The 46th Annual Meeting of the Japanese Society of Toxicology

Axcelead

Drug Discovery Partners Inc.

Solution providing for innovative drug discovery

Non clinical study outcomes of fasiglifam discontinued prior to approval
医薬品事例紹介:承認前に開発中止になったファシグリファムの非臨床薬物動態試験成績

Drug Disposition & Analysis,
Yoshihiko Tagawa, PhD, DVM.

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My Background

- ✓ Main task of DMPK researcher at early and late development stage in pharmaceutical companies

A: Absorption
D: Distribution
M: Metabolism
E: Excretion

→ **"ADME"**

✓**We use radiolabeled clinical candidates (^{14}C, ^{3}H, ^{125}I etc.) in ADME study.**
✓**ADME means DMPK activities using radio isotope (RI) labeled compounds.**

- ✓ **Essential data package for preparing NDA.**
- ✓ **FDA require human ADME for the essential data package for NDA.**

Pre-clinical	IND	Ph I	Ph II	Ph III	NDA
		・tolerability ・PK	・POC ・DDI ・hADME	・large scale clinical study ・combination therapy ・ethnic variation	

Notice

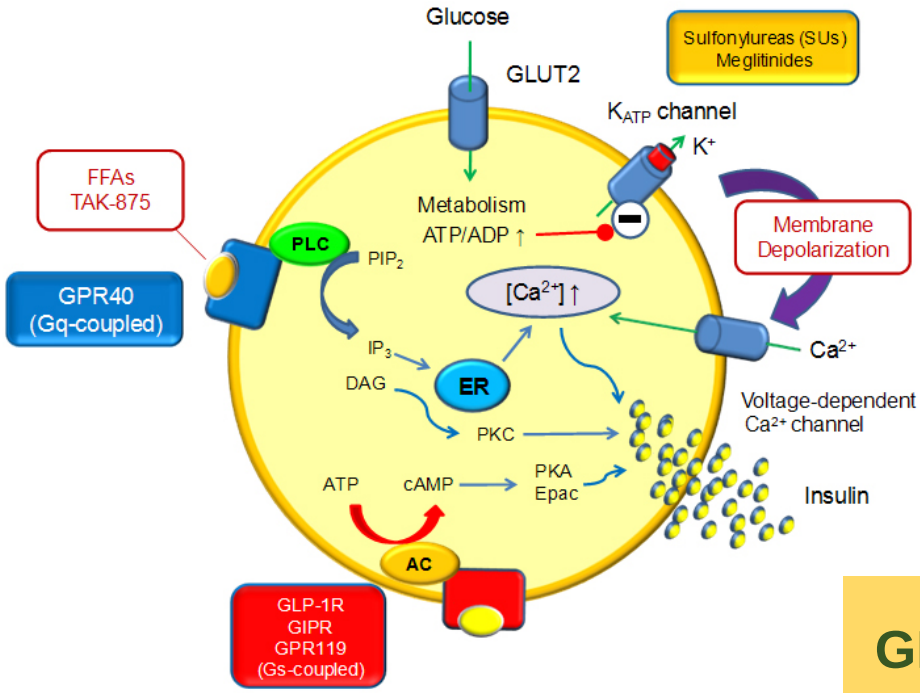
The conclusion in this presentation is the view of the team involved in the writing of the paper below, not the consensus of Takeda and Axcelead.

Title: Characterization of fasiglifam-related liver toxicity in dogs
Authors: A. Kogame, Y. Moriya, I. Mori, L. Pan, A. Morohashi, T. Ebihara, H. Fukui, Y. Tagawa, L. Z. Benet

MOA of GPR40

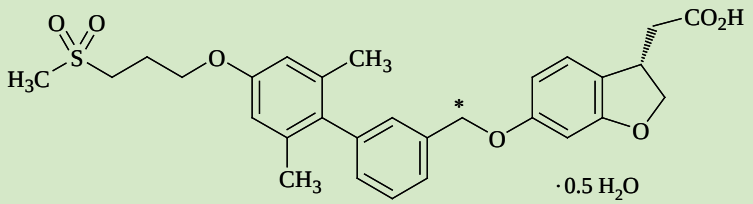
Role of GPR40: Insulinotropic Targets/Signaling Pathways in Pancreatic β-cells

Pancreatic β cells



Itoh et al. Nature 2003;422:173-6.
Briscoe CP et al. JBC 2003;278,11303-11.

Diagram adapted from Winzell and Ahrén. Pharmacol Ther 2007;116:437-48.



[^{14}C]Fasiglifam (^{14}C-TAK-875)

Asterisk denotes the radiolabeled position.

GPR40 activation triggers promoting insulin secretion through different mechanisms from existing agents.

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Background

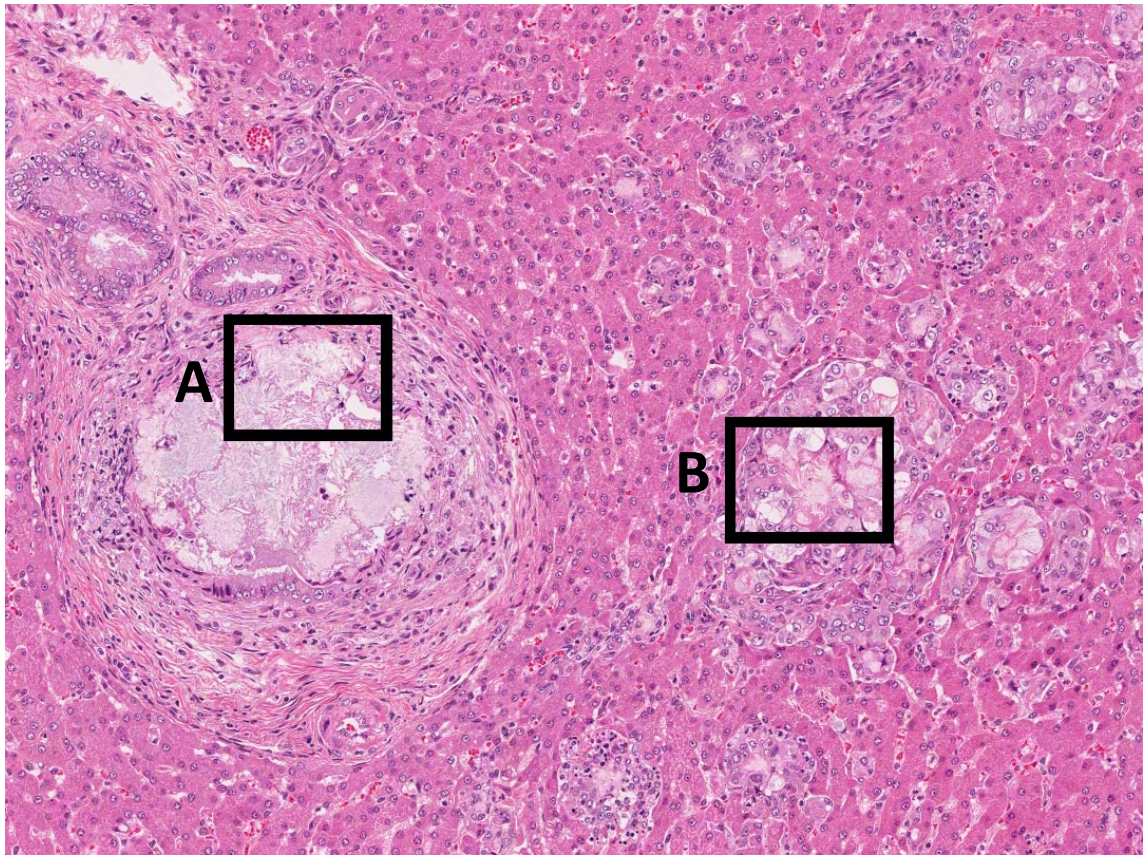
- ✓ Dog liver toxicity was observed in repeated dose toxicity studies of fasiglifam (TAK-875). Characteristic findings were follow.

