Histopathological evaluation of the teeth in regulatory studies: what are the needs and what can we learn from it

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• Teeth represent not a standard protocol organ, not required by any guideline

• Considered as “by-product” when looked at slides of the nasal cavity

• Pathologists feel somewhat uncomfortable when gross lesions occur or teeth are target

• More focus on teeth due to increased investigation of anticancer compounds
Overview of the Presentation:

1. Introduction
   (Physiology/Anatomy/Histology)

2. Non-proliferative Lesions
   (Examples from ToxPath)

3. Proliferative Lesions (Terminology only)
Embryology:

Teeth formed by unique series of reciprocal inductive phenomena occurring between odontogenic epithelium and mesenchymal cells of the dental papilla and dental follicle

Crown formation: continuous process in rodent incisors:
Inner dental epithelium → induction of mesenchymal cells of dental papilla → differentiation into odontoblasts → dentin deposition → reciprocal induction of inner dental epithelium → differentiation into ameloblasts → deposition of enamel

Root formation: Molar roots formed by Hertwig’s root sheath i.e. fusion of inner and outer dental epithelium
Formation of Ameloblast (A) and Odontoblast (O) Layers, Mouse, longitudinal
Formation of Ameloblast and Odontoblast layers, longitudinal cut, Rat
Base of upper incisor, transverse cut (rat)

Courtesy of Dr. H. Ernst
Neck region of upper incisor, transverse cut, Rat

Dentin

Enamel

Ameloblasts

Dentin

Odontoblasts

Pulp

Courtesy of Dr. H. Ernst
Rodents: Anatomy of the tooth

- Only permanent, no deciduous/temporary teeth
- 1 incisor / 3 molars per quadrant 2 (I1/1, M3/3) = 16
- Incisors: Continuous growth / wearing
- Renewal within 40 to 50 days (rats)
  35 to 45 days (mice)

- Malocclusion or fracture of teeth may accelerate growth by a factor of 2 - 3!
Tooth substance composed of:

- **Enamel:**
  1% organic material (glycoproteins),
  99% inorganic material (hydroxyapatite crystals)

- **Dentin:**
  ~ 20% organic material (collagen, glycoproteins)
  ~ 80% inorganic material (hydroxyapatite)

- **Mineral content of rat incisor (mg/g dry weight):**
  Ca: 275, P: 140, Mg: 5.5, Na: 5.3, K: 2.6
Decalcification:
EDTA (3 weeks +)
Citric Formic Acid (2 weeks)
Microwave (few days)

Disadvantage: loss of calculus, loss of enamel, tissue shrinkage and artifacts, suboptimal staining if over decalcified

- Plastic embedding
- Precision cutting
- Grinding methods

Disadvantage: High costs
Routine trimming of the nasal cavity*

Routine trimming of the nasal cavity*

* From: Ruehl-Fehlert CI, B Kittel, G Morawietz et al.: Revised guides for organ sampling and trimming in rats and mice - Part 1
A joint publication of the RITA and NACAD groups, Exp Toxicol Pathol. 2003 Sep; 55(2-3):91-106
Modified trimming of the nasal cavity / jaws

Additionally, one transverse section through the 2nd incisor of the lower jaw possible.
Modified trimming of the nasal cavity with incisors of the upper jaw

* From: Kuijpers MHM et al. (1996): The rat incisor in toxicologic pathology. Tox Path 24, 346 - 360
A  Bone
B  Vasculature
C  Periodontal Ligament
D  Ameloblasts
E  Enamel
F  Dentin
G  Odontoblasts
H  Pulp
Congenital lesions:
  • Deviations from normal number or position, malocclusion

Degenerative lesions:
  • Ameloblast and odontoblast degeneration/necrosis
  • Tooth resorption
  • Attrition

Inflammatory and vascular lesions:
  • Periodontal disease, pulpitis, dental abscesses, fistulae

Neoplastic lesions
Non-neoplastic Terms
Degeneration
Necrosis
Periodontal pocket
Dentin niches
Dentin, decreased
Dentin matrix alteration
Fracture
Resorption
Denticle(s)
Pulp concretion
Cyst(s)
Thrombus

