Outline

- Examples
  - ACE Inhibitors
  - Endothelin antagonists
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ACE inhibitors

- Angiotensin – converting enzyme (ACE) inhibitors
  - ACEI
- Class of drugs for the treatment of hypertension and congestive heart failure
  - First in class: Captopril approved April 6, 1981 (US)
  - Second in class: Enalapril approved December 24, 1985 (US)
- Example Enalapril:
  - Characterised in a complete set of studies
  - “old study designs” – before the implementation of ICH!
  - More comprehensive design particularly for “Segment I”

Enalapril: rat findings

- Not teratogenic or embryolethal
- Decreased foetal weight in the high dose
  - Could be prevented with supplementation of pregnant dams with physiological saline
- Postnatal effects in “old Segment III” study in mid – high dose range evidenced as
  - Reduced maternal (also low dose) and foetal weight gain (mostly mid-dose and higher)
  - Developmental delay (considered secondary to impaired foetal weight)
  - No malformations
- “Old Segment I” study (incl. a C-section and rearing phase)
  - Increase in skeletal variations (retarded ossification) in F1-foetuses from C-section but not in pups delivered naturally
  - More pronounced effects during lactation than in Segment III study incl. increased pup mortality
Enalapril: rabbit findings

- Not teratogenic
- Maternal and foetal toxicity down to the low dose
  - Could be prevented with supplementation of physiological saline in the low – mid dose range but not high dose

Enalapril reprotox: interpretation?

- There is some noise but
  - Maternal toxicity confounds the interpretation
  - Saline supplementation is preventive
    - Seems to point to a pharmacologically mediated effect
  - The signal is mostly in the F1 generation from the “old” Segment I study
  - The findings are unspecific and not uncommon
  - This type of combination of findings often found in these studies and could be interpreted to indicate a pattern of “developmental delay secondary to maternal toxicity and/or pharmacological effects”

- Conclusion?
  - For further detail, refer to Vasotec US FDA Pharmacology review
ACE inhibitors: safety in humans

- Human evidence demonstrates serious concerns
  - Particularly during 2nd and 3rd trimester
    - Intra-uterine growth retardation (IUGR)
    - Increased risk of foetopathy
      - Renal dysplasia, renal failure, anuria, death
      - Oligohydramnios
      - Specific adverse outcomes, secondary to reduced amniotic fluid volume i.e.
        - Limb deformities, cranial ossification deficits, lung hypoplasia
    - Neonatal renal failure
  - Fetal urine production starts towards the end of the 1st trimester (humans)
  - For further detail, refer to Briggs 2002, Tabacova 2003, Cooper 2006

ACE inhibitors: animal findings

Clinical treatment conditions
- Best reflected in „old Segment III“ study design
- Treatment from DG 15 - lactation

Why is there such a difference?

But, remember:
- There was no indication for such severely adverse outcomes!
Tabacova (2001)

- Human foetus has higher vulnerability
- Earlier (relative to animal species) intrauterine development of
  - Kidney
  - Renin-angiotensin system
  - Develop prior to calcareous ossification at the end of the 1st trimester
- Enalapril (and other ACEI) = specific PD action on these systems
- In most "toxicological species" target systems not developed until close to term
  - At this stage, animal foetuses relatively more mature (less vulnerable)
  - Window is narrower than in human
  - Best concordance in foetal PD compared to human = rhesus monkey
  - Least concordance = rat
    - Greater disparity in enalapril availability to the foetus and the relative development of
      the kidney and skeletal ossification

Tabacova (2003)

Exposure to enalapril after the first trimester of pregnancy was strongly associated with oligohydramnios and specific adverse outcomes thought to be secondary to reduced amniotic fluid volume (limb deformities, cranial ossification deficits, lung hypoplasia), as well as with neonatal renal failure.

The relationship did not change after taking numerous potential confounders into account, including duration of exposure, concomitant drug use, maternal age, concurrent disease, neonatal gender, and gestational age at birth. Such a pattern of abnormalities is considered to be a consequence of the effect of ACE inhibition on fetal renal function that develops after the first trimester.