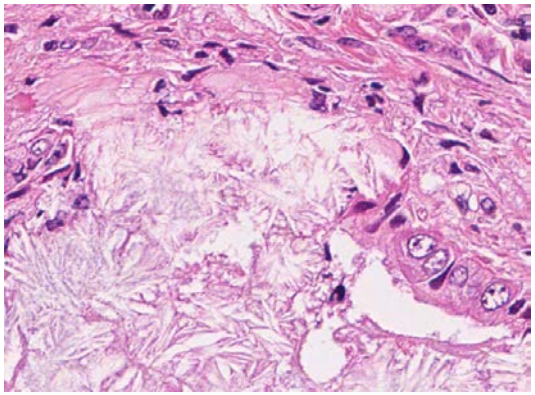
Dog :	Dose- and time-dependent liver damage with jaundice and foreign body granulomas
Rat :	No pathological finding in the liver

- ✓ There was no safety concern in Phase 2 trial. However to initiate Phase 3 trial, the factor for the dog liver toxicity and the possibility of extrapolation from dog to humans with regard to the hepatotoxicity were asked by FDA.

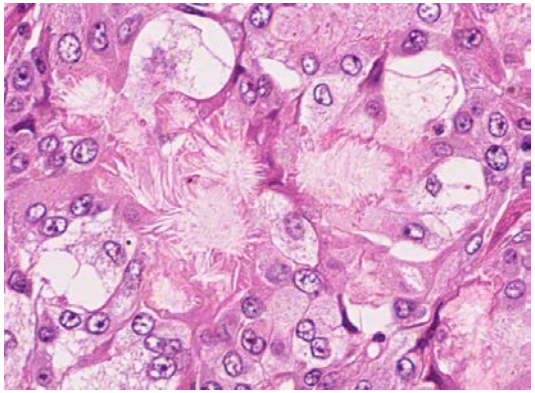
Foreign body granuloma in dog liver

Dose: 600 mg/kg of fasiglifam dose





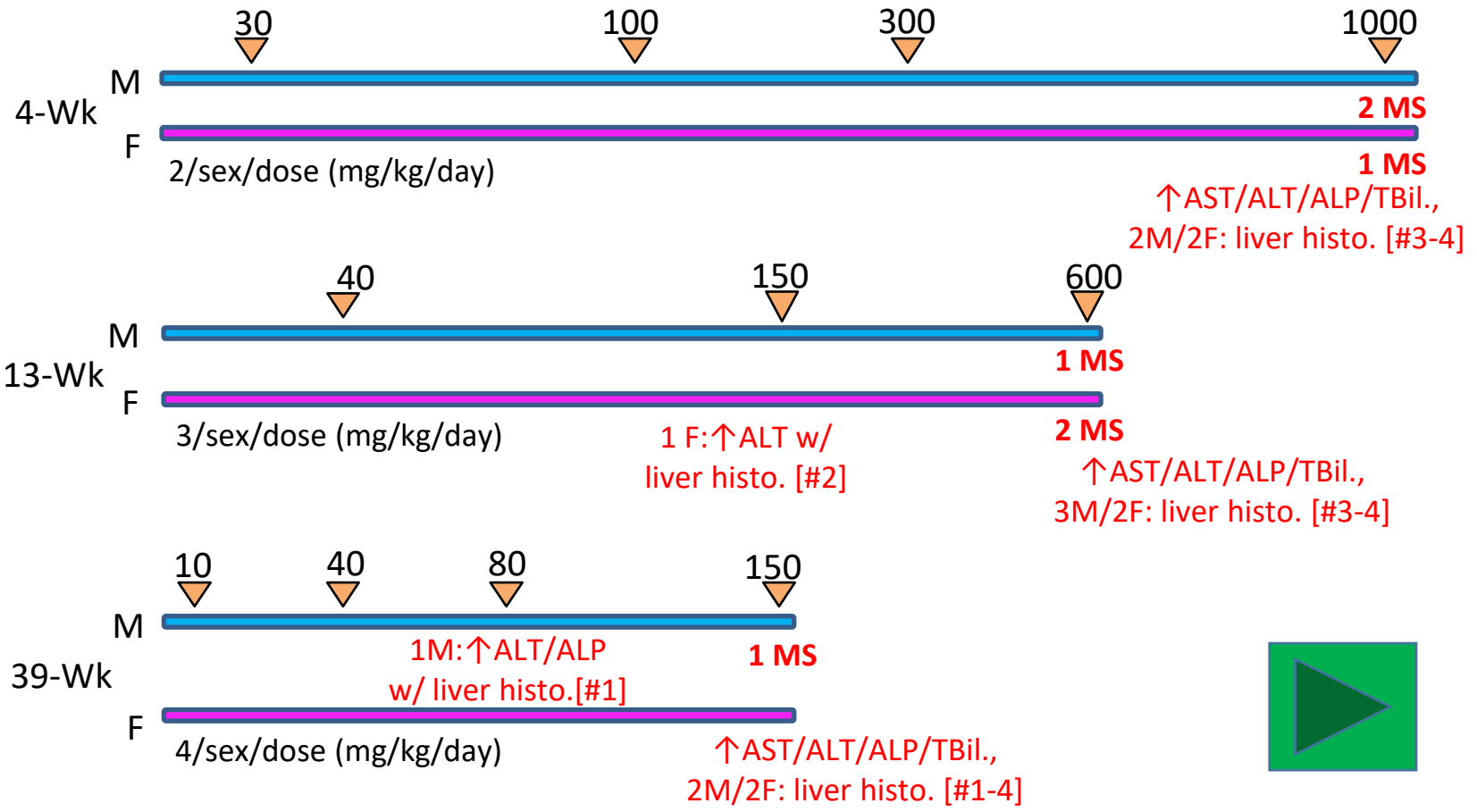
A. bile duct



B. parenchyma

The toxicity was observed in dogs but not in rats.

Dose- and time-dependent liver damage in dogs



liver histo.=portal/periportal granulomatous inflammation w/ crystal formation, number in [ ] indicates severity grade(s); **MS**: moribund sacrifice.

Trigger of this work

- Purpose
  - ✓We try to explain the species differences of hepatotoxicity through the evaluation of species differences in vivo and in vitro DMPK/ADME properties of fasiglifam in rats and dogs.
  - ✓We examine the extrapolation of dog hepatotoxicity to humans, explain the human safety, and propose the initiation of Phase 3 trial.

Content

- **ADME properties of rats and dogs at pharmacological and toxicological dose of [^{14}C]TAK-875**
  - ✓ Confirmation of elimination route (metabolism and excretion)
    - Species difference ?
  - ✓ Confirmation of radioactive concentrations in liver
    - Dogs >>> Rats ?
  - ✓ Metabolite composition in liver of rats and dogs
    - Detection dog unique and/or reactive metabolite(s) in the liver?
  - ✓ Contribution of biliary excretion in rats and dogs
- **Transporter assay (inhibition of BSEP?)**
- **Conclusion**

Pharmacokinetics of Fasiglifam in Rats and Dogs (1/2)

PK parameters

Species	Route	Dose (mg/kg)	Cmax (μg/mL)	Tmax (hr)	T1/2 (hr)	Vd (L/kg)	CL (L/hr/kg)	AUC (μg·hr/mL)	BA (%)
Rat	Oral	3	5.8	1.0	4.1	-	-	65.0	76.0
	IV	1	-	-	4.7	0.2	0.03	28.5	-
Dog	Oral	1	3.3	2.0	7.5	-	-	29.5	92.4
	IV	0.5	-	-	5.9	0.2	0.03	15.8	-

AUC (0-24) in rats and dogs, BA=bioavailability
N. Negoro et al. ACS Med. Chem. Lett 2010, 1. 290-294.

