Full Length Research Paper

Gamma oryzanol loaded microspheres with improved bioavailability

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Gamma oryzanol is a phytosterol that is extracted from rice bran oil. It is an antioxidant that possesses a curative effect for many diseases. Gamma oryzanol is a water insoluble compound supporting the idea that it has a low bioavailability due to low intestinal absorption. In this study, γ-oryzanol was loaded in biodegradable microspheres. Intestinal absorption of γ-oryzanol from microspheres was compared with that absorbed from triolein solution in rabbits. After oral administration of 150 mg/kg of body weight, plasma was collected at intervals and analyzed for γ-oryzanol content using high performance liquid chromatography (HPLC). Results showed that the highest plasma concentration of γ-oryzanol, detected from triolein solution, was 6.37 ± 1.48 µg/ml, whereas the highest plasma concentration from γ-oryzanol microspheres was 130.30 ± 30.40 µg/ml. This concentration was significantly higher than the amount absorbed from triolein solution (p<0.01). In conclusion, microspheres offer an alternative dosage form for γ-oryzanol solution with improved bioavailability.

Key words: Gamma oryzanol, solution, microspheres, bioavailability.

INTRODUCTION

Gamma oryzanol is a natural antioxidant that is extracted from rice bran oil. Crude rice bran oil contains 1599 to 1666 mg γ-oryzanol per 100 g (Pattong and Parichat, 2014). It has been found that γ-oryzanol has a curative effect for many human diseases, such as improving the symptoms of dementia (Masahiko et al., 2018), enhancing glucose uptake by insulin-resistant cells (Chang et al., 2015), inhibition of platelet aggregation (Cicero and Gaddi, 2001), and reduction of plasma cholesterol level (Wilson et al., 2007). In a study on rats with colon cancer, it was found that γ-oryzanol has the capability to improve immunity by increasing the activity of both natural killer and macrophages cells (Kim et al., 2012). It was also found that γ-oryzanol is able to reduce melanin concentration by decreasing its synthesis in melanoma cells (Jun et al., 2012). In addition, γ-oryzanol

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promotes skin capillary, so it has been used in cosmetics industry (Aladedunye and Przybylski, 2013). It is known that plant sterols have limited bioavailability due to their poor water solubility. As such, the effect of γ-oryzanol could be limited by its low bioavailability. Oral route of medicine administration is the most preferable one. However, oral administration limits the bioavailability of medicines to certain degrees according to their physicochemical properties. Gamma oryzanol occurs in a powdered form with low water solubility (Nauman et al., 2017). It is a mixture of ferulic acid esters of triterpene alcohols and plant sterols that are called phytosterols (Patel and Naik 2004). Gamma oryzanol chemical structure indicates that it has a bioavailability problem.

Emulsified γ-oryzanol rich fraction proved to enhance its effect in decreasing plasma low density lipoprotein (LDL) and increasing high density lipoprotein (HDL) levels (Aminu et al., 2016). Bulkiness of emulsion dosage forms could be inconvenient for some patients. However, solid carriers may be useful to improve the therapeutic efficacy of lipophilic compounds. Microspheres are small solid envelopes that can carry lipophilic compounds (Zhang et al., 2014). They are spherical particles with diameters ranging from 1 to 1000 µm with an ideal particle size less than 200 µm (Alagussundaram et al., 2009). Even small particle size of microspheres can provide a large surface area that can enhance the bioavailability of poorly soluble drugs making them good carriers for poorly such drugs. Modified natural compounds like starches, gums, fats, waxes and protein, natural polymers such as albumin and gelatin, and biodegradable synthetic polymers including poly lactic acid and polyglycolic acid are used as carriers; nevertheless, certain polymers are preferable due to their biocompatibility and biodegradability.

MATERIALS AND METHODS

Chemicals and instruments

Gamma oryzanol was obtained from Tokyo Chemical Industry (TCI) (Tokyo); triolein and poly (D,L-lactide-co-glycolide) (PLGA) from Sigma-Aldrich (St. Louis, MO, USA); chloroform and methanol from BDH (BH15 1TD2, England); sodium lauryl sulfate (SLS) from USA (Fluka chemical, USA); other reagents and solvents either HPLC or analytical grade were purchased from Merck (Darmstadt, Germany). High Performance Liquid Chromatography (HPLC) (HP 1100, Palo Alto, CA); Ultracentrifuge, Beckman L7-65 was purchased from Beckman Instruments (Palo, Alto, CA); Electronic balance, Fy-350, (A&D Company, Ltd, Japan).

Animals

Animal handling was in accordance with the ethical guidelines of the University’s Institutional Animal Care Committee (Approval No. UPM/FPSK/PADS/BR-UUH/00477). Female New Zealand white rabbits, weighing around 1 to 1.5 kg were purchased from Molekular Saintifik Enterprise (Malaysia). They were individually housed in stainless steel cages. Rabbits were acclimatized for two weeks, receiving 100 g a day of standard commercial feed purchased from Federal Flour Mills (FFM) Berhad (Malaysia).

Preparation of γ-oryzanol solution

Gamma oryzanol is a water insoluble compound. Henceforth, triolein, a triacylglycerol carrying three oleic acid molecules, was used as a solvent. Gamma oryzanol solution was prepared by weighing 100 mg of γ-oryzanol on electronic balance and dissolved in 2 g of triolein according to Fujiwara et al. (1983).

