Practical approaches for endocrine toxicity in preclinical safety assessment

Akira Inomata, D.V.M., Ph.D., D.J.S.T.P.

Tsukuba Drug Safety, Global Drug Safety,
Biopharmaceutical Assessments Core Function Unit

Eisai Co., Ltd.

hhc
human health care

Agenda

- **Evaluation of endocrine toxicity in non-clinical safety studies**
- **Practical cases 1 to 4**
- **Points to consider for safety evaluation**

EVALUATION OF ENDOCRINE TOXICITY IN NON-CLINICAL SAFETY STUDIES

Endocrine Toxicity

Hallmarks

Endocrine glands work cooperatively, responding to internal and external stimuli to maintain homeostasis.

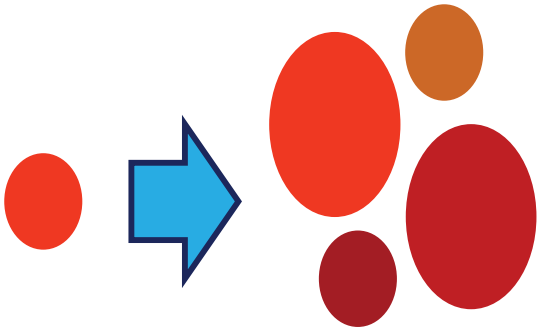
Functional modulation in an endocrine gland would cause pathophysiological responses in multiple tissues as well as the target tissue.

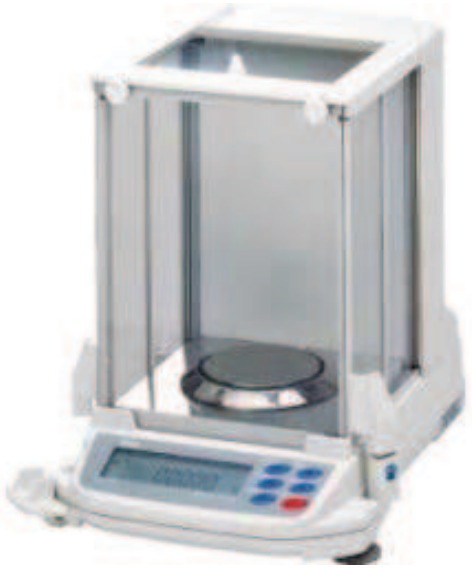
Points to be taken into account

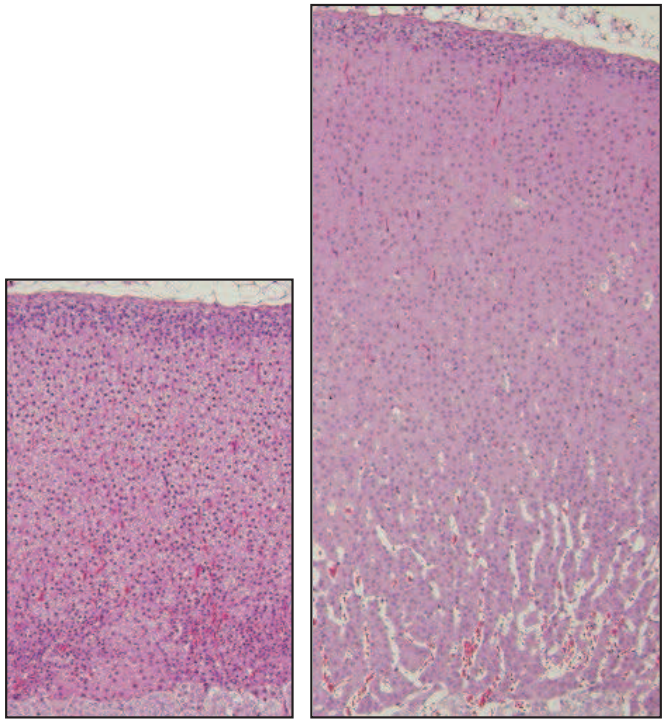
1. **Direct effect or other associated factors**
2. **Species and gender differences in hormone transport proteins**
3. **Sensitivity of target cells**

Evaluation of endocrine toxicity in non-clinical studies (1)

– In general, the effect of a test article on the endocrine system is first recognized as a change in size/color/organ weight at necropsy or in histopathologic examination.







Evaluation of endocrine toxicity in non-clinical studies (2)

- **Measurement of serum hormone levels or special staining or immunohistochemical analysis of endocrine organs is very helpful, however, it would be inadvisable to perform these examinations in routine toxicity studies in terms of time and cost.**
- **It would be more practical to examine endocrine organs in a step-by-step manner from early studies and to add test parameters as needed.**

Ex) Slight follicular cell hypertrophy was noted in an early shorter-term study (a few days to 2 weeks).

↓

Measurements of thyroid hormones, TSH, or hepatic enzyme activities can be considered in pivotal studies (4 or 13 weeks).

Evaluation of endocrine toxicity in non-clinical studies (3)

- Results from measurements of hormone levels or enzyme activities would provide useful information to explain the mode of toxicity of the test article.
- If it is difficult to examine these additional parameters in main groups for reasons of sample volume or timing, etc., to add a satellite groups in the study or conduct a separate study focused on a target organ can be optional.

Other approaches for endocrine toxicity evaluation

Since endocrine organs are under control of upper organs (hypothalamus – pituitary system) *in vivo*, it often becomes difficult to explain the mode of toxicity.

- **In such cases, an *in-vitro* system using specified cell-line or primary culture which is independent from the control of associated organs or hormones, or reporter gene assay would be helpful.**
- **Prior to conducting *in vivo* studies, it may be supportive for the future toxicity evaluation to have some information about structure activity correlation or receptor binding activity by *in silico* systems .**

PRACTICAL CASES 1 TO 4

Case 1.

Pigmented thyroid

Case 2.

Renal and bone lesions in rats (renal osteodystrophy)

Case 3.

Renal and parathyroid lesions in a carcinogenicity study in rats

Case 4.

Hypoadrenocorticism in monkeys

Pigmented thyroid

CASE 1.

Preface 1

Summary of Nonclinical Safety Profile

- Toxicology studies
  - MTD/DRF studies in mice, rats and monkeys
  - 4-week GLP toxicity studies in mice and monkeys
  - 4-week and 4/13-week reversibility study in mice

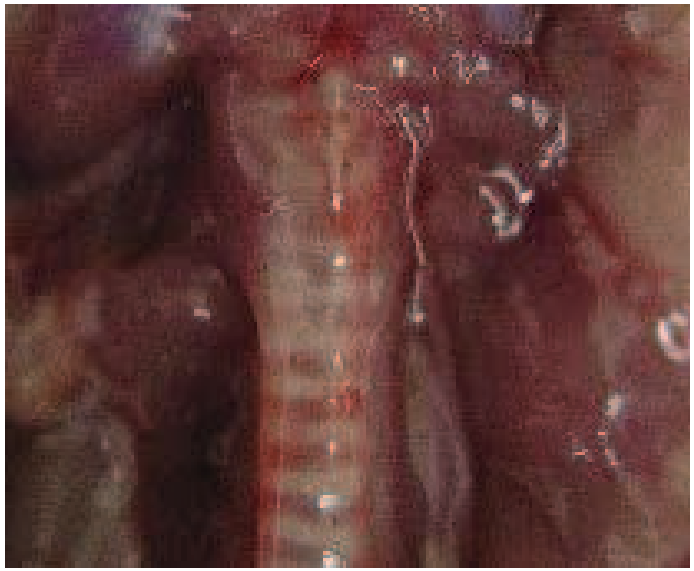
  Target organs
    - Thyroid *>> next slides*
    - Liver: Hepatic enzyme induction
- No effects on cardiovascular/CNS/respiratory system
- No genotoxicity

MTD: maximum tolerated dose, **DRF**: dose range finding
GLP: good laboratory practice, **CNS**: central nervous system

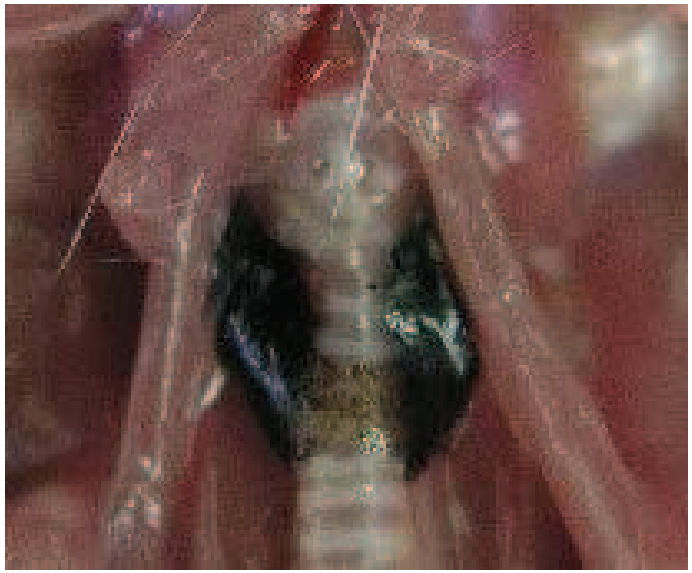
Thyroid Change

- Macroscopic findings
  - **Pigmentation/Enlargement**: mice, rats, monkeys

4-week study in mice



Control

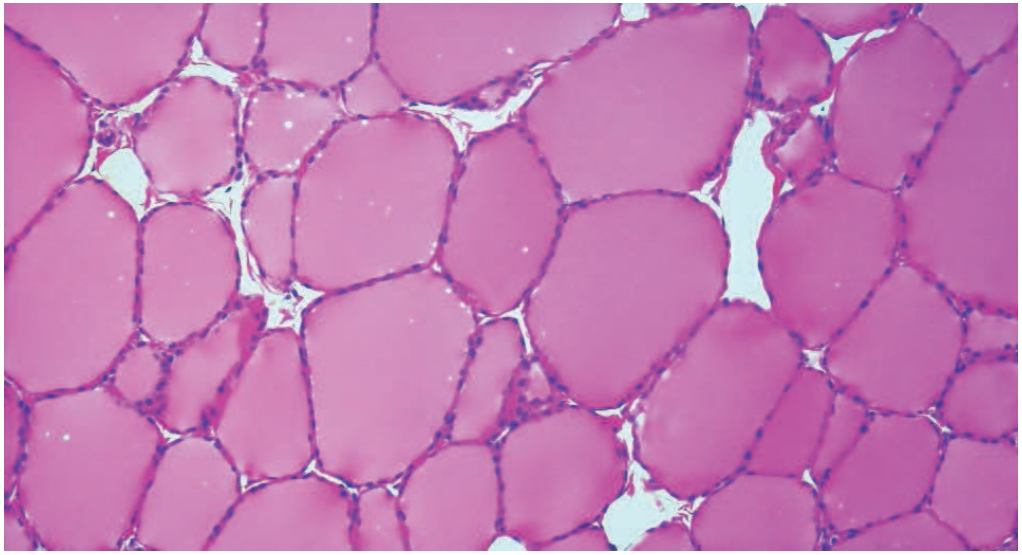


Treated

Thyroid Change

- Microscopic findings
  - **Follicular cell hypertrophy**: mice, rats, monkeys

4-week study in monkeys



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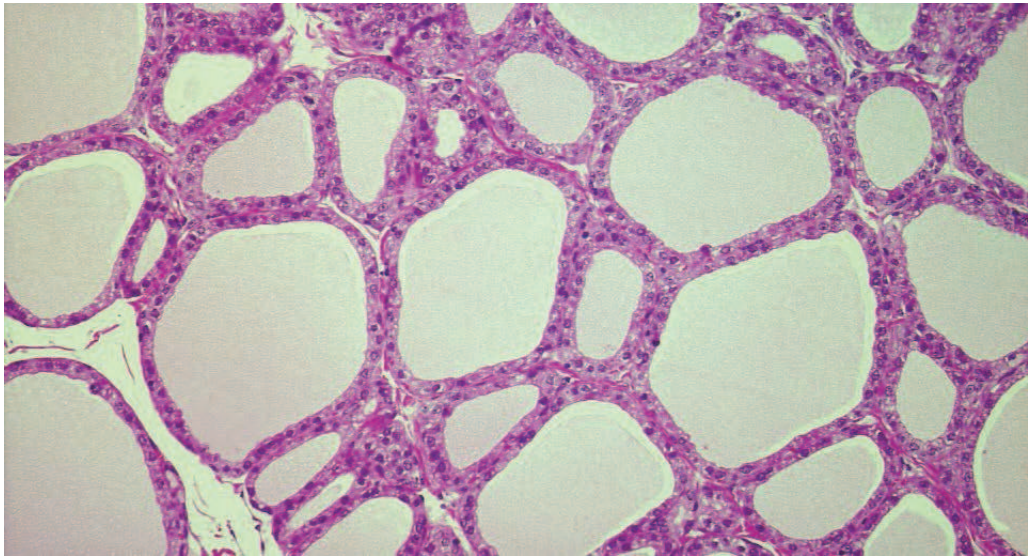