- ✓ TAK-875 has small Vd and low CL in rats and dogs.
- ✓ High plasma protein binding (>99%) across species was observed.
- ✓ BA of human was about 100%.
- ✓ Low CL in humans (0.01 L/hr/kg).
- ✓ Moderate Vd in humans (0.87 L/kg).

Pharmacokinetics of Fasiglifam in Rats and Dogs (2/2)

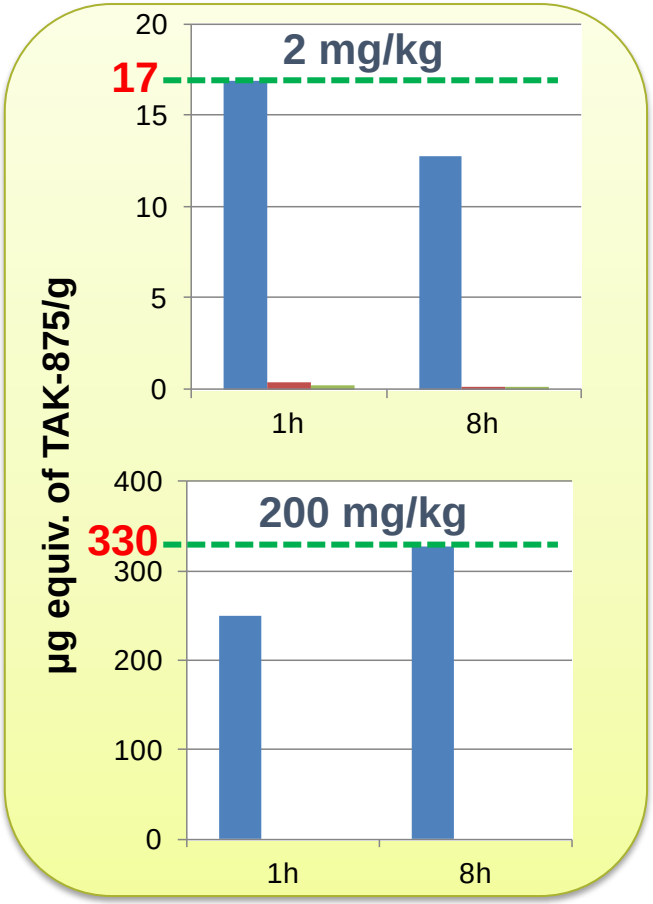
Metabolic Fate (Dose: 1 mg/kg)

Compound	% of dose				
	Rats			Dogs	
	Urine (0-24h)	Feces (0-24h)	Bile (0-24h)	Urine (0-24h)	Feces (0-72h)
Total ^{14}C	0.2	84	93	0.1	97
TAK-875	LOQ	48	7	LOQ	72
M-I	LOQ	3	LOQ	LOQ	1
TAK-875-G	LOQ	2	46	LOQ	1
Others	0.2	31	40	0.1	23

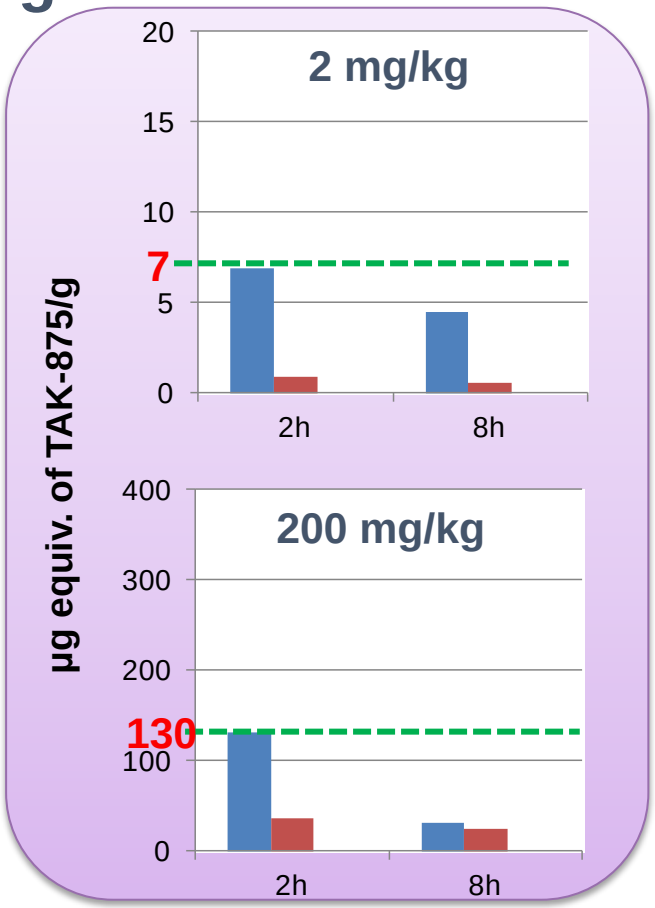
- ✓ Biliary excretion is main elimination route in both rats and dogs
- ✓ Glucuronidation is main metabolic pathway in rats and dogs(??)
- ✓ **No clear species difference in ADME property**

Concentrations of TAK-875 and its metabolites in the liver

Rat



Dog



- ✓ No dog unique metabolite was observed.
- ✓ Concentration of TAK-875 in dog liver were lower than those in rat.
- ✓ High concentration of TAK-875 in rat liver indicated that TAK-875 might not relate to the hepatotoxicity.

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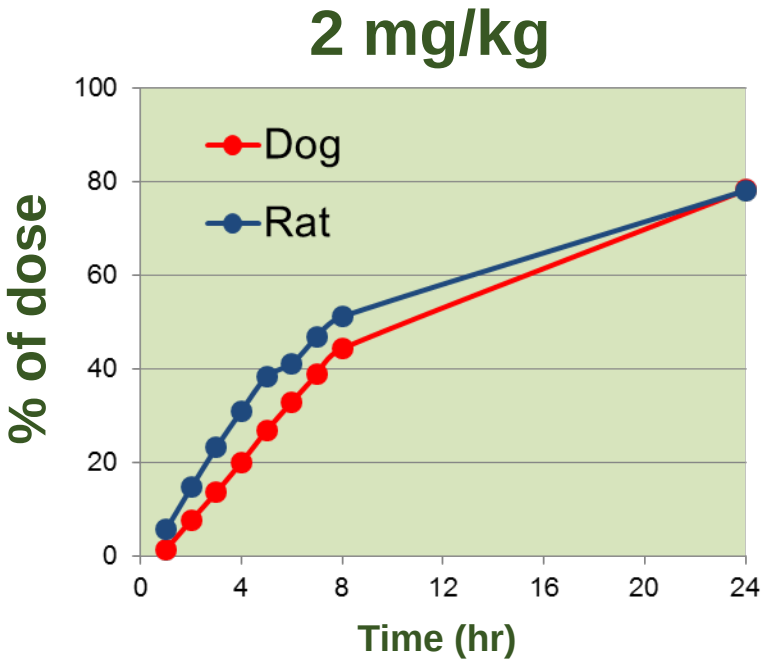
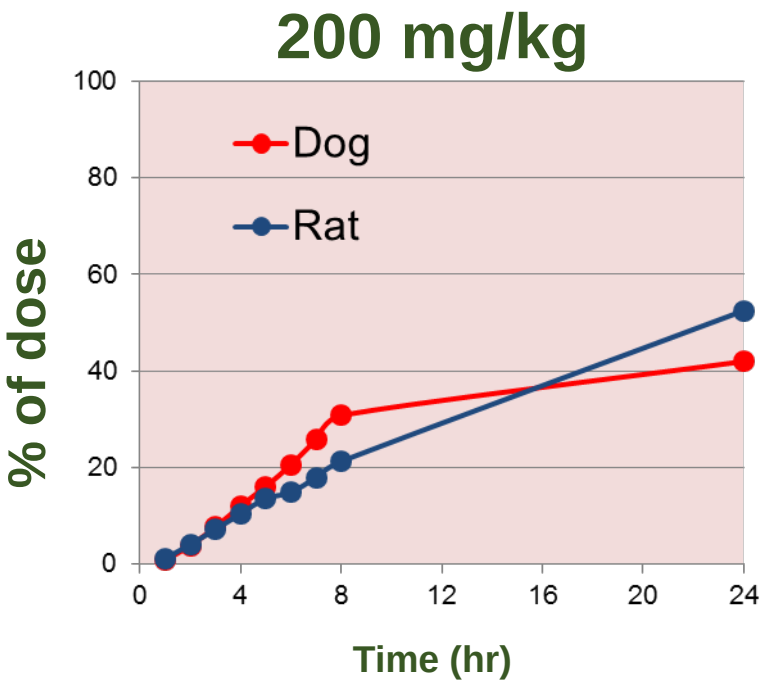
Cumulative excretion of ^{14}C into the bile

