Mini Review

Absorption and Metabolism of γ -Oryzanol, a Characteristic Functional Ingredient in Rice Bran

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Summary γ -Oryzanol (OZ), a functional substance found in rice bran, consists of multiple molecular species. In both in vitro and in vivo studies, the researches exploring the various function of rice bran OZ have been conducted for a long time, and it has become clear that OZ has a lot of pharmaceutical activities. It is assumed that each type of OZ molecular species may have different effects. In contrast, the profile behaviour of OZ inside the body has not been fully understood. This article reviews the previous studies about the digestion, absorption, metabolism, and effects of rice bran OZ and also introduces the new method to evaluate the OZ metabolic fate by using high-performance liquid chromatography (HPLC) combined with tandem mass-spectrometry (MS/MS) which has higher selectivity and sensitivity.

Key Words γ -oryzanol, absorption and metabolism, rice bran oil, HPLC-MS/MS

Rice Bran OZ

γ -Oryzanol (OZ), a compound first isolated in 1954 from rice bran oil, has a chemical characteristic as a tasteless, odorless, pale-yellow colored powder, non-hygroscopic, stable to heat, and insoluble in water. OZ was named originally from the plant (*Oryza sativa* L., the scientific name of rice) from which it is extracted. The structure of OZ is a mixture of ferulic acid esters of triterpene alcohols and plant sterols. Up to date, about 10 types of OZ molecular species have been reported. Cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, campesteryl ferulate, and β -sitosteryl ferulate are known to be the major components of OZ (Fig. 1). Cycloartenyl ferulate and 24-methylenecycloartanyl ferulate are classified as triterpene alcohol type, and campesteryl ferulate and β -sitosteryl ferulate are classified as plant sterol type. The major compound contained in rice bran is triterpene alcohol type (1, 2).

Function of OZ

The antioxidant activity of OZ comes from its phenolic hydroxyl group of ferulic acid that is present in the molecule, acting as a hydrogen donor. It was reported that the increase of peroxide value can be suppressed by adding OZ in rice bran oil samples which have been treated by removing OZ and vitamin E beforehand (3).

OZ is also used as an ultraviolet absorber since it has a maximum absorption wavelength at 292 nm and 320 nm in n-heptane solution, derived from the intramolecular ferulic acid (4). Other activities of OZ such as an anti-inflammatory activity and inhibition of the secretion of adiponectin have been reported in animal experiments (5).

In Japan, OZ has been accepted as a pharmaceutical ingredient. As a medicine, OZ has been proved to ameliorate physical symptoms, anxiety, tension, depression in psychosomatic disorders (such as a menopausal symptom and irritable bowel syndrome) and hyperlipidemia condition (6). OZ has also been used as an antioxidant agent in food (7). In the study focused on brown rice, rich in OZ, its potency as antiobesity and antidiabetic have been reported in human study (8).

These evidences suggested that most of the OZ effects are derived from ferulic acid, triterpene alcohol, plant sterol, and their metabolites, which might be produced by hydrolysis of OZ inside the body. However, as described below, there are also reports suggesting that OZ itself works as the active substance. Therefore, it is important to clarify the behavior of OZ in vivo and thereby the reason that OZ has various effects can be clearly understood.

The Behavior of OZ In Vivo

In the initial studies, digestion, absorption, and metabolism of OZ were evaluated using radioactively

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Fig. 1. Chemical structures of the major components of OZ in rice bran.

Fig. 2. Representative MRM chromatograms of OZ prepared from rice bran oil.

^{14}C -labeled OZ. OZ which has been labeled on the ferulic acid site was orally administered to animals, then blood and organs were extracted with solvent by extracting a hydrophobic fraction (presumed to be OZ) and a hydrophilic fraction (presumed to be ferulic acid). The radioactivity of each fraction was measured, and the components were confirmed by TLC. Looking at the initial studies, it was assumed that OZ was absorbed and distributed to blood and each organ in its initial form and then rapidly metabolized to ferulic acid in the body (9–12). Aside from that, utter data were not obtained regarding the distribution of OZ to the brain (9, 10, 13). Since in these previous studies OZ has been considered to be rapidly metabolized to ferulic acid in the body, the research focusing on ferulic acid starts increasing (14, 15).