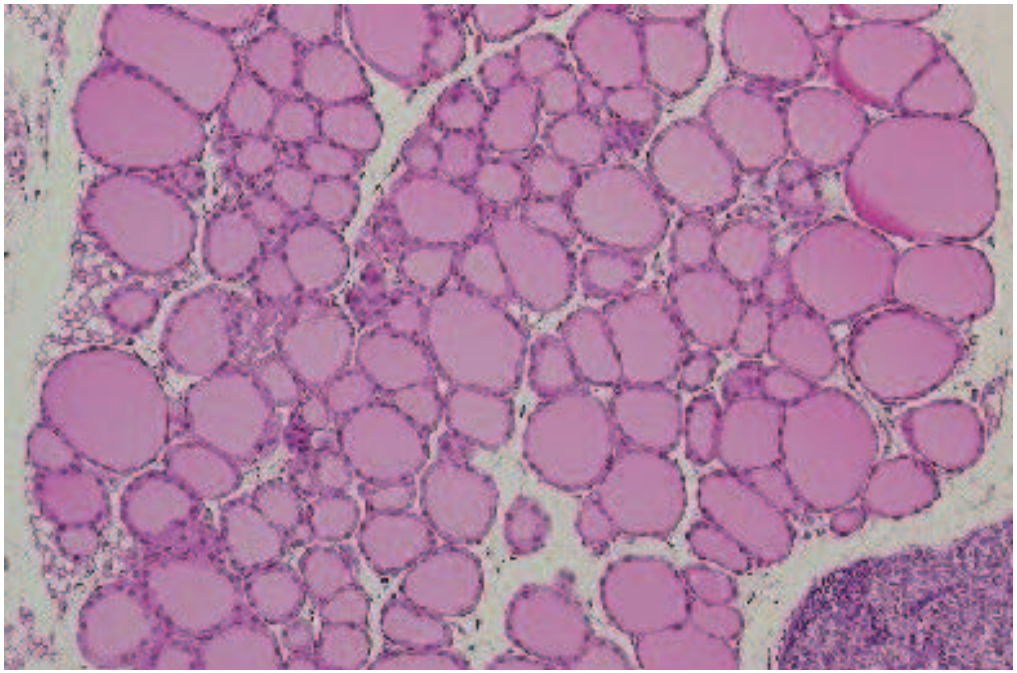
Treated

Thyroid Change

- Microscopic findings
  - Additional lesions: mice

    Interstitial inflammation, hyperchromatic colloid, nucleated exfoliated follicular cells

4-week study in mice



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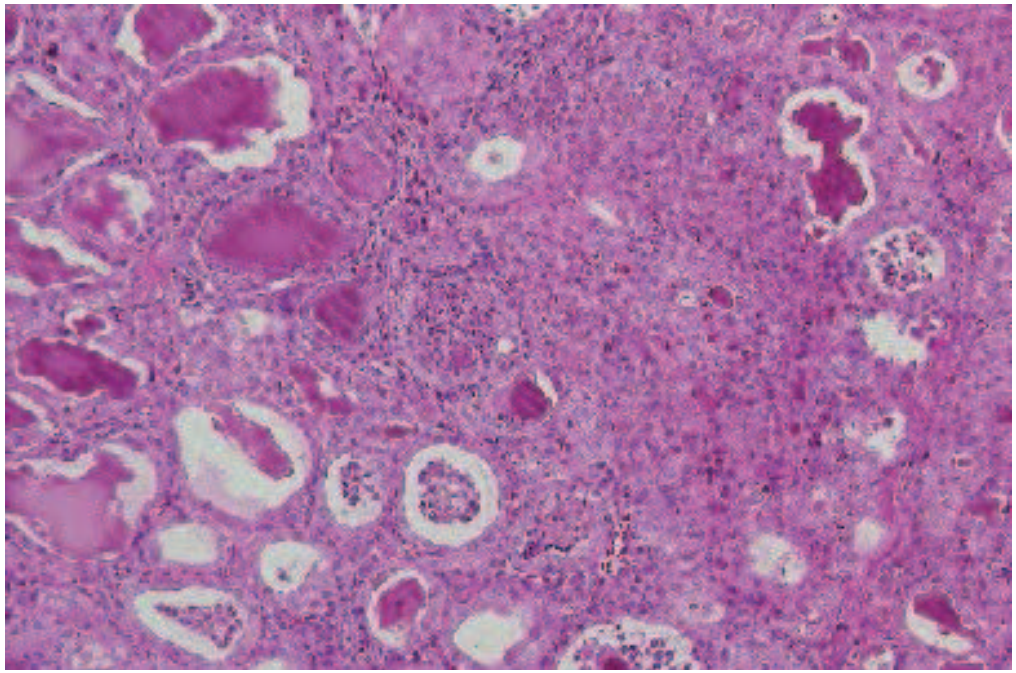
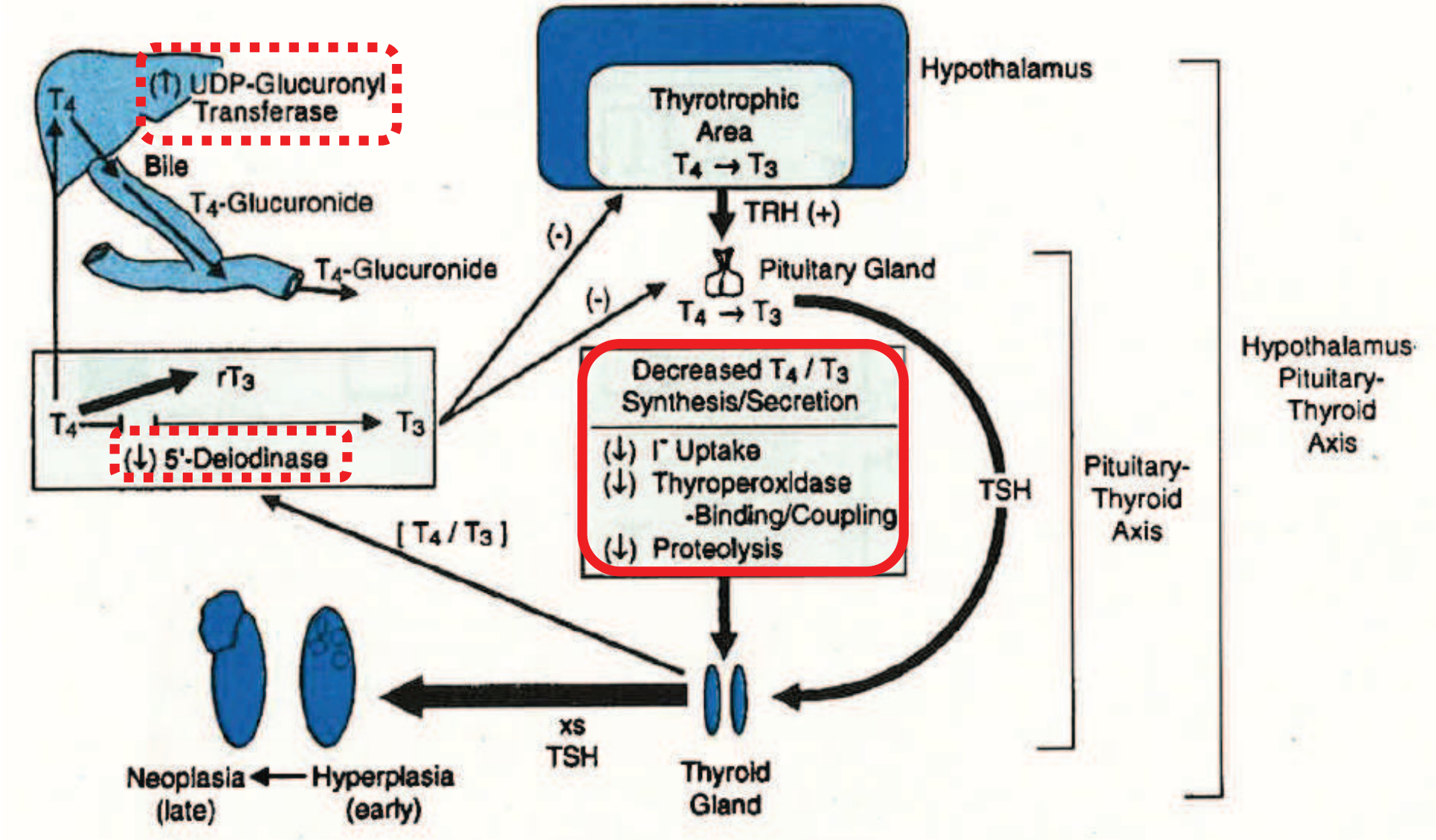


Treated

Hypothalamus – Pituitary – Thyroid Axis

Effects on hormone economy (Indirect)

Effects on hormone synthesis (Direct)



Casarett and Doull's Toxicology: The Basic Science of Poisons. 2001. p. 711-759

Species Differences in Thyroid Hormone

Thyroxin (T_4) and serum binding protein

Dohler et al., Pharmacol Ther 5:305-313, 1979.

Animal	TBG	Post albumin	albumin	Pre albumin
Mouse	-	++	++	-
Rat	-	+	++	+
Dog	++	-	++	-
Monkey	++	-	++	+
Human	++	-	++	+

TBG: T_4-binding globulin

Affinity to T_4 : TBG >>> (pre) albumin

Half-life of T_4 : 12-24 hrs (rat), 5-9 days (human)

Bile excretion of T_4 : 50% (rat), 10-15% (human)

Serum TSH level : rat > human, male rat > female rat

TRH sensitivity : rat > human

Rodents are susceptible to thyroid hormone economic changes.