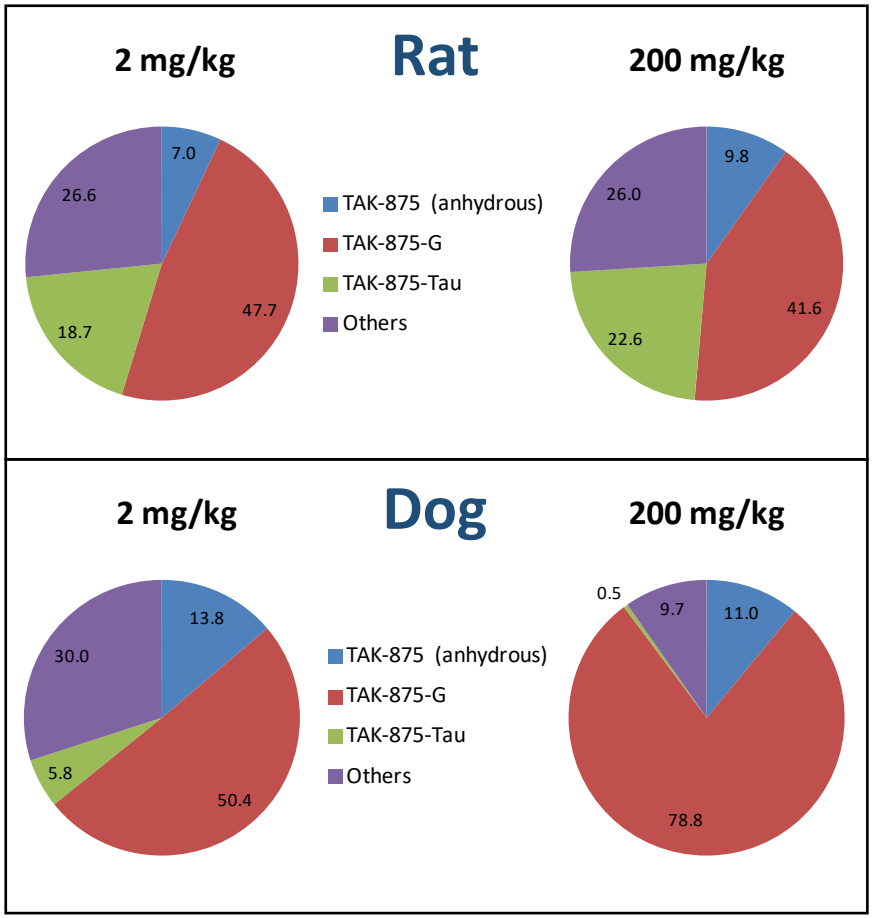




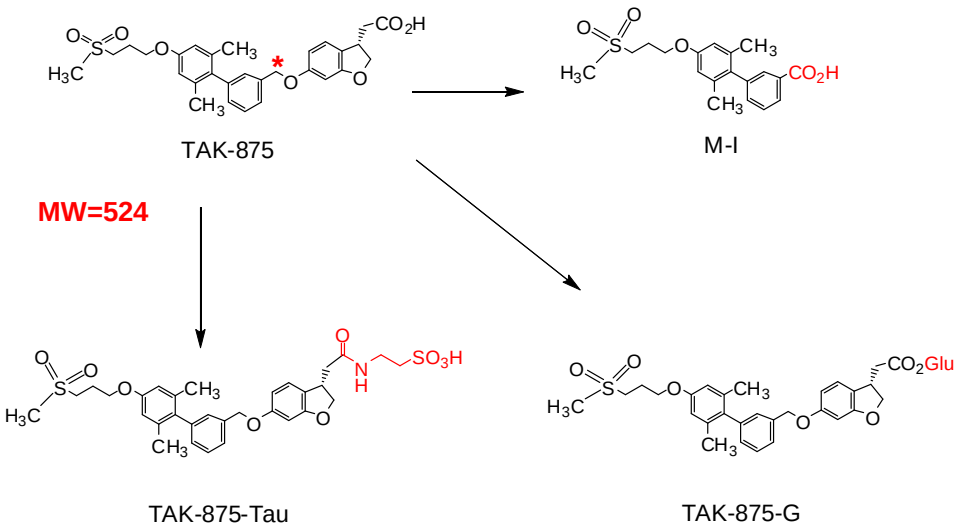
✓ The rate and cumulative excretion of ^{14}C was the same between rats and dogs.

Metabolite profiles of [^{14}C]TAK-875 in the bile

Single oral administration of [^{14}C]TAK-875



0 - 24 h



TAK-875 Metabolic Pathway

- ✓ TAK-875-G was the major component in dog and rat bile.
- ✓ The metabolite profile was similar between rat and dog.

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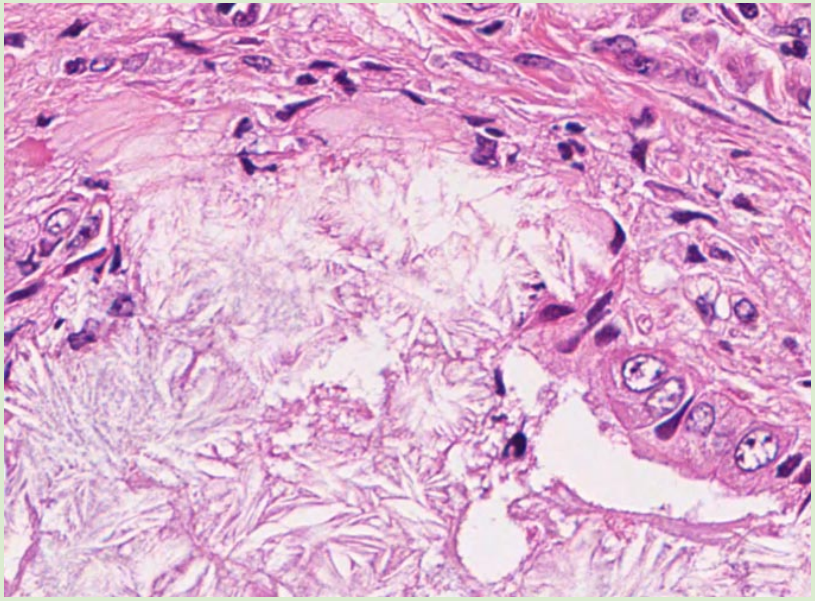
Analysis of granule foreign body in the liver in dogs



- ✓ Direct imaging mass clarified that the crystalline materials were composed by TAK-875 and its glucuronide.
- ✓ Hepatotoxicity in dogs is considered due to the precipitation of TAK-875-related materials in the bile duct.