Actual INHAND Nomenclature
Fossey S. et al. 2016, JTP 29, 3 (supplement)
Supernumerary tooth

Courtesy of Dr. H. Ernst
‘Root’ Resorption: perforation of dentine layer by inflammatory tissue

Dental cyst

Courtesy of Dr. H. Ernst
Dental folds

Intrapulpal denticles

Courtesy of Dr. H. Ernst
Example 1:
Antiparasitic compound, 13 weeks

Inlife observation:
Accelerated growth of incisors
Y-shaped growth

Malocclusion or fracture of teeth may accelerate growth by factor of 2 - 3!
Yellow color of teeth due to thin iron-containing layer.
Example 1: Antiparasitic compound, rat, 13 weeks

A. Normal appearance, upper jaw
B. Proliferation of gingival epithelium and periodontal ligament, inflammation
Example 1: Antiparasitic compound

A. Normal appearance of the gingiva and periodontal ligament, lower jaw
B. Periodontal ligament proliferation, gingiva hyperplasia
Example 1: Antiparasitic compound

A. Gingivitis and hyperplasia

B. Periodontal ligament proliferation, multifocal karyomegaly in cementoblast layer
Example 1: Antiparasitic compound

A. Normal appearance of ameloblast layer, mid part
B/C. Atrophy and degeneration of ameloblast layer
Example 1: Antiparasitic compound, transverse, lower jaw

Control

Control

Mid Dose

High Dose

A

B

C

D
Example 2: Anticancer compound 1, rat, 4 weeks

In-life Observation: Accelerated growth of incisors, fractures

A. Normal appearance
B. Degeneration of odontoblasts and dentin matrix alteration
Example 2:

A. Normal appearance
B/C. Degeneration of odontoblasts and dentin matrix alteration with pulp concretions (pulp stones)
Example 2: Anticancer compound 1

A. Cystic lacunae formation within dentin, close to basis of incisor
B. Accelerated growth, cellular remnants in normally acellular dentin
Example 2: Anticancer compound 1

A. Normal Appearance
B. Penetration of periodontal ligament into dentin (Tooth resorption)
C. „Fracture“ of cementum
Example 3: Anticancer compound 2, mouse, 13 weeks

Inlife observation: Deformation and fracture of incisors

A. Altered dentin composition, hyperdentinosis
B. Altered dentin, periodontal ligament and cementum within normal range
C. Altered dentin, odontoblast and ameloblast layer without alterations
Example 3: Anticancer compound 2, mouse, 13 weeks

In-life observation: Deformation and fracture of incisors

A./B. Fracture and pulp necrosis - Attention: Septicemia possible
Example 4: Anticancer compound 3, rat, 13 weeks

In-life observation: Discoloration and occasional fracture of incisors

A./B. Fracture and pulp necrosis, Necrosis of ameloblast layer
Example 4: Anticancer compound 3, rat, 13 weeks

Early changes of ameloblast layer vacuolization
Example 4: Anticancer compound 3, rat, 13 weeks

Enamel defects and dentin matrix alterations
Example 4: Anticancer compound 3, rat, 13 weeks

Degeneration of periodontal ligament
Example 4: Anticancer compound 3, rat, 13 weeks

Severe vascular degeneration of dental growth zone
Example 4: Anticancer compound 3, rat, 13 weeks

Degeneration of odontoblast layer and protrusion into dentin
Example 4: Anticancer compound 3, rat, 13 weeks

Sequestration and production of dentin (denticles?) within pulp cavity
Example 4: Anticancer compound 3, rat, 13 weeks

Early fractures with necrosis
Example 5: Anticancer compound 4, rat, 3d on 3d off 3d on

A. Necrosis of odontoblast layer at basis of odontogenic epithelium, d 3
B. Regeneration of odontoblast layer during drug holiday, d 6
Example 5: Anticancer compound 4, rat, 3d on 3d off 3d on

A. Odontoblast degeneration, B. Pulp necrosis, loss of dentin
Example 5: Anticancer compound 4, rat, 3d on 3d off 3d on

A./B. Odontoblast degeneration and sequestration of dentin
Neoplastic Terms

Ameloblastoma (TgAC)
Ameloblastic odontoma
Odontogenic fibroma
Odontoma (no tumor/hamartoma)
Cementifying/ossifying fibroma
Tumor, odontogenic, benign
Tumor, odontogenic, malignant
What about Non-rodents and Relevance of Rodent Findings for Humans?
## Dentition in Beagle dogs