TSH: thyroid-stimulating hormone, **TRH**: thyrotropin-releasing hormone

Issues about the thyroid lesion for IND

- **Reversibility**
  - Autoimmune response → chronic
- **Direct or Indirect**
  - Hepatic UGT induction (indirect) → rodent-specific
- **Characterization of pigments**
  - Involvement in the thyroid lesion

Timeline

Mouse

4-day
Liver: hepatocyte hypertrophy
Thyroid: pigmention /FC hypertrophy

4-week
Liver: hepatocyte hypertrophy
Thyroid: pigmention/FC hypertrophy/inflammation

Reversibility

4-week + 4/13-week recovery
Thyroid function tests

Monkey

7-day
Liver: hepatocyte hypertrophy

4-week
Liver: hepatocyte hypertrophy
Thyroid: pigmention /FC hypertrophy

Others

Direct or Indirect

In vitro system
Primary FC spheroid culture

Pigment research

Characterization of pigments

IND

FC: follicular cell

Reversibility study in mice

Animal : ICR mice

Drug administration : oral, daily

Group	4w		4w recovery		13w recovery	
	M	F	M	F	M	F
Control	5	5	5	5	5	5
LD	5	5	5	5	5	5
MD-L	5	5	5	5	5	5
MD-H	5	5	5	5	5	5
HD	5	5	5	5	5	5

Measurements : CYP activity/contents,

UGT activity, T3, T4, TSH

Reversibility study in mice

≥ mid-high dose	: pigmented thyroid (brown ~ dark brown)
High dose	: enlarged thyroid, increased liver weight[#], thyroid changes*

[#] : CYP3A↑, CYP contents↑, UGT (male)↑, liver weight↑,
* : enlarged thyroids, follicular cell hypertrophy/degeneration, hyperchromatic colloid, interstitial inflammation

- **Thyroid lesions after 4 and 13-week recovery showed clear reversibility. However, it was not completed and hyperchromatic colloid and interstitial macrophages remained in the HD group.**
- **All the changes including thyroid hormones had already been reversed following the 4-week recovery period.**
- **Thyroid pigmentation was still observed after the 13-week recovery; however, there was a time-dependent decrease in color (black-dark brown-brown).**
- **In the liver and pituitary, there were no histologic changes at any dose throughout the experimental periods.**

Thyroid Change

- Microscopic findings (reversibility)

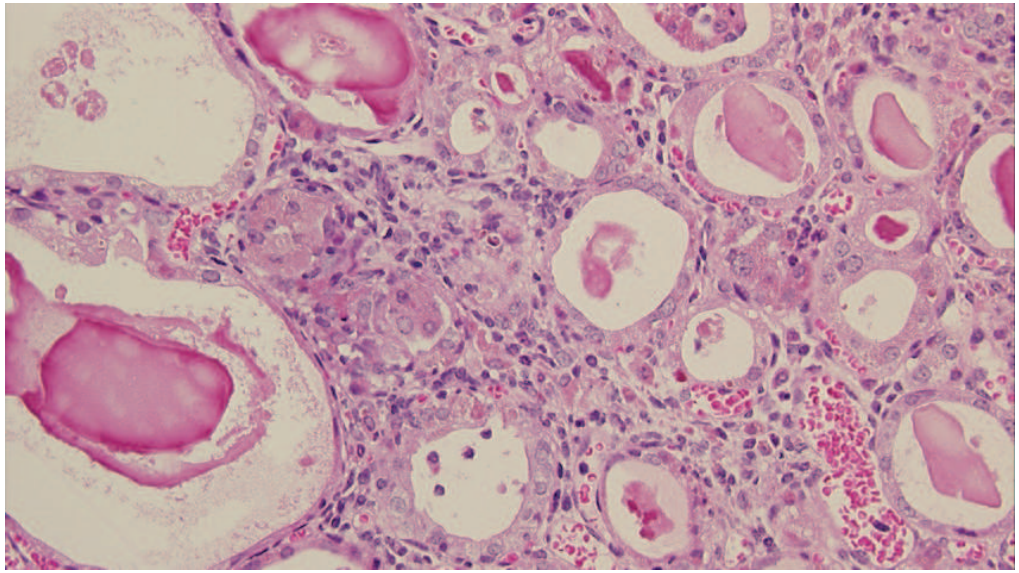
  Interstitial inflammation, hyperchromatic colloid

  nucleated exfoliated follicular cells

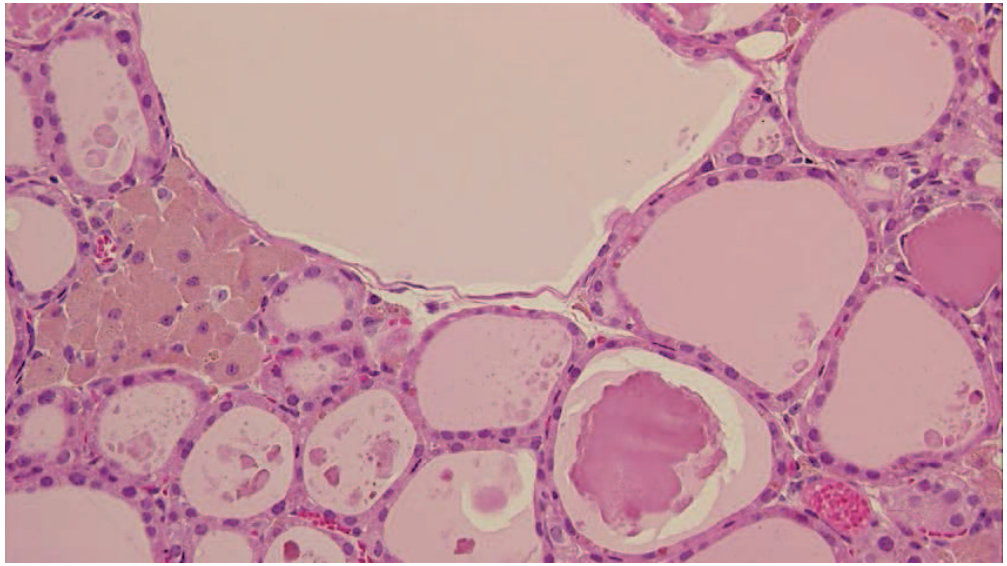
  ↓

  hyperchromatic colloid, interstitial macrophages

Reversibility study in mice

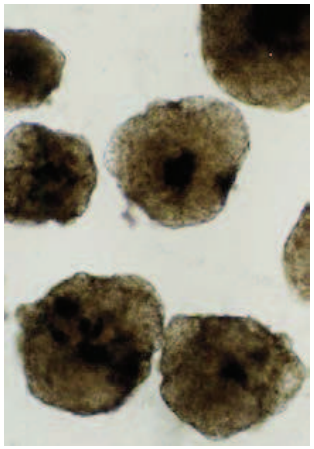


After 4-week dosing



After 13-week recovery

In vitro system
– thyroid spheroid culture -



STUDY DESIGN

The test article (free form) and 6 metabolites

Concentration : 1 and 10 µM

Positive control : methimazole

Negative control : vehicle (dimethyl sulfoxide)

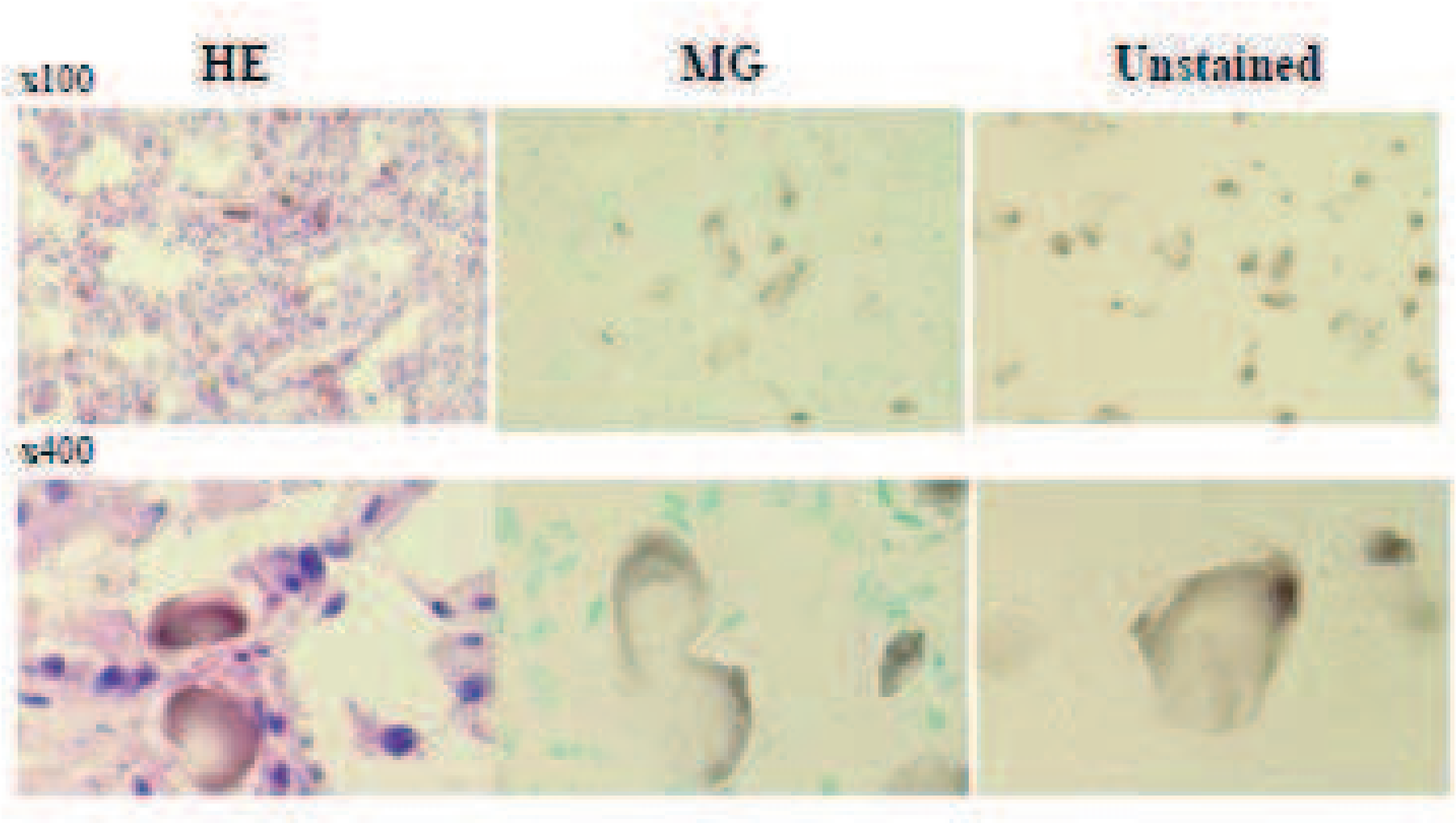
Procedure

1. 6-day pre-culture of thyroid cells obtained from 2-week-old rats to allow thyroid cell spheroids formation
2. Thyroid cell spheroids exposed to each condition for 24 hours, and sodium iodide-125 was added during the last 3 hours of treatment.
3. At the end of the 24 hours of treatment, iodide uptake and organification were measured.

CONCLUSION

The test article (free form) and its metabolites showed no specific effects on iodide uptake and organification.