Bile duct
Dose: 600 mg/kg

✓What is different between rats and dogs?
✓Is human being rat or dog?

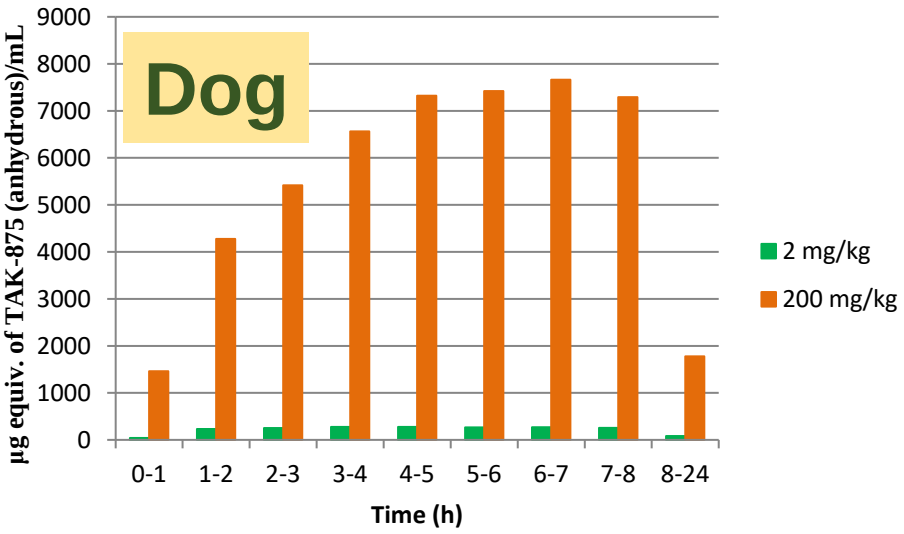
This phenomena was also presented by J. Marcinak et al. at American Association for the Study of Liver Diseases (AASLD) 2016 Drug Induced Liver Injury Annual Conference Proceedings. SESSION IV Hot new breakthrough findings and viewpoints. "Fasiglifam: Preclinical and clinical safety considerations"

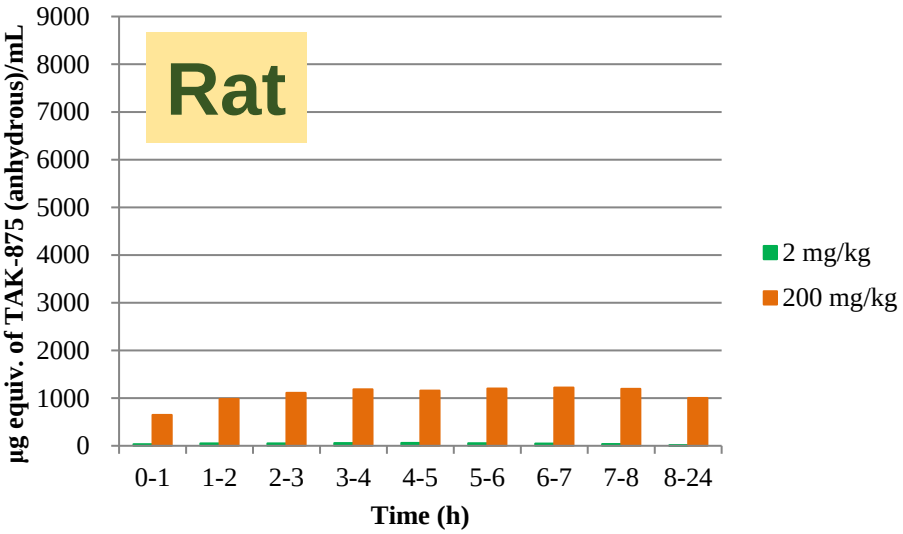
Summary 1

- **Why did dogs show hepatotoxicity ?**
  - ✓ No species difference in ADME properties of TAK-875 was observed.
  - ✓ From rat liver data, TAK-875 and TAK-875-G were not critical factors of the hepatotoxicity.
  - ✓ No dog unique metabolite(s) was confirmed.
- **Is human being close to rat or dog?**
  - ✓ How do we explain the human safety?

Give UP?

The conc. of ^{14}C in bile after single po dose





✓^{14}C concentrations in dogs bile were much higher than those in rats.

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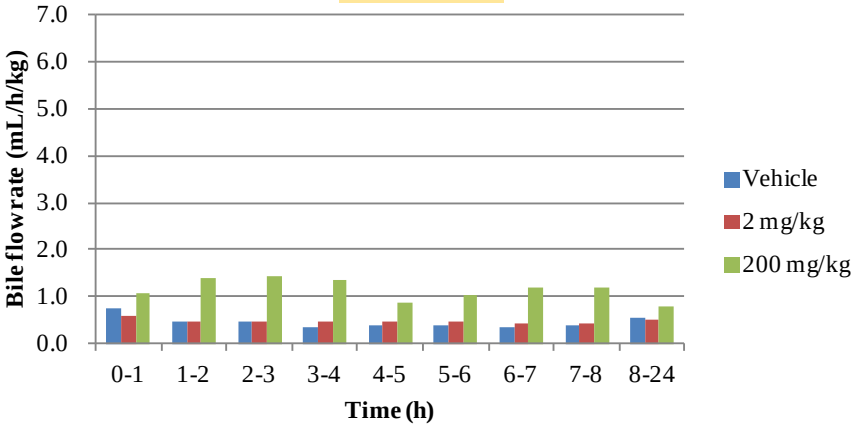
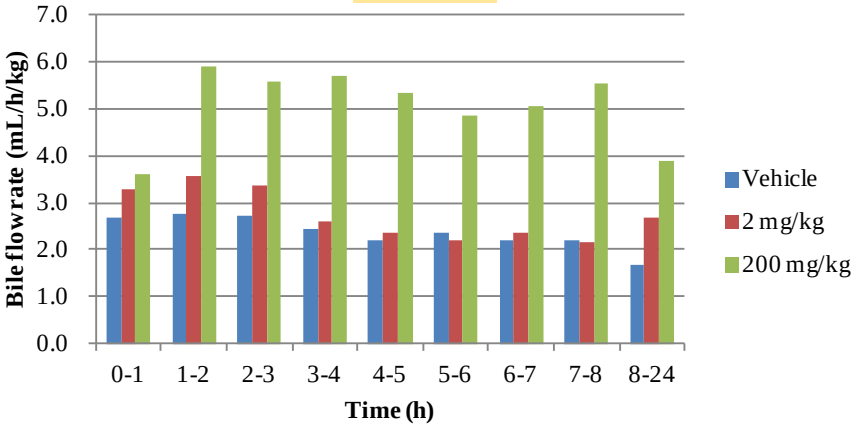
Comparison of bile flow rate

Dog



Rat



➢**Bile flow rate in dogs was lower than that in rats.**

- ✓ **Because the bile flow rate of dogs was lower than that of rats, the concentrations of ^{14}C in dog bile was much higher than that in rat.**
- ✓ **Dog specific liver findings were suspected to be due to precipitation of TAK-875 and its metabolites caused by the low bile flow rate in dogs.**

