Responses to the events associated with Beni-koji containing foods produced by Kobayashi Pharmaceutical Co., Ltd. (National Institute of Health Sciences)

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Ingredients (rice, rice germ, water, *Monascus* fungus) → Fermentation /Drying → **Fermentation batch** → Mixture of different fermentation batches → **Raw material batch** → Formulation/ Tableting → **Product batch (in tablet form)**

Active compounds "red yeast rice polyketides ≒ monacolin K" are produced

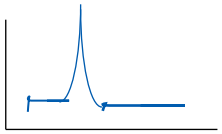
(Produced from June to August in 2023)

Puberulic acid is found in some product batches shipped after July 2023

Batches associated with adverse events

Analysis using LC/MS *etc.*

Batches **not** associated with adverse events



Unique peaks observed in the batches associated with adverse events

①-1 Detection of compounds

- Puberulic acid
- Compound Y
- Compound Z

①-2 Identification of compounds

Identification and characterization of the compounds

②Clarification of mechanism

- Verify whether identified compounds can be produced by co-incubation of *Monascus* fungus and *P. adametzioides*
- Verify at which step in the production contamination of the compounds occurred

③ Verification of renal toxicity

Verification of the toxicity of the identified compounds in animal testing

Current information on possible causative substance(s)

①－２） Identification of the two compounds detected other than puberulic acid

- Compound Y : isolation→chemical formula ($C_{28}H_{42}O_8$)
- Compound Z : isolation→chemical formula ($C_{23}H_{34}O_7$)

- The basic molecular structures of both compounds are similar to that of monacolin K, and they are assumed to be derived from monacolin K through the involvement of blue mold.
- No information on either compound is found in Mass spectral libraries or scientific literatures as of today, and they are probably not already-known natural compounds.

② Clarification of mechanism

A: Wakayama factory) Incubation room for seed fungus, Drying room, tubes, filters *etc.*
B : Wakayama factory) the inner surface of the lid of the fermentation tank
C : Osaka factory) Incubation room for seed fungus *etc.*

I. Presumption about contamination stage

- Confirmed that compounds (puberulic acid, Compound Y and Compound Z) are contained in the fermentation batches.
- Confirmed that fermentation batches constituting raw material batches are not completely homogeneous (demonstrated by the fact that monacolin K concentrations are different in the samples taken from the same fermentation batch)

- Contamination at the stage of fermentation is suspected.

II. Verification of production (direct) of compounds by blue molds

- blue molds collected at Wakayama factory (A) → Puberulic acid detected when incubated in rice medium
- blue molds collected at Wakayama factory (B) → Puberulic acid detected when incubated in rice medium
- blue molds collected at Osaka factory (C) → Puberulic acid detected when incubated in rice medium

- Blue molds (*Penicillium adametzioides*) were collected at both Osaka and Wakayama factories.
- *P. adametzioides* alone produces puberulic acid but does not produce either Compound Y or Z.

III. Verification of production (indirect) of compounds by blue molds

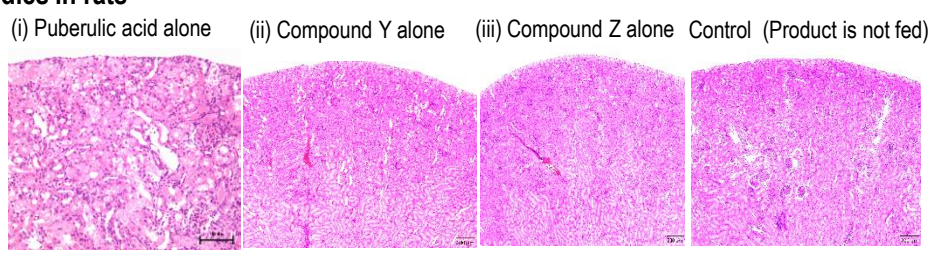
- Experiments on co-incubation of *Monascus* fungus and blue molds (B) → They can co-exist
- When blue molds (B) were incubated in the existence of monacolin K, the production of Compound Y and Z was confirmed.