Characterization of pigments
- localization of pigments in thyroids -



Counterstain: **HE**: hematoxylin and eosin, **MG**: methyl green

Characterization of pigments - microautoradiography -

Test Article

^{14}C-labeled test article

Animal

8-week-old SD rat (n=1)

Administration

Route : Oral
(14.8MBq/kg, Specific activity 0.069MBq/mg)

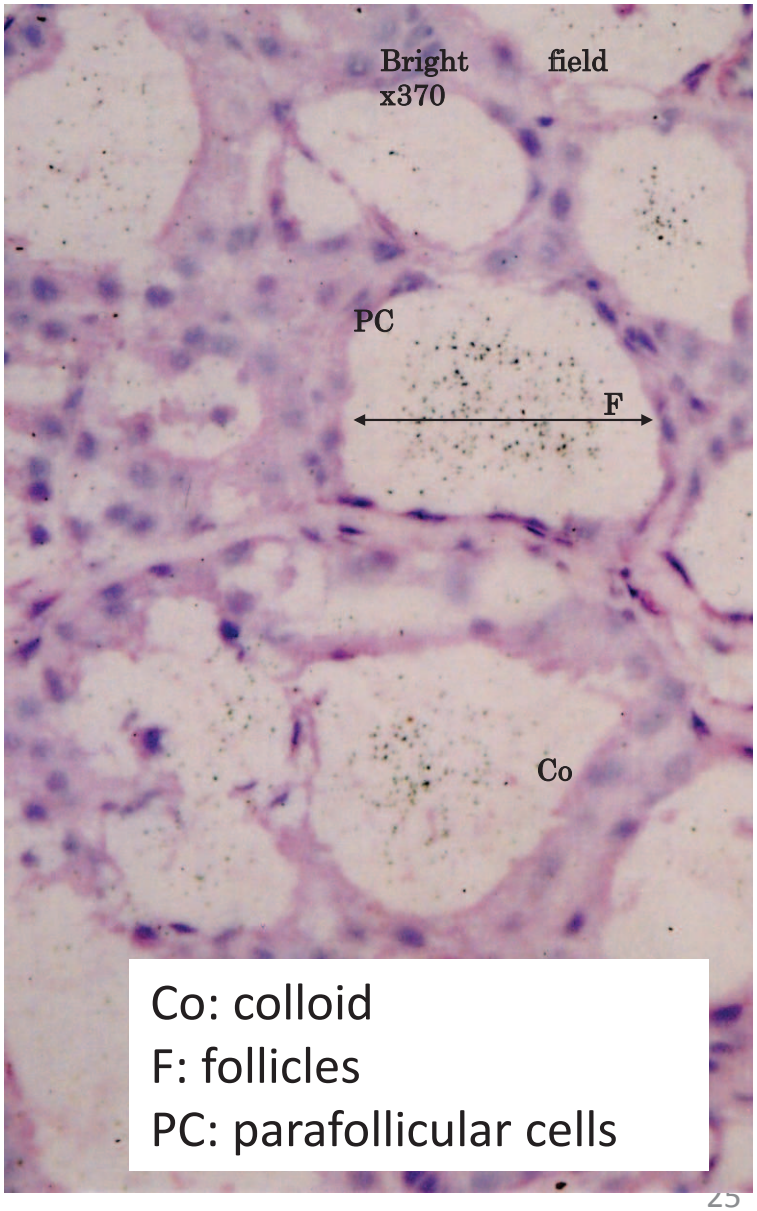
Duration : 4 days

Observation

Auto-radiography of thyroid after 24 hr post-dosing

Results

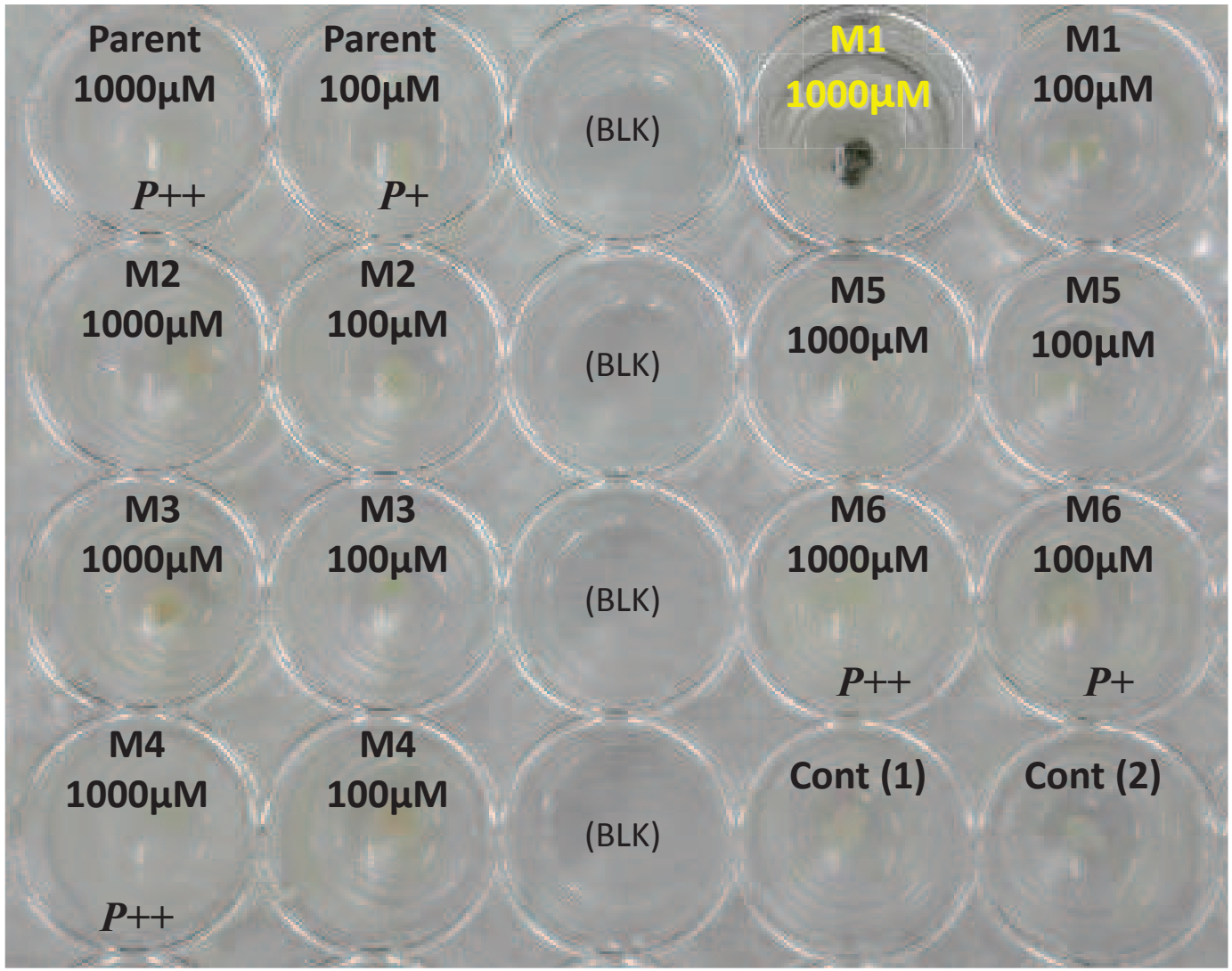
Test article or test article-related compounds existed in colloid.



Co: colloid
F: follicles
PC: parafollicular cells

Characterization of pigments
- in vitro thyroid pigmentation -

6 hr-treatment



Tissue:

Adult rat thyroids

Carrier:

10% DMSO in PBS

Culture condition:

37˚C, 5% CO_2

P: Precipitate

Parent: unchanged form

M1-M6: Metabolites

Summary of Thyroid Change

- **Reversible but not completely**
  - **Fast and complete** (within 4 weeks withdrawal period)
    - Hepatic enzyme induction
    - Follicular cell hypertrophy
    - Increased TSH, T4, FT4
  - **Slow and incomplete** (more than 13 weeks required)
    - Pigmentation/hyperchromatic colloid
    - Interstitial inflammation → interstitial macrophages
- **No direct effects on primary thyrocytes *in vitro***
  - **No specific effects** of test article and metabolites on iodide uptake and organification
- **Characterization of pigments**
  - **A metabolite or metabolite-protein complex** was mainly considered to attribute to pigment formation.
  - Many pigmented materials may be produced in pigmented thyroid but most of the chemical structures and contents are still unclear.

Renal and bone lesions in rats (renal osteodystrophy)

CASE 2.

Preface 2

Animal : Sprague Dawley rats

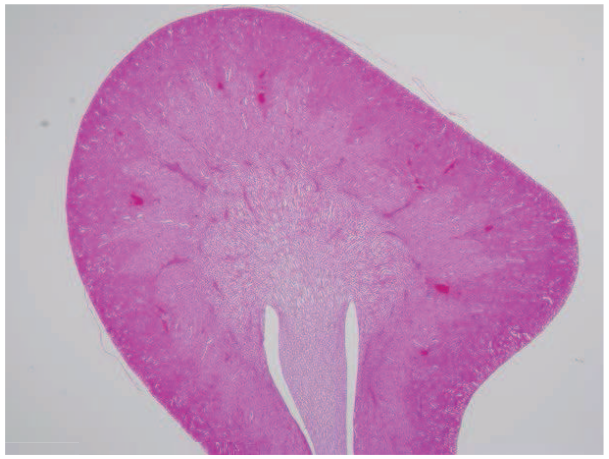
Drug administration : oral, daily

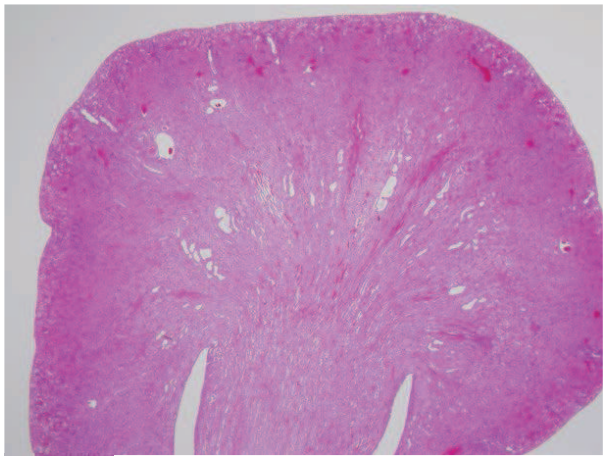
Dose levels : control, low, mid, high doses