<table>
<thead>
<tr>
<th>Tooth Type</th>
<th>Deciduous Teeth</th>
<th>Permanent Teeth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eruption on day</td>
<td>Exfoliation on day</td>
</tr>
<tr>
<td>Incisors</td>
<td>23 - 25 (+/- 3)</td>
<td>114 - 133 (+/- 6)</td>
</tr>
<tr>
<td>Canines</td>
<td>21 - 22 (+/- 2)</td>
<td>140 - 160 (+/- 23)</td>
</tr>
<tr>
<td>Premolars</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molars</td>
<td>29 - 35 (+/- 4)</td>
<td>137 - 155 (+/- 7)</td>
</tr>
</tbody>
</table>
Beagle dog: Trimming of the teeth / Upper jaw
Example 6: Anticancer compound
Beagle Dog, Age at study start 119 - 147 days, 4 wk + 4 wk Recovery
Inlife Observations: none

A. Control, incisor, normal appearance, straight dentinal canaliculi
B. Treated, incisor, altered dentin composition
Example 6: Anticancer compound, dog

A. Treated, incisor, base of tooth with altered dentin matrix (4 wk)
B. Treated, progressed dentinal alteration, followed by normal growth (4 wk R)
Example 6: Anticancer compound, Beagle dog, 1 year

Inlife observation: persisting canines

A. Control, incisor, normal appearance
B. Treated, incisor, more or less normal, straight dentinal canaliculi
Example 6: Anticancer Compound 2, Beagle Dog, 1 year

A. Treated, found dead on day 232, incisor, undulated dentinal canaliculi
B. Treated, same animal, canine tooth, altered dentin composition
### Known reasons for incisor lesions:

<table>
<thead>
<tr>
<th>Vitamin Disorder</th>
<th>Effect Description</th>
</tr>
</thead>
</table>
| Hypovitaminosis A      | Increased thickness of enamel covered dentin  
                        | Reduced thickness of cementum-covered dentin  
                        | Pulp invasion by odontogenic epithelium                                                             |
| Hypovitaminosis D      | Wide zone of predentin, marbled dentin  
                        | Hypocalcified cementum                                                                              |
| Hypovitaminosis E      | Degeneration of enamel and fibrous replacement                                                                 |
| Hypervitaminosis D     | Hypercalcified dentin and cementum  
                        | Ossification of periodontal membranes                                                                |
## Known reasons for incisor lesions:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
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<tbody>
<tr>
<td>Ca and P Deficiency</td>
<td>Wide zone of predentin, Vascular inclusions in dentin</td>
</tr>
<tr>
<td>Mg Deficiency</td>
<td>Disturbed dentin calcification (stratification), Enamel atrophy and hypoplasia</td>
</tr>
<tr>
<td>Fluorosis</td>
<td>Disturbed dentin calcification, Enamel hypoplasia</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Enamel defects</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Necrosis of pulp mesenchyme and odontoblasts</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>Necrosis of pulp mesenchyme and odontoblasts</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Growth interruption, supernumerary teeth</td>
</tr>
<tr>
<td>VEGF Inhibitors</td>
<td>Degeneration/necrosis of odontoblasts</td>
</tr>
<tr>
<td>Tubulin Inhibitors</td>
<td>Enamel hypoplasia and discoloration</td>
</tr>
</tbody>
</table>
Rodent: Conclusions

- Unique model of dental pathological processes
- Continuous growth and differentiation
- Odontogenic tissue remains functional during life span
- Sensitive recorder of altered mineral metabolism
- Modify level 1 of the nasal cavity slide
- Additional use of longitudinal cuts of the incisor of lower jaw is proposed
Dog: Conclusions

- Sensitive recorder of altered mineral metabolism
- Incisor teeth are easy to process
- Always think of age of the animals and duration of study, renewal of the tooth takes some time
Közsönöm szépen!

Some day, we will all die, Snoopy!

True, but on all the other days, we will not.