- *Monascus* fungus and *P. adametzioides* can co-exist.
- Assumed that monacolin K is modified to be Compound Y and Z mediated by *P. adametzioides*.

③ Verification of renal toxicity (in animal testing)

- **7-day repeat-dose toxicity studies in rats**
  Kidney pathological findings

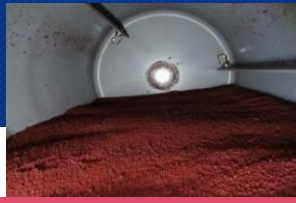
(i) Puberulic acid alone
(ii) Compound Y alone
(iii) Compound Z alone



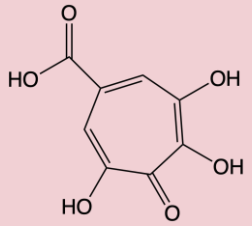
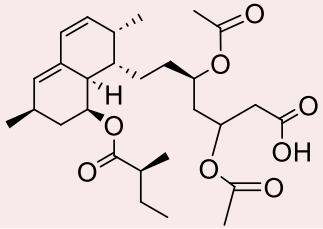
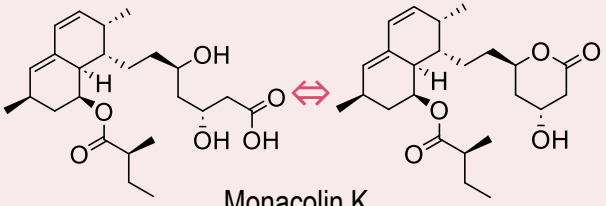
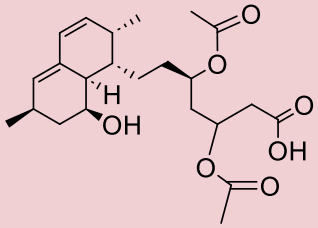
<Representative tissue specimens : preliminary results>

Degeneration or necrosis of proximal tubules were observed when rats were fed (i) Puberulic acid alone, while no toxicity findings were observed in kidney when rats were fed (ii) Compound Y alone or Compound Z alone.

Characteristics of the compounds contained in the products associated with adverse events



Facts and hypotheses

Compounds	Characteristics	Mechanism	Renal toxicity
①Puberulic acid 		Produced by blue molds (*Penicillium adametzioides*), which were collected from both Osaka and Wakayama factories, growing on rice medium	Determined that ① (Puberulic acid alone) causes renal dysfunction in animal testing (in rats)
②Compound Y ($C_{28}H_{42}O_8$) 	The basic molecular structure resembles monacolin K's.  (Acid form) Monacolin K (Lactone form) They are probably not already-known natural compounds.	**NOT** produced by *P. adametzioides* alone, which were collected from both Osaka and Wakayama factories, but when *P. adametzioides* and *Monascus* fungus are co-incubated, monacolin K is modified to be Compound Y.	Determined that ② (Compound Y) or ③ (Compound Z) does not cause renal dysfunction in animal testing (in rats)
③Compound Z ($C_{23}H_{34}O_7$) 		Confirmation of the mechanism similar to that of Compound Y.	

The following conclusions can be inferred at this time:

- Contamination of *P. adametzioides* dwelling in the factory occurred at the stage of fermentation, and puberulic acid was produced by *P. adametzioides* grown on rice medium.
- Due to co-incubation of *P. adametzioides* and *Monascus* fungus, monacolin K was modified to be Compound Y and Compound Z.
- It was determined that puberulic acid causes renal dysfunction while Compound Y or Z does not.

Future actions

Since it was confirmed that pubrulic acid can cause renal dysfunction, appropriate measures under Food Sanitation Act (e.g., establishment of standards or implementation of stricter hygiene control) to prevent similar incidents will be considered while continuing to gather relevant scientific information.