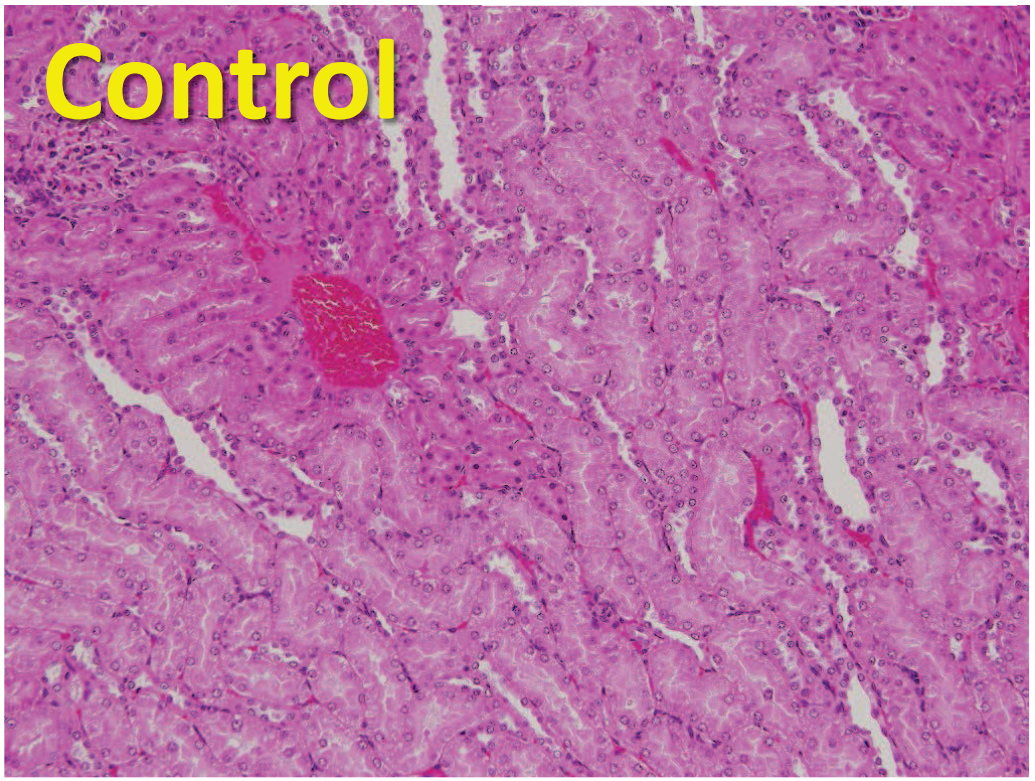
Duration : 14- or 26-week dosing

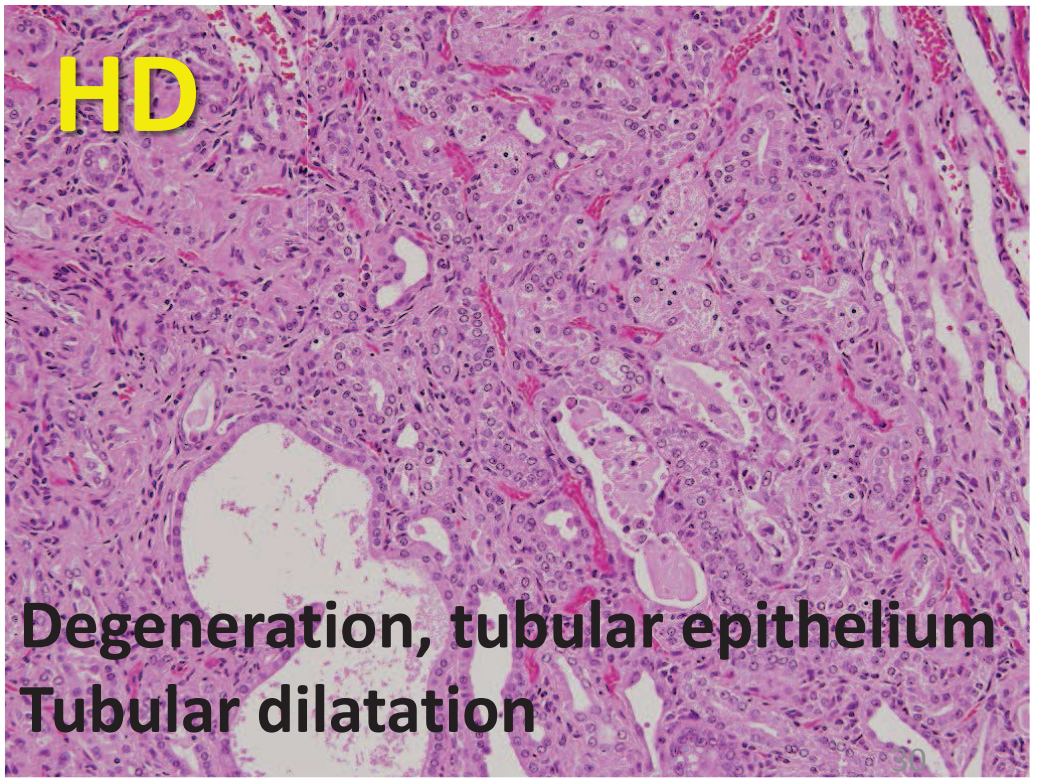
Group	Female	
	14w	26w
Control	10	10
LD	10	10
MD	10	10
HD	10	10

Histopathology (kidney)

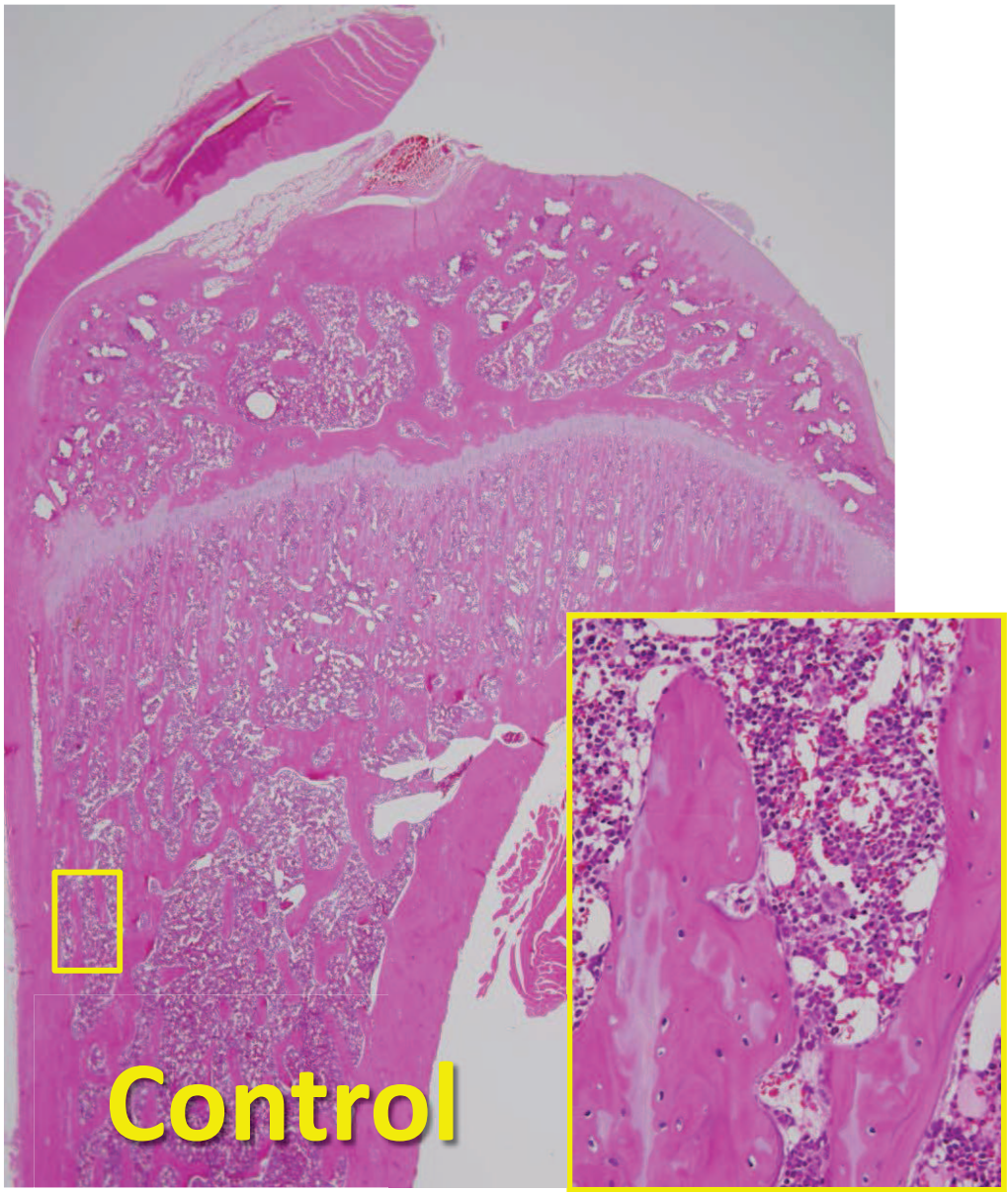


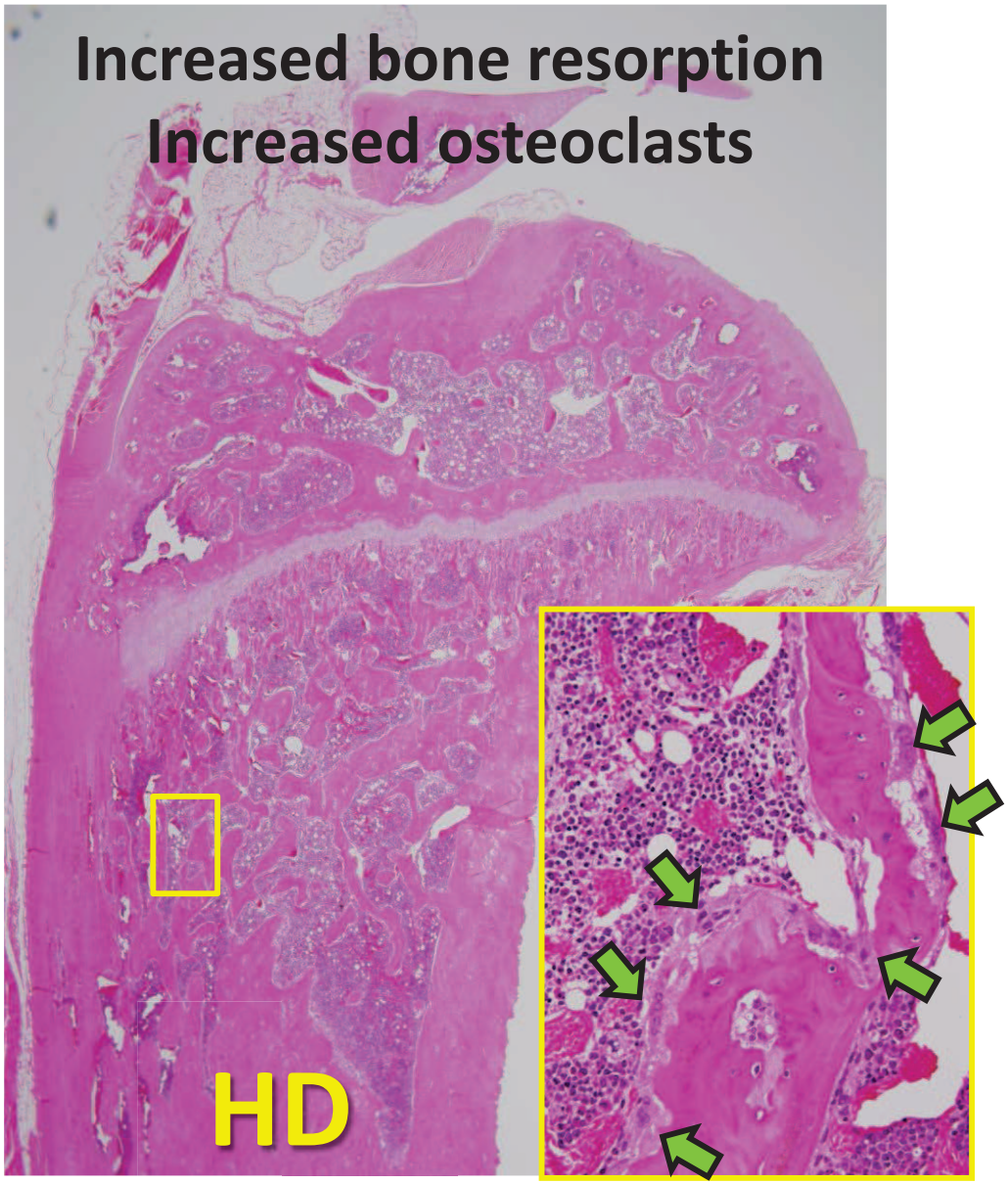






Histopathology (tibia)