The specificity and temporality of the observed adverse manifestations suggest a causal relationship to enalapril exposure.
Tabacova (2005) Conclusion

“[...] animal studies that follow standard protocols and evaluate developmental toxicity only for exposures during embryogenesis will miss developmental effects arising secondary to disruption of target systems that develop after the period of major organogenesis. Thus, although the animal mode of action (MOA) for enalapril and other ACEI is plausible in humans, differences in the timing of development of critical target organ systems, particularly the renal system and RAS, explain the absence of definitive structural abnormalities in test animals.”

Take home message

Beware! A negative result may not be predictive and stimulate treacherous self-assurance!

Absence of evidence is not evidence of absence!
Are ACEI teratogenic when taken during the 1st trimester?
- Angiotensin II receptors widely expressed in fetal tissues
- Playing a part in the early development of the heart, kidneys, and brain?
- Refer to Arch Dis Child 2006 and Cooper 2006

Previous pregnancy label (e.g. Vasotec)
- C (1st trimester)
- D (2nd and 3rd trimester)

Cooper (2006)
- Cohort = 29'507 infants
  - Born between 1985 - 2000
  - 209 exposed to ACEI (1st trimester only)
  - Few malformations
  - Mainly cardiovascular (9), CNS (3) and renal (2)
  - 202 exposed to other anti-hypertensive medications (1st trimester only)
  - No increase in malformations

Authors conclude that there is a risk
Comments to editor (2006)

- **Scialli and Lione**
  - Obesity may be a confounding factor
  - Independent risk factor for neural-tube defects and cardiac malformations in infants
  - Requires multiple medications

- **Sealey**
  - Unknown whether effect specific for ACEI or applicable to other classes of drugs that block the RAS (beta-blockers, ACE receptor blockers, renin inhibitors)
  - The oocyte, embryo, and developing fetus are bathed almost continuously in prorenin, the precursor of renin, from just before ovulation until parturition
  - *Remember – there were more pronounced effects in the "Seg I" study for Enalapril*

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**Li (2011)**

**Cohort of 465’754 mother/infant pairs**
- CHD increase in cohorts treated during 1st trimester only with
  - ACEI or
  - Other anti-hypertensives
- Compared to normal (healthy) controls
- However, not different from hypertension controls without treatment

**Authors conclude:**
- Risk profile ACEI similar to other anti-hypertensives
- Apparent increased risk of malformations likely due to the underlying hypertension
Risk of ACEI during 1st trimester

Conflicting evidence from 2 retrospective cohort studies and a literature review/meta-analysis
- Cooper (2006) – there is a risk
- Li (2011) – there is no increased risk compared to other anti-hypertensives
- Walfisch (2011) – The Motherisk program, conclusion agrees with Li

Matter of debate in the scientific community

e.g. Vasotec
Pregnancy label D since 2012
Appraising a risk for drugs that act on the RAS

Enalapril label (2008)

- Boxed warning

**USE IN PREGNANCY**
When used in pregnancy during the second and third trimesters, ACE inhibitors can cause injury and even death to the developing fetus. When pregnancy is detected, **Vasotec** should be discontinued as soon as possible. (See WARNINGS, Fetal/Neonatal Morbidity and Mortality).

- Precautions:
  - Pregnancy Categories C (first trimester) and D (second and third trimesters): see WARNINGS, Fetal/Neonatal Morbidity and Mortality.
Enalapril label (2012)

- Boxed warning

**WARNING: FETAL TOXICITY**
*See full prescribing information for complete boxed warning.*
- When pregnancy is detected, discontinue Vasotec as soon as possible.
- Drugs that act directly on the renin-angiotensin system can cause injury and death to the developing fetus. See Warnings: Fetal Toxicity.

Take home message

*Our apparent knowledge of today might be our errors of tomorrow!*
Outline

- Examples
  - ACE Inhibitors
  - Endothelin antagonists
  - Thalidomide
  - Triptanes (Sumatriptan)
  - Epigenetic modification
- Summary and conclusions
- Take home messages

Endothelin antagonists

Example: Bosentan (Tracleer)
- Approved 2001 (US)
- Orphan drug in treatment of pulmonary hypertension

Discovered to be a teratogen in a regulatory reproductive toxicity programme
Findings in regulatory studies