On the other hand, there are not so many studies examining the digestion, absorption and metabolism of OZ in human. When the yoghurt containing OZ was ingested (once a day, OZ 3.4 g, 3 d), most of the OZ form were detected in feces (16). Since the recovery of OZ in feces was about 80%, it was suggested that some

amount of OZ was absorbed in the human body. Further research is needed to know the absorption and metabolism of OZ in human.

Since the effects of each type of OZ molecular species may be different, it is very interesting to have further research about the digestion, absorption, and metabolism referring of each type of OZ molecular species (17–20). One in vitro study has examined the influence of digestive enzymes on the digestion of OZ, showing that OZ was partially hydrolyzed by pancreatic cholesterol esterase and the plant sterol type were more easily degraded than triterpene alcohol type (21). In the study using yoghurt containing OZ that was described above, it is reported that the recovery rate of OZ from feces was different depending on the molecular species (16). Thus, digestion, absorption, and metabolism in the body may be different depending on the OZ molecular species, implying the possibility that the action and strength thereof may also differ depending on the molecular species.

Fig. 3. Representative MRM chromatograms and content (ng/mL, cycloartenyl ferulate equivalent concentrations) of cycloartenyl ferulate, 24-methylenecycloartenyl ferulate isomers, campesterol ferulate, and β-sitosterol ferulate in mouse plasma 5 h after the oral administration of the OZ prepared from rice bran oil (A) or vehicle control (B).

Fig. 4. Behavior of OZ in vivo during the oral administration (authors' estimation).

HPLC-MS/MS Evaluation of Absorption and Metabolism of Rice Bran OZ

Nowadays, the researches about functional ingredients (e.g., OZ) in foods are conducted all over the world. Although the physiological function of the target component has been confirmed in in vitro study, it is considered that its chemical structure is often involved in in vivo metabolism process. Therefore, it is important to understand the digestion and absorption of functional ingredients in the body. For this purpose, it is essential to develop a highly selective and sensitive method to analyze functional ingredients in vivo.

High-performance liquid chromatography-mass spectrometry (HPLC-MS) and HPLC-tandem mass-spectrometry (MS/MS) are useful for structural identification and determination of the concentration of specific elements, even in the presence of background contaminants (22–24). Recently, we developed an HPLC-MS/MS analysis method to measure OZ molecular species in rice bran oil (Fig. 2) (25). This method has highly selectivity by using multiple reaction monitoring (MRM) and the limit of detection was 300 femtograms (cycloartenyl ferulate) per injection at a signal-to-noise ratio of 3.

We prepared OZ concentrate (cycloartenyl ferulate equivalent concentration; 52.2%) from purified rice bran oil and then carried out analysis of plasma samples from mice after oral administration of OZ concentrate (600 mg kg⁻¹) by the developed HPLC-MS/MS method (25). OZ was detected in MRM chromatograms of the plasma 5 h after administration (Fig. 3A). Interestingly, trace amounts of OZ components, such as cycloartenyl ferulate, were also detected in MRM chromatograms of the plasma from the control mice (vehicle-treated mice; Fig. 3B). This suggests that the substances might originate from a small amount of OZ contained in commercially available diet, suggesting that OZ always exists in the mouse blood. These results showed, unlike traditional theory, part of orally administered OZ is directly absorbed into the bloodstream and remains as its intact form in the plasma.

Based on our research and literature, the conjectures about in vivo behavior of OZ when orally ingested are shown in the following explanation (Fig. 4). A part of OZ is hydrolyzed to the ferulic acid, triterpene alcohols and plant sterols inside the body. However, some of OZ is still absorbed into the body as its intact form in sufficient amount. The intact form of OZ and its metabolites might both contribute to physiological activities, but probably in different ways.

Conclusion

Considering the human diet, OZ (and its metabolites) may possibly exist in the body all the times. Based on its firm activity and pharmacological action, it is crucial to understand the actual profile of OZ in the human body. It is necessary to gather the data about OZ absorption path into the body, distribution to the organs, metabolic form and so on. It is expected that various actions and mechanisms of OZ will be clarified by researching about the analysis of the behavior of each type of OZ molecu-

lar species. Such studies will lead to expand the range of utilization of OZ.

Disclosure of State of COI

No conflicts of interest to be declared.

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