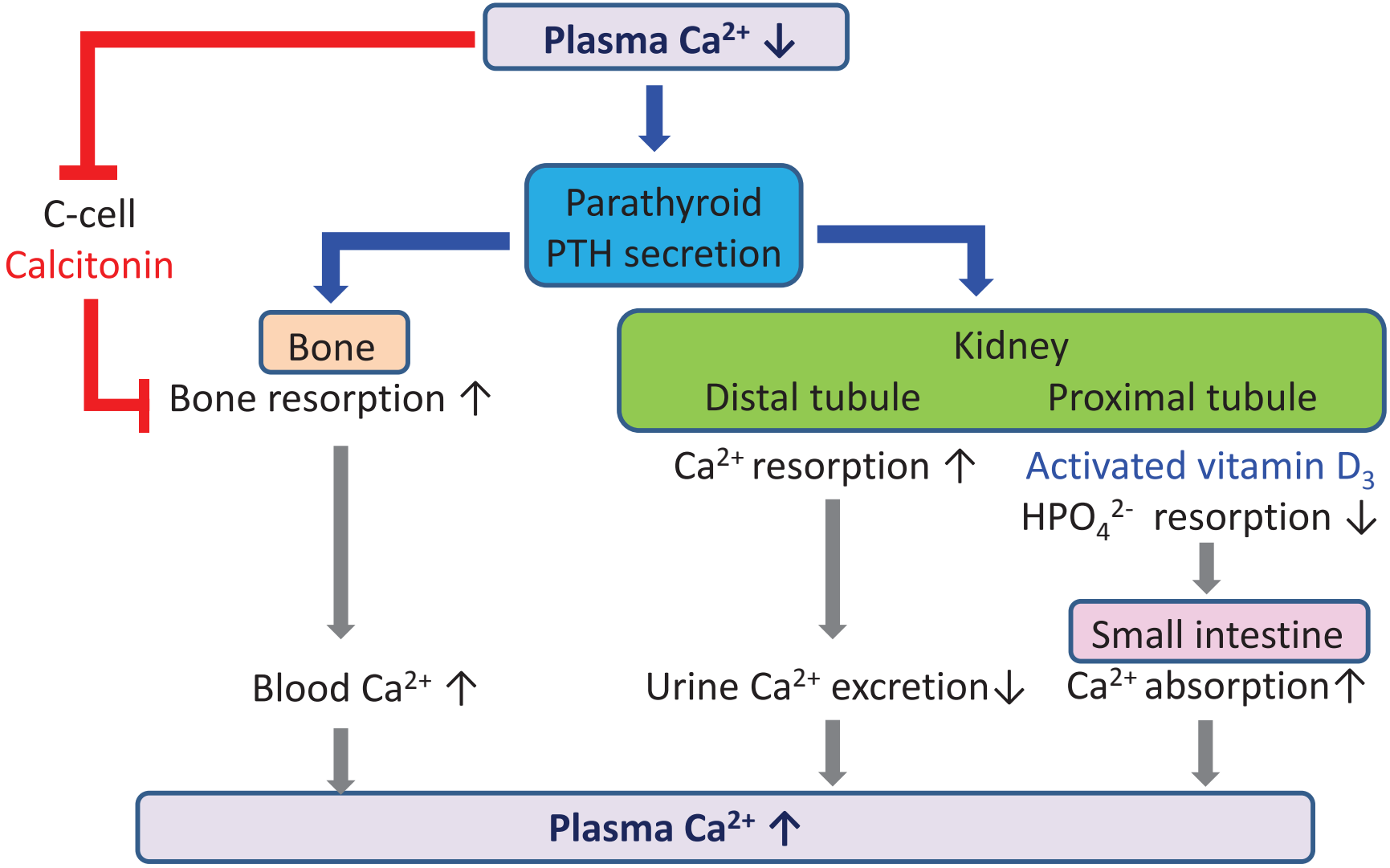


Correlation between renal lesion and increased bone resorption

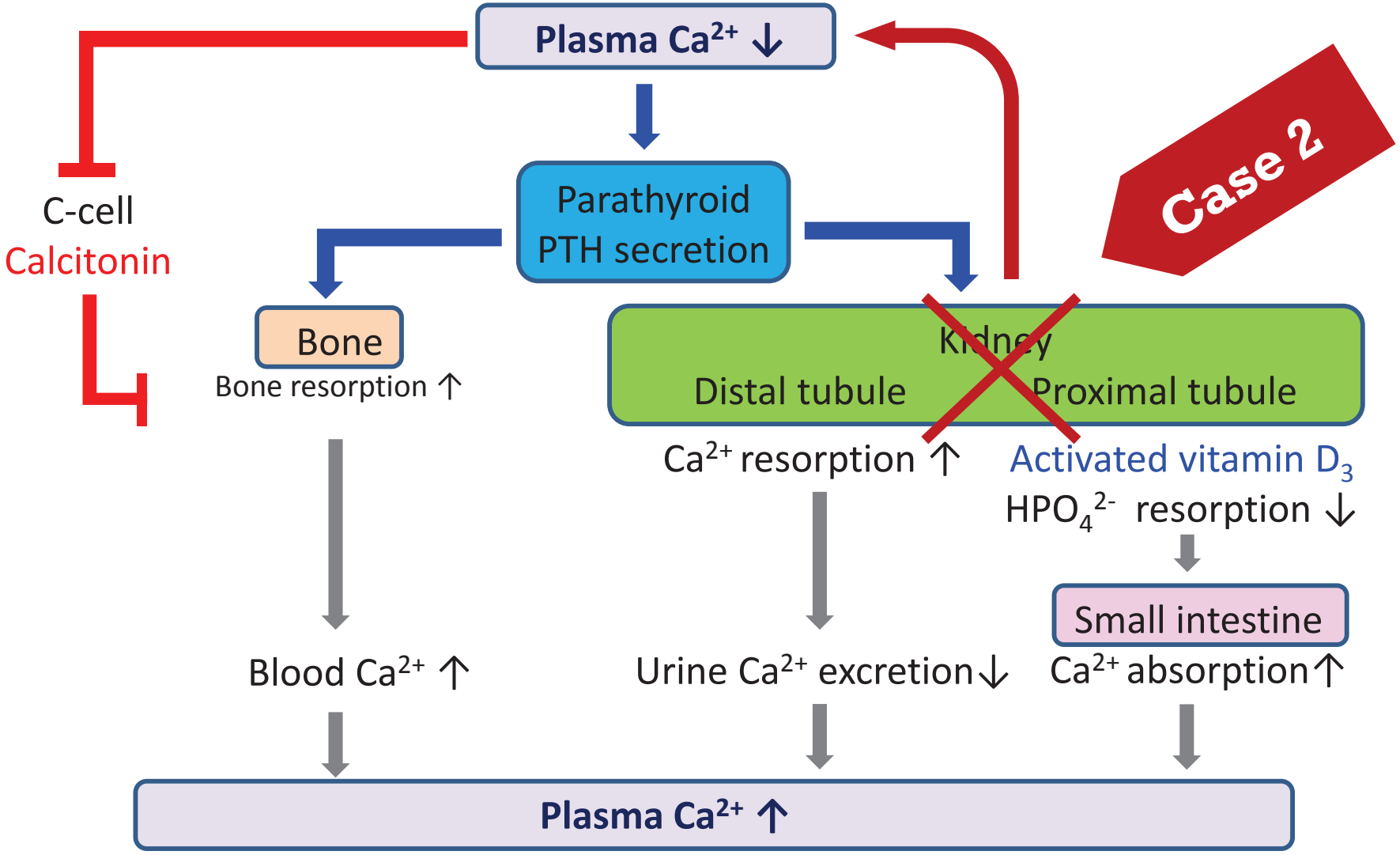
W14			
Animal No.	Bone Increased bone resorption		Kidney Degenera-tion tubules
	Tibia	Alveolar	
03F01	1+	1+	2+
03F02	-	-	-
03F03	-	-	1+
03F04	-	-	1+
03F05	-	-	-
03F06	1+	1+	3+
03F07	-	-	1+
03F08	1+	1+	3+
03F09	-	-	3+
03F10	-	-	2+

W26			
Animal No.	Bone Increased bone resorption		Kidney Degenera-tion tubules
	Tibia	Alveolar	
03F11	1+	1+	2+
03F12	1+	1+	2+
03F13	-	-	3+
03F14	1+	1+	3+
03F15	-	-	2+
03F16	1+	1+	3+
03F17	-	-	1+
03F18	-	-	1+
03F19	-	-	-
03F20	1+	1+	2+