Next Step

➢**Is human being rat or dog?**

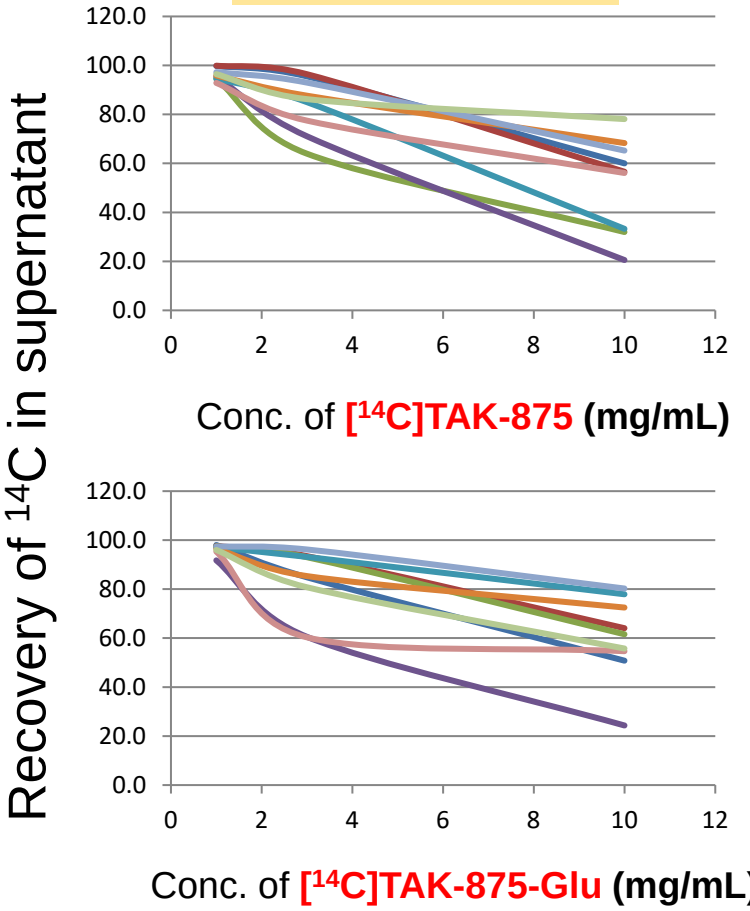
✓How do we explain the human safety?

<Action plan>

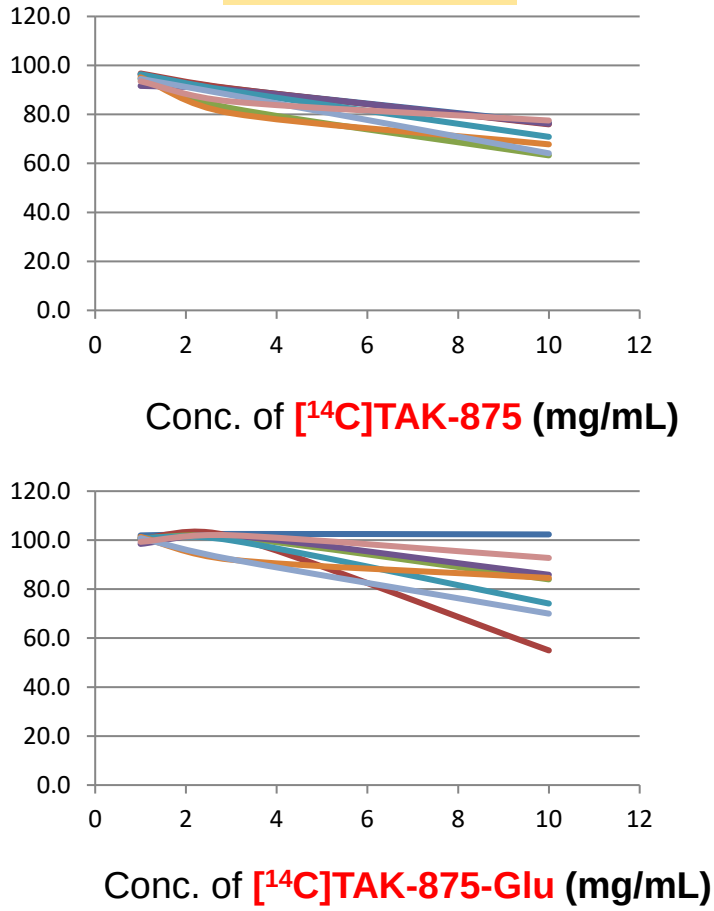
1. **Species difference in the solubility of TAK-875 and TAK-875-G in dog and human bile**
2. **Correspondence to FDA requirement**
   - ✓ **Transporter assay to avoid the possibility of drug induced liver injury (DILI) by TAK-875 medication**
3. **Prediction of the concentration for TAK-875 and its metabolites in vivo human bile**

In vitro solubility in the bile spiked withTAK-875 and TAK-875-Glu

Human bile



Dog bile



- **No clear difference in the solubility of TAK-875 and TAK-875-Glu in the bile of dogs and humans.**

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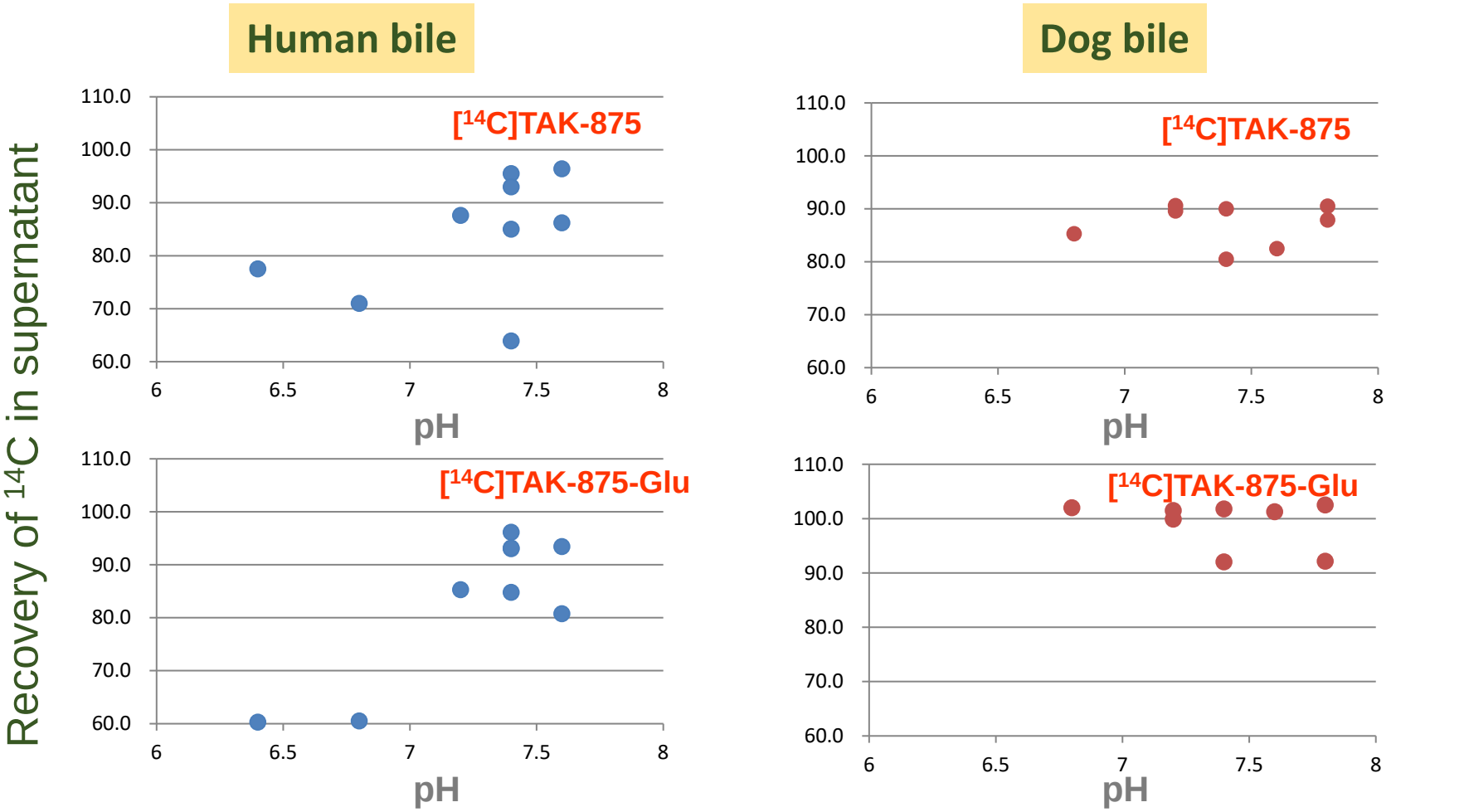
Relationship between pH and solubility in the bile TAK-875 and TAK-875-Glu (in vitro at 3 mg/mL)



- The no clear correlation was observed between pH and solubility of TAK-875 and TAK-875-Glu in the dog and human.