- **Rat: dose-related pattern**
  - Teratogenic and fetotoxic when given orally to rats during organogenesis at doses as low as the MRHD
    - Agenesis of the palate
    - Craniofacial abnormalities
      - Including shortened, misshapen mandibles, fusion of the pterygoid process with the tympanic annulus, abnormal zygomatic arch, shortened tongues, anophthalmia and microphthalmia
    - Blood vessel variations (abnormal origin of the right subclavian and innominate arteries)

- **Rabbit**
  - Impaired foetal body weight in the presence of maternal toxicity only
  - Some skeletal variations more common in the high dose group
  - No evidence of teratogenicity

Conclusion (FDA)

Other endothelin antagonists and a knockout mouse model show similar findings

**Likely class effect**

ET-1 / knock-out mice die of respiratory failure at birth and have morphological abnormalities of the pharyngeal-arch-derived craniofacial tissues and organs (Kurihara 1994)
Risk mitigation and management

- Label boxed warning/Pregnancy category X

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Experimental evidence

Therefore, testing is required in a rodent and a non-rodent model

But still: Rat and rabbit findings are not concordant

Endothelin has been demonstrated to be involved in embryo-foetal development

Convincing
**Take home message**

We must postulate that adverse reproductive findings in preclinical species are predictive for humans and consequently mitigate potential risks - but we will hopefully never know for sure!

Inter-species sensitivity is not necessarily the same for any type of finding!

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**Outline**

- **Examples**
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  - Endothelin antagonists
  - **Thalidomide**
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- **Summary and conclusions**
- **Take home messages**
Thalidomide (Schardein 3rd edition)

- Pattern of adverse effects very variable across species
  - Mostly increased resorptions in the rat
  - Rabbit and primates best concordance with human phocomelia
- Profoundly teratogenic in humans
- Far less potent in animals
  - Extensively characterised in numerous species (strains/breeds/species)
    - Rats (10), mice (15), rabbits (11), dogs, hamsters (3), primates (9), cats, armadillos, guinea pigs, swine, ferrets

Thalidomide (2)

- More toxic to the embryo than to the mother
  - Especially hazardous
- Teratogenic mechanism is still a mystery
- Re-approved by Celgene 2006
  - Multiple myeloma
  - Erythema nodosum Leprosum
  - Extensive REMS in place
- Similar strategies have been implemented for other known human teratogens
  - E.g. Isotretinoin (Accutane)
The response in a biological system A may differ significantly from the response in a biological system B and yet still reflect a similar reaction to a common insult.

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- Examples
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Sumatriptan (anti-migraine)

- Approved 1995
  - Embryolethal in rabbits when given in daily IV doses approximately equivalent to the maximum recommended single human SC dose of 6 mg on a body surface area basis (MRHD) (1). The doses were at or close to those producing maternal toxicity.
  - Fetuses of rabbits administered oral sumatriptan (at doses greater than 50 times the MRHD) during organogenesis had an increased incidence of cervicothoracic vascular and skeletal anomalies (1).
  - In contrast, embryo or fetal lethality was not observed in pregnant rats treated throughout organogenesis with IV doses approximately 20 times the MRHD. Moreover, no rat embryo/fetal lethality or teratogenicity was observed with daily SC doses before and throughout gestation (1).
  - Shepard described a study in which no fetal adverse effects were observed in rats given up to 1000 mg/kg orally during organogenesis (2)


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Sumatriptan pregnancy registry

- Established in 1996
  - To monitor pregnancy outcomes following treatment with Sumatriptan/Naratriptan/Treximet

- Data do not indicate a signal for major teratogenicity
  - Pregnancy category C

- The Sumatriptan/Naratriptan/Treximet Pregnancy Registry Interim Report 1 January 1996 through 31 October 2011 Issued: May 2012
Conclusions

A new class of compounds was approved with major benefit for the patients

Many drugs have a preclinical reproductive profile similar to the triptanes

The risk was carefully and successfully managed

There is a clear need to distinguish the hazardous compounds from those which are benign

The ultimate risk/benefit ratio will depend on the medical need (indication) and the inherent risk of a new drug

Take home message

Careful risk management will support the development of innovative and beneficial drugs
Outline

- **Examples**
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Species sensitivity and prediction of teratogenic potential (Schardein 1985)