Calcium homeostasis



Calcium homeostasis

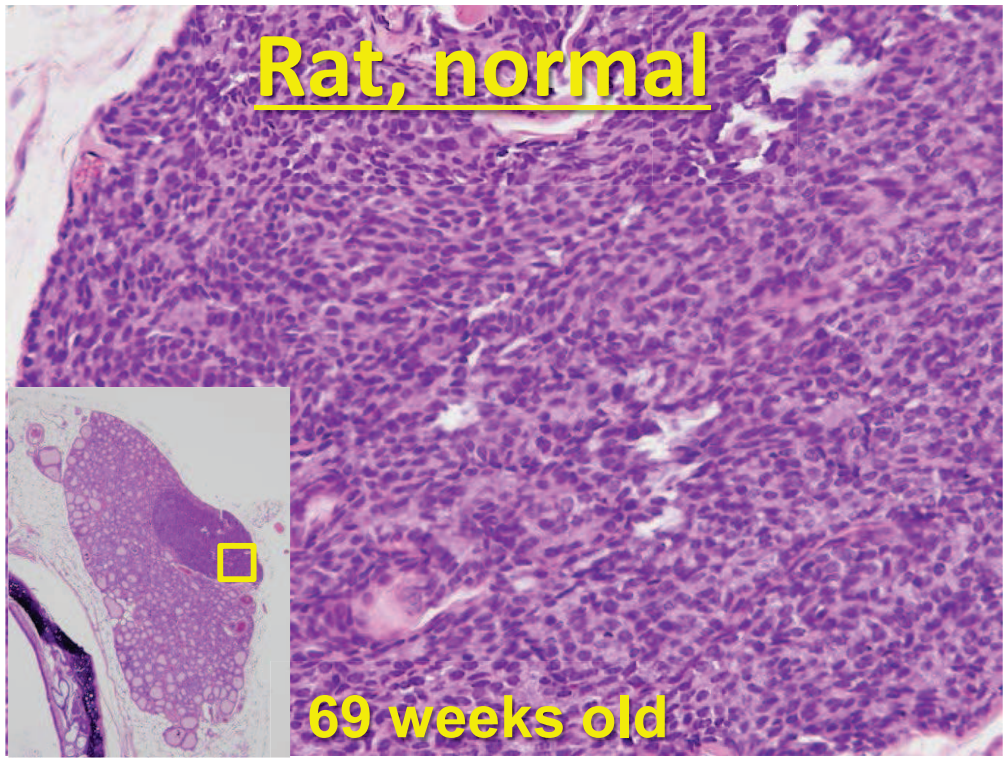


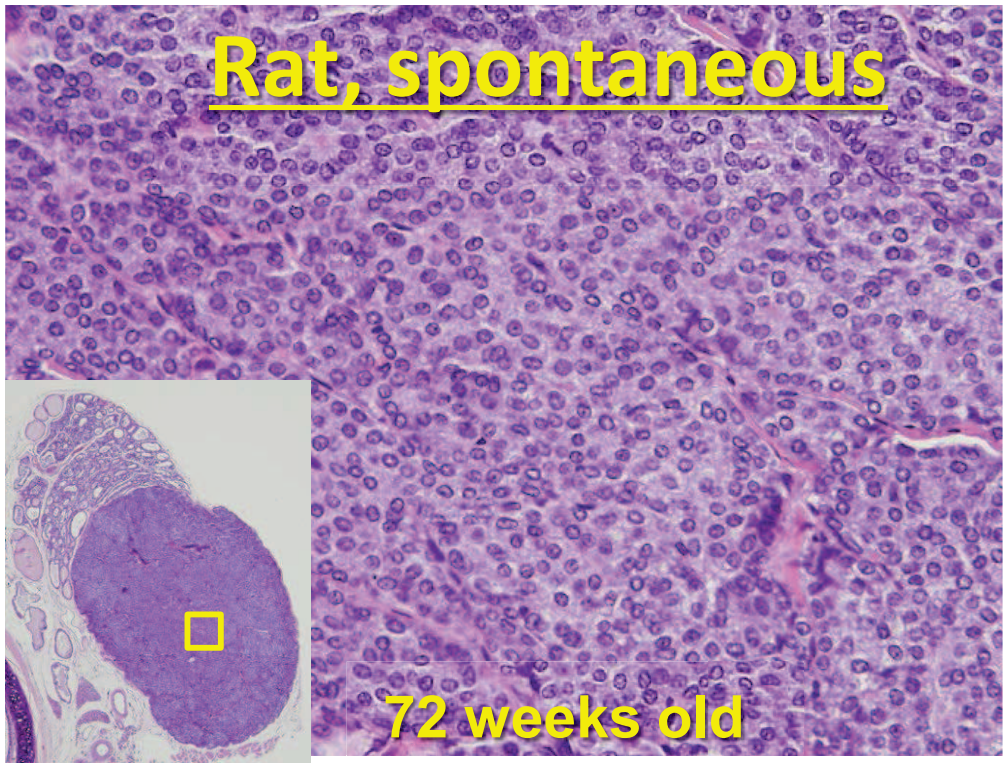
Renal and parathyroid lesions in a carcinogenicity study in rats

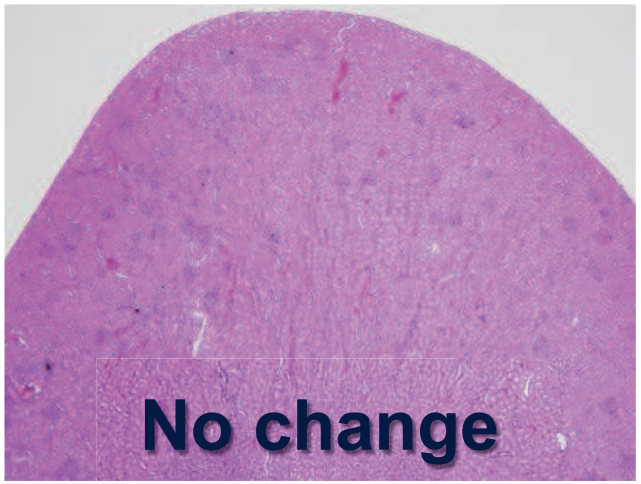
CASE 3.

Histopathology (parathyroid)

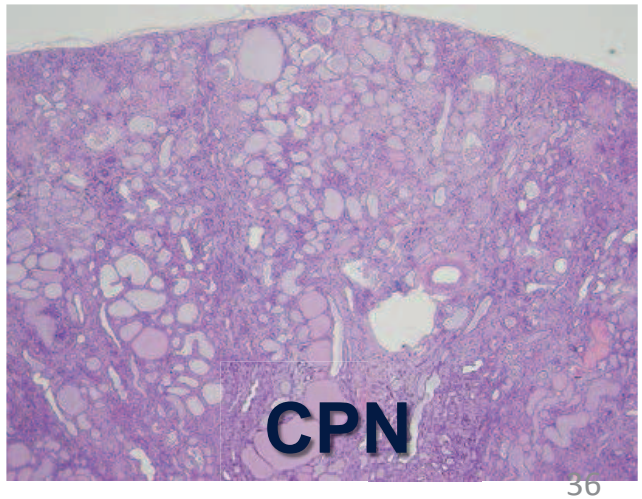
Diffuse hyperplasia





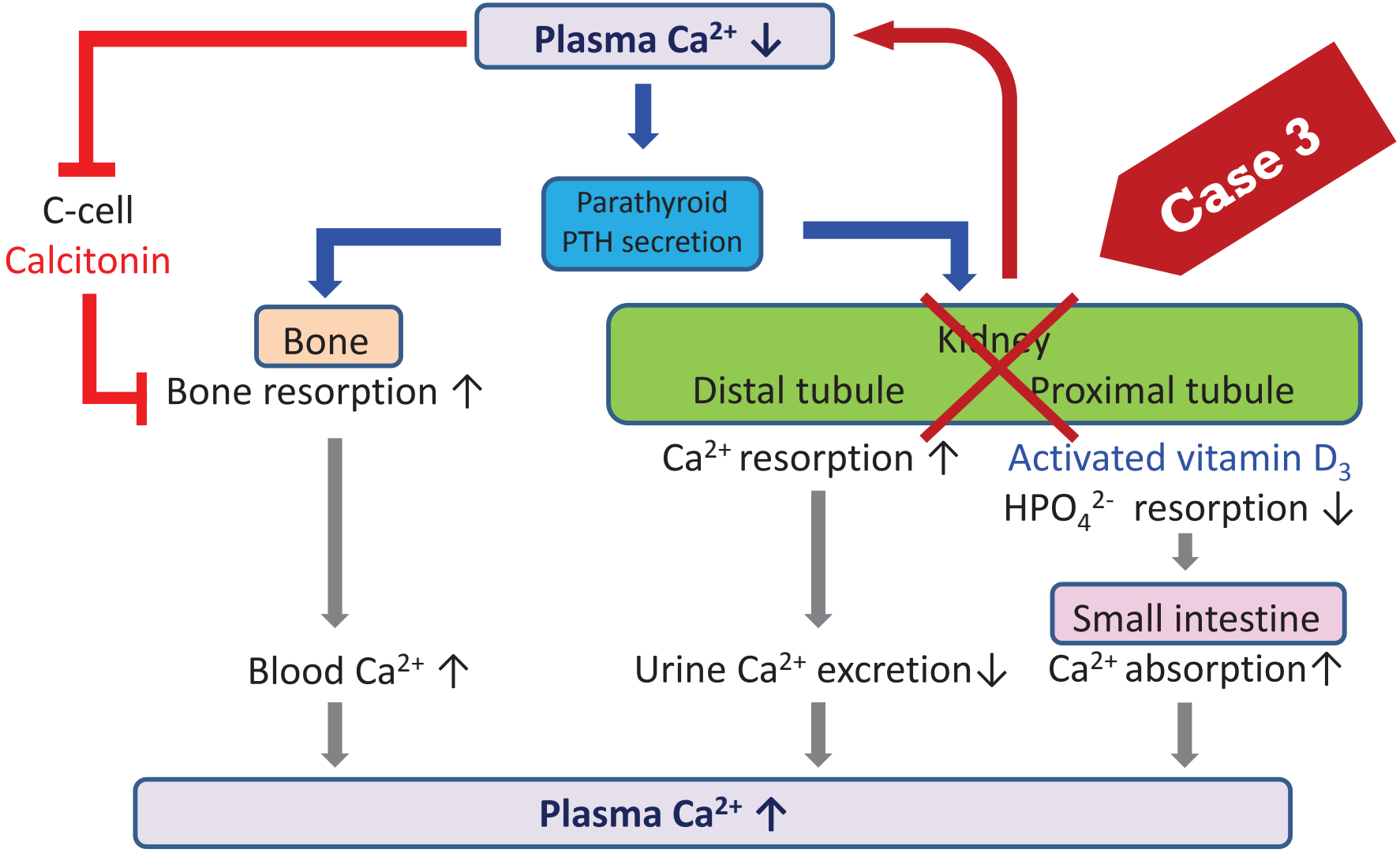


Associated change
Kidney



CPN: chronic progressive nephropathy

Calcium homeostasis



Hypoadrenocorticism in monkeys

CASE 4.

Preface 4

Animal : cynomolgus monkeys, female

Drug administration : oral, daily

Dose levels : control (n=4), 1 dose (n=24)

Duration : 13-week dosing at maximum

Subjects : **4 moribund animals**

Day 9 (n=1), 14 (n=1), and 43 (n=2)

In-life data and clinical tests

Animal No.	#210	#205	#206	#214
Necropsy (Day)	9	14	43	43
Clinical signs before moribund	decreased activity prone position shiver	decreased activity prone position shiver	lateral position vomiting	lateral position vomiting
Body weight	-	-	↓ (2.9 → 2.5 kg)	↓ (3.2 → 2.9 kg)
Food consumption	↓	↓	No change	↓
Hematology	↑: Neu, Mon ↓: Lym	-	-	-
Blood chemistry	↑: AST, **ALT**, **CK**, **UN**, Cre, **K** ↓: **ALP**, **T-Bil**, Glu, T-Cho, TP, Alb, A/G, Ca, **Na**, Cl	↑: AST, **ALT**, **CK**, UN, **K** ↓: **ALP**, **T-Bil**, T-Cho, TP, A/G, **Na**	↑: **ALT**, **CK**, **UN**, **K** ↓: **ALP**, **T-Bil**, Glu, T-Cho, **Na**	↑: AST, **ALT**, **CK**, **UN**, Cre, **K** ↓: **ALP**, **T-Bil**, Glu, Ca, **Na**, Cl

In-life data and clinical tests

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Food consumption	↓	↓	No change	↓
Hematology	↑: Neu, Mon ↓: Lym	-	-	-
Blood chemistry	↑: AST, **ALT**, **CK**, **UN**, Cre, **K** ↓: **ALP**, **T-Bil**, Glu, T-Cho, TP, Alb, A/G, Ca, **Na**, Cl	↑: AST, **ALT**, **CK**, **UN**, **K** ↓: **ALP**, **T-Bil**, T-Cho, TP, A/G, **Na**	↑: **ALT**, **CK**, **UN**, **K** ↓: **ALP**, **T-Bil**, Glu, T-Cho, **Na**	↑: AST, **ALT**, **CK**, **UN**, Cre, **K** ↓: **ALP**, **T-Bil**, Glu, Ca, **Na**, Cl