Correspondence to FDA requirement

- ✓ **Transporter assay to avoid the concern of drug induced liver injury (DILI) by TAK-875 medication**

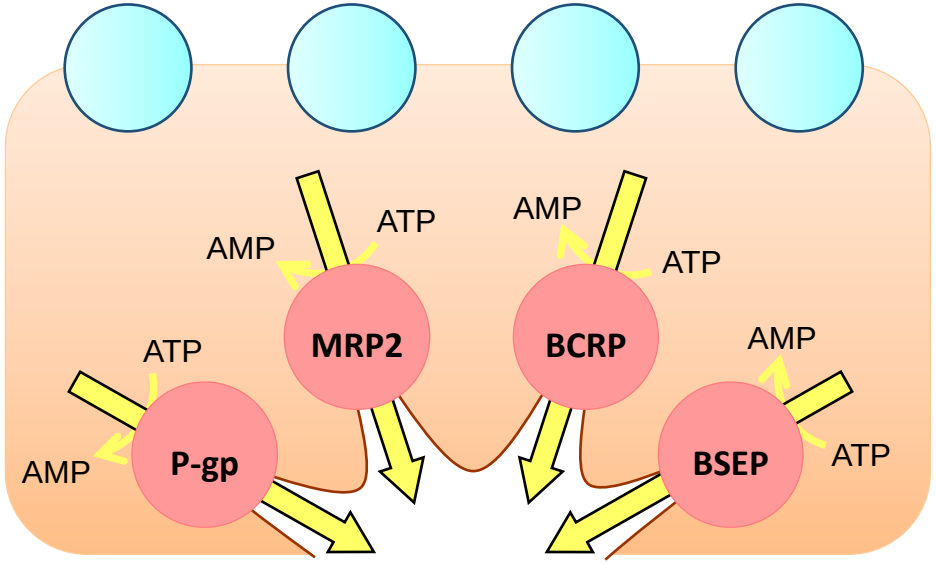
Drug-Induced Liver Injury(DILI)

- ✓ **The most potential factors of withdrawal from the market and of use restriction for approved drugs, and those of discontinuation for drug development with regard to the safety.**
- ✓ **One proposed mechanism of DILI is inhibition of bile acid transport, leading to necrotic and/or apoptotic cell death due to increased hepatocellular concentrations of bile acids.**
- ✓ **Appropriate approaches to distinguish between drugs that cause severe DILI and those that do not, are being investigated.**

Drug Metab Dispos 42:665–674, 2014
toxicological sciences 136(1), 216–241 2013

In vitro transporter studies

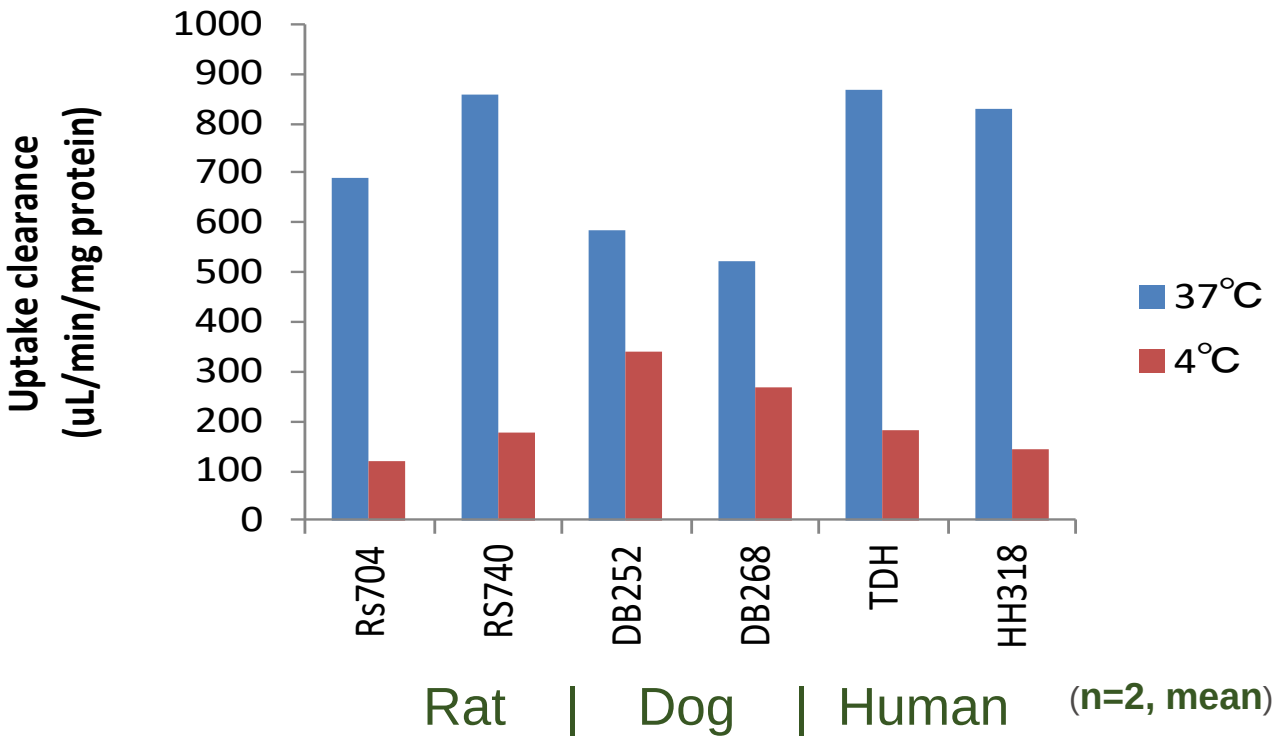
ABC transporters expressed in the hepatocyte



ABC (ATP binding cassette) transporters

P-gp : digoxin, vinblastin, etc.
MRP2 : pravastatin, cerivastatin, valsartan, olmesartan, methotrexate, Glucuronide conjugates, GSH conjugates, etc.
BCRP : Fluoroquinolone, pitavastatin, rosuvastatin, sulfasalazine, methotrexate, Sulfate conjugates, etc.
BSEP : Bile acids

Uptake of TAK-875 into rat, dog and human hepatocyte



- **Obvious species differences were not observed in the uptake of TAK-875 into rat, dog and human hepatocytes.**

→The hepatic uptake of TAK-875 would have no relevance with the dog-specific liver tox.