- "Many chemicals shown to be teratogenic in laboratory animals are not known to be teratogenic in humans.
- However, it remains to be determined if the unresponsiveness of humans is due to lessened sensitivity, to generally subteratogenic exposure levels, or to the lack of an appropriate means of identifying human teratogens.
- [...] those agents well accepted as human teratogens have been shown to be teratogenic in one or more laboratory species.
- Yet, no single species has clearly distinguished itself as being more advantageous in the detection of human teratogens over any other.
Species sensitivity and prediction of teratogenic potential (2)

- Among the species used for testing, the rat and mouse most successfully model the human reaction, but the rabbit is less likely than other species to give a false positive finding.
- Among species less commonly used for testing, primates offered a higher level of predictability than others.
- Regarding concordance of target malformations, the mouse and rat produced the greatest number of concordant defects, but they also were responsible for the most nonconcordant responses as well.
- Since no other species is clearly more predictive of the human response, it is concluded that safety decisions should be based on all reproductive and developmental toxicity data in light of the agent's known pharmacokinetic, metabolic and toxicologic parameters.”

Studies on fertility and early embryonic development

Testing paradigm:
- Yes-or-No answer of the very early stages of the conceptus
- Damage at this stage not compatible with life
- Surviving conceptus = healthy

Is this true?
Epigenetic modification

Emerging area of science

Mediated by several mechanisms
- DNA Methylation
- Histone modification
- RNA interference
- ...

Regulation of the activity of genes

Jirtle (2013, presented at EUROTOX)

- Human epidemiological and animal experimental data
  - Risk of developing adult onset disease and neurological disorders
    - Influenced by persistent adaptations to prenatal and early postnatal environmental exposures
  - One group of epigenetically regulated genes that potentially link environmental exposures early in development to adult diseases
    - Metastable epialleles = identical alleles but variably expressed due to epigenetic modifications
    - Highly variable expression
      - Stochastic allelic changes in the epigenome
      - Rather than mutations in the genome
Dolinoy (2007/8): experimental evidence

Viable yellow agouti (A\textsuperscript{vy}) mouse

- Metastable A\textsuperscript{vy} allele
- Upstream insertion of a transposable element

**Phenotype**

- Yellow (rather than brown) fur
- Adult-onset obesity
- Diabetes
- Tumorigenesis

**Principle**

- **Yellow mice**
  - hypomethylated at the transposable element upstream of the Agouti gene
  - allowing maximal ectopic expression
  - Mice that are predominately yellow are also clearly more obese than brown mice

- **Brown mice** (pseudoagouti animals)
  - hypermethylation of this site silences ectopic agouti expression
  - Healthy, lean and longer lived mice are brown (agouti coat color)
Avy mouse model as an epigenetic biosensor

To characterize nutritional and environmental factors

• affecting epigenetic gene regulation and
• subsequent adult phenotype

Bisphenol A (BPA)

Endocrine disruptor

Used widely in manufacture of polycarbonate plastic and epoxy resins

Present in many commonly used items

Food, beverage containers, baby bottles (!), dental sealants, etc....
**Study design**

- 2 weeks prior to mating, throughout gestation and lactation
- Females a/a (non-agouti genotype) fed with:
  - Phytoestrogen-free diet
  - Phytoestrogen-free diet + 50 mg BPA/kg
- Mating with A<sup>av</sup>/a males

**Results**

- No effect on:
  - Litter size, litter survival, mean weight, sex ratio = routine endpoints in reprotox studies
  - Genotypic ratio
- But: coat colour of A<sup>av</sup>/a offspring shifted towards yellow due to decreased methylation
Results (2)

Hypomethylation of the foetal epigenome induced by BPA abolished by maternal dietary supplementation with Methyl donors (folic acid, betaine, vitamin B12, choline) or the phytoestrogen Genistein.

Dutch famine (Hoek 1998)

During 2nd world war (1944/45)

Unlike other famines

Dutch famine struck at a precisely circumscribed time and place.

Society able to document the timing and severity of the nutritional deprivation.

Effects on fertility and health.