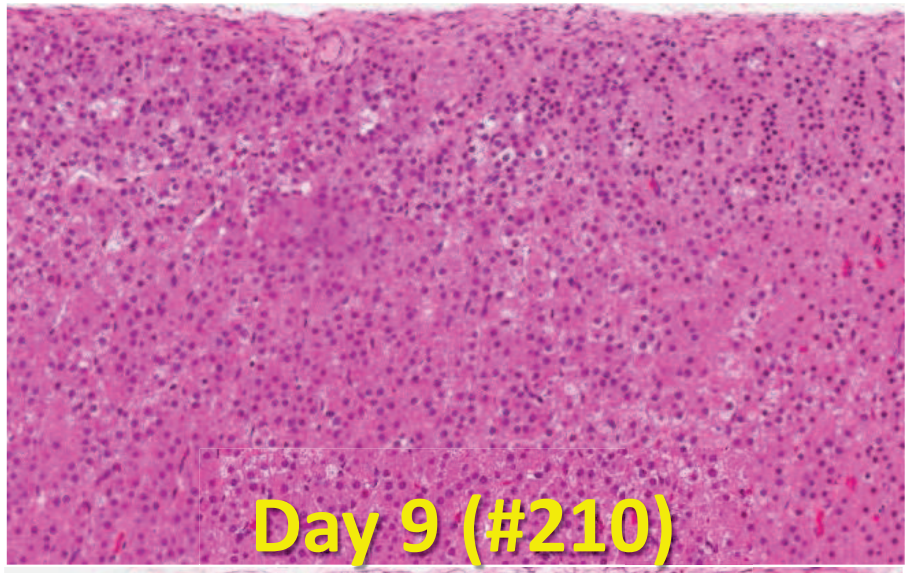
Common changes

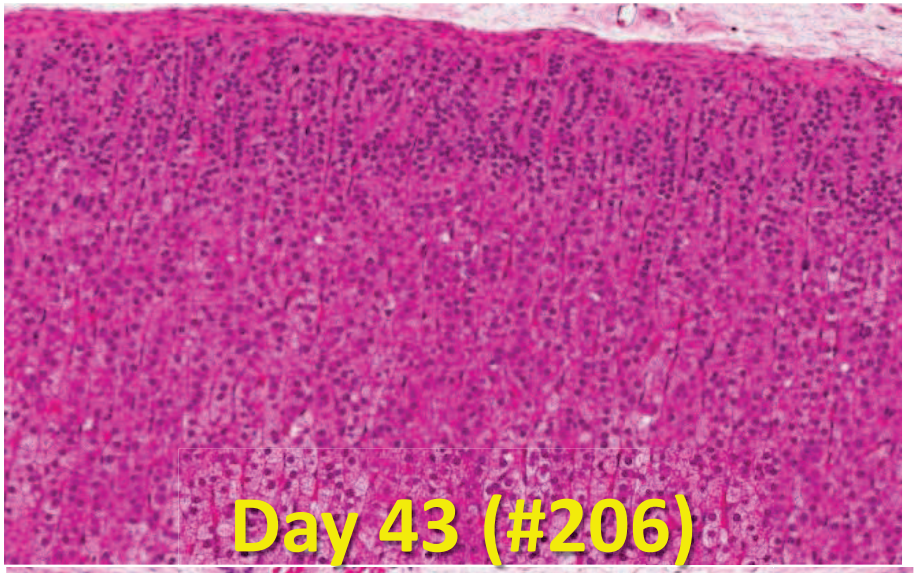
Summary of common findings

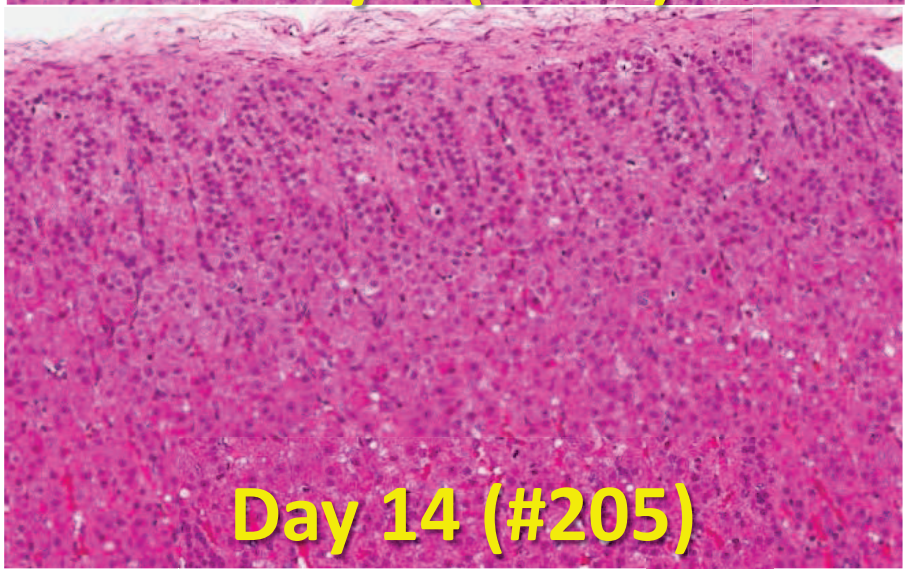
Animal No.	#210	#205	#206	#214
Necropsy (Day)	9	14	43	43
HPA-related hormone levels (vs. pre-value)				
ACTH ↓ (pg/mL)	844.7 → 21.6 (-97%)	159.0 → 74.2 (-53%)	992.7 → 70.1 (-93%)	285.8 → 159.4 (-46%)
Aldosterone ↓ (pg/mL)*	227 → 186 (-18%)	658 → 178 (-27%)	642 → 61 (-90%)	440 → 380 (-14%)
Clinical examination parameters (vs. historical background data)				
Na ↓	85%	88%	90%	73%
K ↑	1.6x	1.8x	1.5x	1.6x

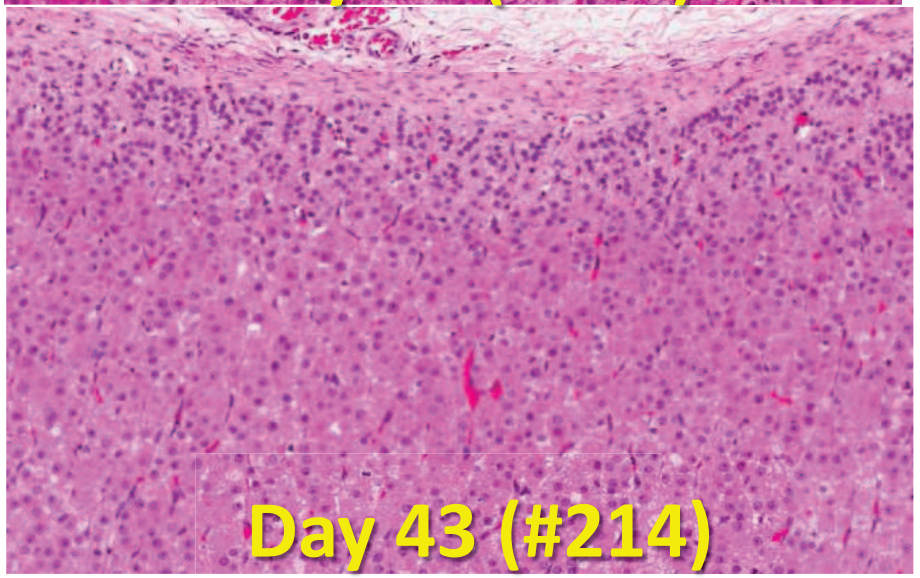
Red < -50% * Ref. 29.9 - 159 pg/mL (lying), 38.9 - 307 pg/mL (standing) in man

Histopathology (adrenal cortex)









Changes suggesting decreased function (atrophy) were not present, while stress-related changes (decreased lipid droplets or hypertrophy) were noted.

Adrenal failure

Veterinary laboratory medicine, Clinical Pathology, 4th edition

Hypoadrenocorticism

Adrenal gland failure : adrenal-dependent, Addison's disease

ACTH secretion failure : pituitary-dependent

Laboratory findings

With/Without histologic lesions

1. **Baseline plasma cortisol concentration may be within the reference interval or decreased.**
2. **There is no response to attempted ACTH stimulation.**
3. **Hyponatremia and hyperkalemia develop from renal loss of Na+ and retention of K+ because of aldosterone deficiency.**
   a. **Na+/K+ ratio < 23:1 is highly suggestive adrenal insufficiency**
   b. **Na+/K+ ration < 26:1 also may be suggestive of adrenal insufficiency**
   c. **Sodium and potassium values that are within the reference interval do not exclude adrenal insufficiency.**
4. **Hypercalcemia, lymphocytosis, or hypoglycemia may be observed.**

Summary of diagnostic criteria for adrenal failure

Animal No.	#210	#205	#206	#214
Necropsy (Day)	9	14	43	43
Pituitary				
ACTH ↓	◎	◎	◎	○
Adrenal				
Aldosterone ↓	◎	○	◎	○
Kidney				
Na+ ↓	◎	◎	◎	◎
K+ ↑	◎	◎	◎	◎
Na+/K+ ratio < 23:1	◎ 18:1	◎ 17:1	◎ 20:1	◎ 15:1
Cortisol ↓	-	-	- (slight increase)	- (slight increase)
Ca ↑	○	-	-	◎
Glucose ↓	◎	-	◎	◎
Lymphocyte ↑	-	-	-	-

Definitive criteria

Case 4. Summary

- **Moribund sacrifice was conducted in 4 animals on Days 9, 14, and 43. The plasma concentrations of the test article were high before necropsy when compared to the pre-value of the surviving animals.**
- **Decreases in plasma ACTH and aldosterone levels were noted in all these animals, and associated low ratio of serum sodium/potassium was also observed.**
- **It was suggested that acute hypoadrenocorticism was induced and caused moribundity, because no significant adrenal changes were observed histopathologically.**

POINTS TO CONSIDER FOR SAFETY EVALUATION

Results

In-life data

- Clinical sign, body weight, food consumption, etc.
- Clinical tests: hematology, blood chemistry (incl. electrolytes), hormones, urinalysis, etc.

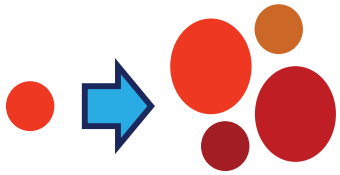
T3, T4, TSH, thyroglobulin
Calcitonin, PTH
Cortisol/Corticosterone, aldosterone

Post mortem data

- Organ weights
- Gross findings
  - size, color, etc.
- Histopathology
  - Changes in associated organs

    Thyroid (parathyroid) : liver, pituitary, kidney, bone, repro. organs, etc.

    Adrenal : kidney, pituitary, thymus, etc.
  - Historical background data
  - Physiological variation
  - Species-/gender difference



Interpretation

Primary or Secondary

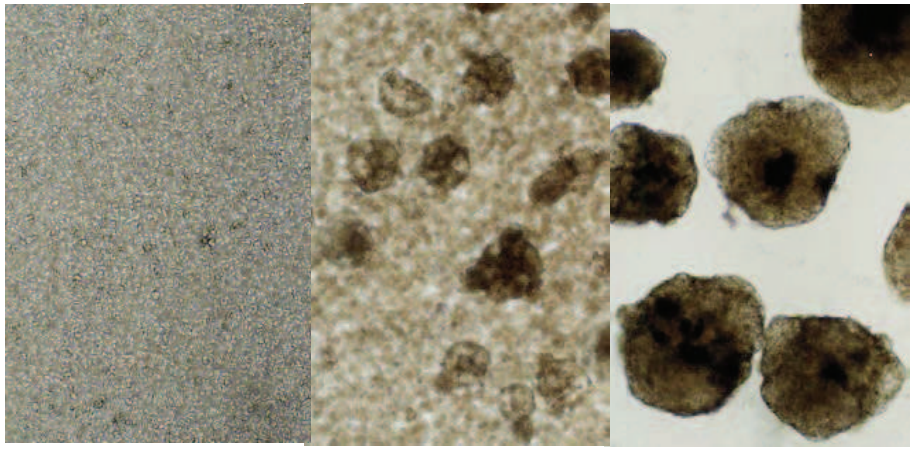
Direct : hormone synthesis/excretion, functional disorder, etc.

Indirect : upper organs, hepatic enzyme induction, stress, etc.

In vitro or other system

Cell culture : FRTL-5 cell, primary culture (monolayer, spheroid), etc.

Animal models : hypophysectomized, adrenalectomized, etc.



FRTL-5: Fischer rat thyroid cell line-5

Thank you.