Hepatobiliary Transporters; Inhibition

	IC50 (μmol/L)/ (μg/mL)							
	P-gp		Bcrp/BCRP		Mrp2/MRP2		Bsep/BSEP	
	TAK-875	TAK-875-G	TAK-875	TAK-875-G	TAK-875	TAK-875-G	TAK-875	TAK-875-G
Rat	ND	ND	**11.8** 6.2	**15.3** 8.0	NC	**2.9** 1.5	**20.6** 10.8	**82.5** 43.3
Dog	ND	ND	NA	NA	NC	**5.1** 2.7	**16.1** 8.5	**18.2** 9.6
Human	**11.8** 6.2	ND	**10.9** 5.7	**27.3** 14.3	NC	**9.0** 4.7	**14.3** 7.5	**41.6** 21.8

NA=not available, NC=not calculated, ND=not determined

- ✓ **No species differences in transporter inhibition.**
- ✓ **Following findings remains unclear.**
  - **•Conc. of TAK-875 and TAK-875-G in hepatocytes was unknown.**
  - **•Whether TAK-875 and TAK-875-G actually inhibits transporter activity in hepatocytes on rats, dogs and humans.**

Summary of experimental activities

Possible factors contributing to the dog specific liver toxicity

Differences in

- Yes ☐ No ☑ ➢ **Metabolite profile?**
- Yes ☑ No ☐ ➢ **Concentrations of TAK-875 and its metabolites in bile?**
- Yes ☐ No ☑ ➢ **Solubility of TAK-875 in bile?**

How about human?

The higher conc. of TAK-875 and TAK-875-Glu in dog bile due to the lower bile flow rate would be involved in the crystal formation.

This difference in the bile flow rate would cause the species difference in the liver toxicity.

Prediction for fasiglifam conc. in human bile

$$Biliary\ excterion\ amount\ at\ T_{max\_bile} = C_{max\_bile} \times Bile\ flow\ rate$$

$$\frac{Biliary\ excretion\ amount\ at\ T_{max\_bile}}{C_{max\_plasma}} = \frac{\frac{dX_{bile}}{dt}}{C_{max\_plasma}} = CL_{bile}$$

$$\frac{C_{max\_bile} \times Bile\ flow\ rate}{C_{max\_plasma}} = CL_{bile}$$

$$C_{max\_bile} = \frac{CL_{bile} \times C_{max\_plasma}}{Bile\ flow\ rate} = \underline{\mathbf{44.5\ \mu g/mL}}$$

$$CL_{bile} = CL_{total} \times Biliary\ excretion\ ratio$$

- C_{max_plasma} in T2DM at 50 mg: 5.3 μg/mL (Leifke et al., 2012)
- CL_{total} in human at 6.25 mg iv: 14.6 mL/hr/kg (Kogame et al., 2018)
- *Human bile flow rate*: 0.24 mL/hr/kg (Davies and Morris, 1993)
- *Biliary excretion ratio*: 0.138 (assuming dog ER = human ER)

Prediction of TAK-875 and its metabolites in human bile

Prediction of TAK-875 and its metabolites in human bile

TAK-875: C_{max_bile} = 44.5 μg/mL

its metabolites: x9 of TAK-875 = 400.5 μg/mL

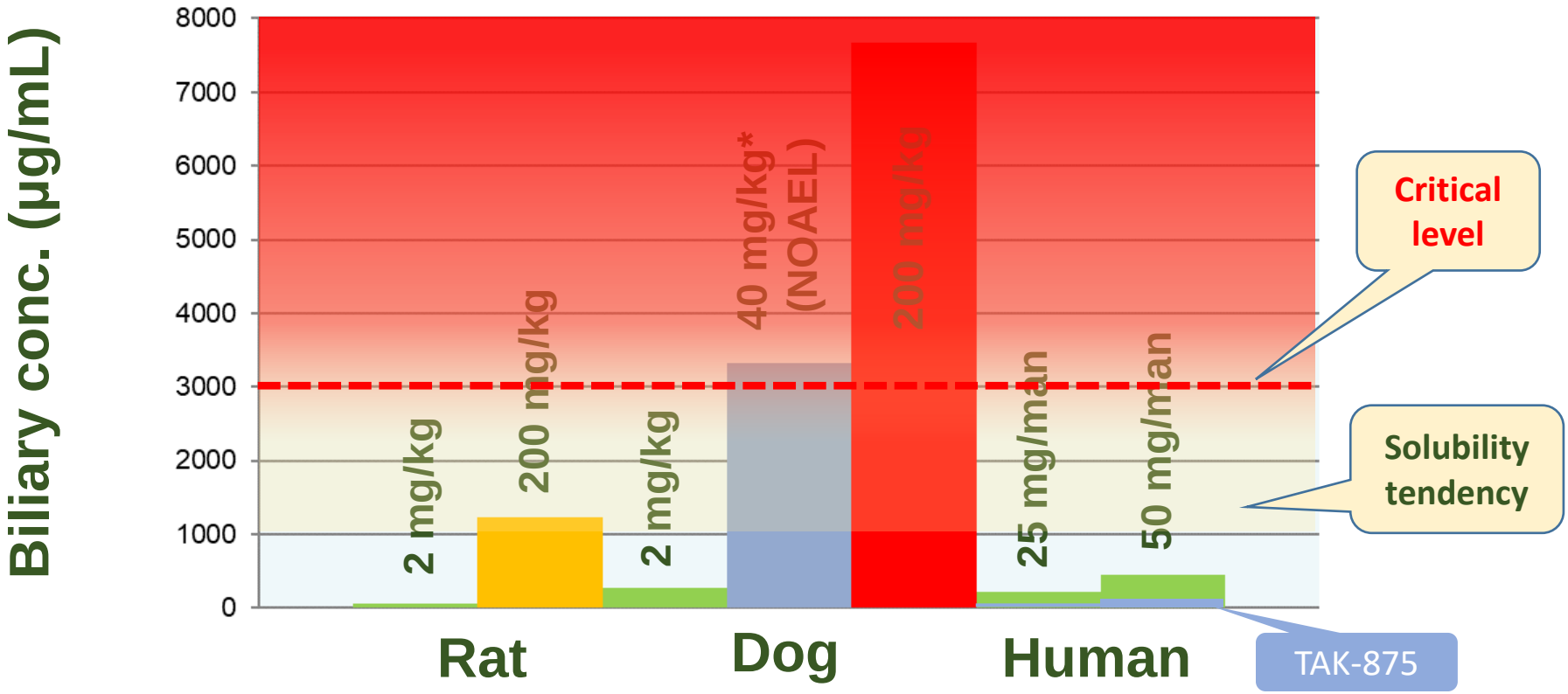
(from rat biliary ^{14}C composition, Worst-case scenario)

Total: about 445 μg/mL

The solubility study in dog and human bile indicated that the decreasing tendency in solubility was initiated from

1 mg/mL (Worst-case scenario)

The comparison of TAK-875 related compounds in bile



*:Dog 40 mg/kg (NOAEL): Calculated value

The conc. of TAK-875 related compounds in human bile at therapeutic doses was much lower than that in dogs at the dose of 40 mg/kg (NOAEL in 39-week dog toxicity study).

Conclusion

1. **The hepatotoxicity in dogs was considered to be mainly caused by precipitation of TAK-875 and its metabolites in bile duct due to low bile flow rate.**

2. **If only the precipitation in dog bile is responsible for liver findings, it is considered that sufficient safety margins will be secured from dose comparison.**

3. **Therefore, the possibility to show the dog type hepatotoxicity in human would be low at clinical relevant dose.**

Final Conclusion of Fasiglifam development

1. We submitted the results, proposed the hypotheses of human safety to FDA and we got approval for initiating Phase 3.

2. However, the elevation of liver enzymes was found in Phase 3, and the development was voluntarily withdrawn just before NDA.

3. Nonclinical researchers believe that the mechanism of the liver enzyme elevation in human observed in Phase 3 is different from that in dogs.

Acknowledgements

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Affiliations at the time of 2011.