AGAH Workshop Expect the Unexpected 2014
Dutch famine (2)

**Dutch maintained comprehensive military and health records**

- Allowed comparing incidence of neurodevelopmental disorders in adulthood for birth cohorts
- Exposed to prenatal famine
- Unexposed to prenatal famine

Dutch famine (3)

- Early prenatal famine specifically and robustly associated with each of three conditions:
  - (1) congenital anomalies of the central nervous system
  - (2) schizophrenia
  - (3) schizophrenia spectrum personality disorders.
- Greatest increase in the risk of schizophrenia spectrum disorder-schizophrenia plus spectrum personality disorder- occurred among males born in the famine cities in December 1945.
- Persons born in December 1945 were generally conceived at the absolute peak of the famine (March-April 1945).
Dutch famine (Carey 2012)

- Long-lived effects on body weight development depending on period of pregnancy affected
  - Famine during 1st trimester
    - Offspring (children) more likely to develop obesity later in life (F1)
    - Even F2 offspring (grandchildren) still are at higher risk to develop obesity
  - The opposite: famine during 3rd trimester
    - Babies likely born small
    - Offspring exhibited lower obesity rates than the general population

Discussion

- Current pre-clinical tools are not designed to evaluate such effects
  - But: consider pragmatic limitations
- ICH Study on fertility and early embryonic development (“Segment I”)
  - Pre-mating treatment
  - Pregnant females are terminated after mid-pregnancy
  - No delivery phase
- Reminder: Enalapril – best match of human results with experimental evidence was found in the (“old”) Segment I study
  - Included a C-section at term and a delivery phase
- Some of the human evidence seems to point into the same direction as experimental data
  - The situation around conception may well have long-term effects
Conclusion?

Reminder: Testing paradigm
- Yes-or-No answer of the very early stages of the conceptus
- Damage at this stage not compatible with life
- Surviving conceptus = healthy

Are we still sure that this is true?

Further reading

http://www.geneimprint.com/
Take home messages

Expect the unexpected!

Always challenge your beliefs and convictions!

Outline

- Examples
  - ACE Inhibitors
  - Endothelin antagonists
  - Thalidomide
  - Triptanes (Sumatriptan)
  - Epigenetic modification
- Summary and conclusions
- Take home messages
Summary and conclusions

- The most challenging situations are those where the experimental data are inconclusive e.g. due to
  - Unspecific patterns
  - Confounders (maternal toxicity)
  - Lack of concordance between species
  - Lack of biological plausibility
- Human evidence may either increase level of concern
  - Example: ACEI
- Or, alternatively, decrease the level of concern
  - Example: Sumatriptan

Summary and conclusions (2)

- Absence of evidence should not be taken for granted
  - It is virtually impossible to confirm the absence of findings in humans
  - Lack of adverse findings is reassuring but the evidence has to be corroborated
    - E.g. pregnancy registries (such as for Sumatriptan)
- Specific patterns of adverse findings in animals must be considered predictive a priori
  - Potential risks must be mitigated to prevent adverse human outcomes
    - Example: REMS for Bosentan, Isotretinoin or Thalidomide (Celgene)
- All established human teratogens have shown teratogenicity in one or more animal species
Summary and conclusions (3)

- Many substances shown to be teratogenic in animals are not known to be teratogenic in humans
  - Reasons not fully understood
  - Difficult to distinguish which animal findings are of concern
- Apparently plausible MoAs do not always result in the expected outcome
  - E.g. Endothelin antagonists not teratogenic in rabbit
  - Suspected key role of prorenin in development not associated with clear adverse effects on organogenesis in animal studies
  - In a majority of cases, the MoA could not be conclusively demonstrated
- Mechanisms like epigenetic modification have just been discovered
  - A much better understanding needs to be developed

Summary and conclusions (4)

Reproduction is a biologically highly complex process

We understand much more than some decades ago but there is still a lot to discover!
Outline

- Examples
  - ACE Inhibitors
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Take home messages

- Beware: a negative result may not be predictive and stimulate treacherous self-assurance!
- Absence of evidence is not evidence of absence!
- Our apparent knowledge of today might be our errors of tomorrow!
- We must postulate that adverse reproductive findings in preclinical species are predictive for humans and consequently mitigate potential risks - but we will hopefully never know for sure!
- Inter-species sensitivity is not necessarily the same for any type of finding.
Take home messages (2)

The response in a biological system A may differ significantly from the response in a biological system B and yet still reflect a similar reaction to a common insult.

Careful risk management will support the development of new and beneficial drugs.

Decision making and risk management will benefit from a timely dialogue with the experts.

Expect the unexpected!

Always challenge your beliefs and convictions!

Thank you very much for your attention!
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