Preparation of γ-oryzanol-loaded microspheres

An oil-in-water solvent evaporation method was used to prepare γ-oryzanol loaded microspheres. This method has been revised from previous reports (Yen et al., 2001; Mirakabad et al., 2014). In order to obtain dispersed phase, 100 mg of γ-oryzanol and 100 mg of poly (D,L-lactide-co-glycolide) (PLGA) is dissolved in glycolide/lactide with a ratio of 50/50 in 5 ml of chloroform. The aqueous phase was prepared by dissolving 201.8 mg of sodium lauryl sulfate (SLS) in 100 ml of water. The dispersed phase was slowly mixed with the aqueous phase while being shaken at 150 rpm. After the addition of the dispersed phase, the speed was increased to 250 rpm and the shaking continued at room temperature for 3.5 h until significant amount of chloroform was evaporated. To obtain solidified microspheres, filtration through filter paper (Whatman 2) was carried out followed by washing with 2 ml of cold water, twice. Collected microspheres powder was dried in the oven at 50°C for 12 h to evaporate the chloroform completely. To calculate loading percentage of γ-oryzanol in microspheres, three batches were used. Triplicates of 5 mg of microspheres powder from each batch was weighed on F-350 electronic balance and dissolved in 1 ml of chloroform. Samples were vortexed for 30 seconds and filtered using 0.22 µm syringe filters. An aliquot of 20 µl from each sample was injected into HPLC. Same conditions used for γ-oryzanol analysis were used to analyze γ-oryzanol loading in microspheres. Loading percentage was calculated according to the following equation:

\[
\text{Loading Percentage} = \frac{\text{weight of } \gamma\text{-oryzanol}}{\text{weight of microspheres}} \times 100
\]

Administration of γ-oryzanol solution and microspheres

Rabbits were acclimatized for two weeks, receiving 100 g a day of standard commercial feed with free access to water. Before starting the study, rabbits were fasted overnight but were given the free water access. The animals were divided into two groups with three animals per group. One group received γ-oryzanol in triolein solution and the other received γ-oryzanol microspheres. Doses equivalent to 150 mg/kg of γ-oryzanol in the forms of triolein solution and microspheres were given via feeding tube connected with a syringe.

Blood Sampling

Before taking blood samples, ears of rabbits were shaved gently, wiped with 70% ethanol and around 1 ml of blood samples were withdrawn from ear veins into K₂ EDTA tubes at 0, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, and 72 h. Blood samples were immediately centrifuged at 3000 g for 10 min and plasma from each sample was collected into Eppendorf tubes wrapped with aluminum foil. Plasma samples were kept at -30°C until they were analyzed for γ-oryzanol content.
Figure 1. HPLC chromatogram of γ-oryzanol. Components were detected at 325 nm with PDA detector. Separation was carried out at 250 × 4 mm column packed with 5 μm ODS (C18) Hypersil silica. Mobile phase was acetonitrile/methanol/isopropanol (50:45:5) with a flow rate = 1 ml/min. The first peak is cycloartenyl ferulate, the second peak is 24-methylene cycloartanyl ferulate, the third peak is campesteryl ferulate and the fourth peak is sitosteryl ferulate and campesterol ferulate.

**Extraction of γ-oryzanol from plasma**

The modified method of Folch et al. (1957) was used to extract γ-oryzanol from plasma. Plasma was extracted twice since one extraction step could be insufficient to extract plasma γ-oryzanol. In this method, 200 µl plasma was diluted with 600 µl distilled water. Plasma samples were then deproteinized by adding 800 µl absolute ethanol and vortexed for 15 s. Aliquots of 800 µl hexane were added to each sample and vortexed for 90 s. Mixtures were then centrifuged at 1000 g for 3 min and hexane layers were collected. Residues were re-extracted with another 800 µl hexane and hexane layers were combined and analyzed for γ-oryzanol content using HPLC (Jasco-Borwin, Tokyo) connected with PU-1580 pump (Jasco), using C18-5 µm, 0.25 × 4 mm column (Hewlett Packard, USA). Peaks were detected by UV detector at 325 nm (UV-1575, Jasco). The mobile phase was a mixture of acetonitrile/methanol/isopropanol (50:45:5) with a flow rate of 1 ml/min for 20 min.

**Preparation of γ-oryzanol standards**

An amount of 50 mg γ-oryzanol was weighed on an electronic balance, transferred to a 50 ml volumetric flask and dissolved in 5 ml chloroform. The volume was made up to 50 ml with γ-oryzanol mobile phases. This concentration (1 mg/ml) was used as the stock solution. Working solutions at concentrations of 0.5, 0.25 and 0.125 mg/ml were prepared by diluting the stock solution with the mobile phase. These concentrations were used to draw the standard curve used to quantify plasma concentrations of γ-oryzanol.

**Statistical analysis**

The data were analyzed using SPSS window program version 11.0. One way ANOVA was used to compare means of γ-oryzanol absorbed from solution and microspheres. Results are given as mean ± SD. P-value < 0.05 was considered as statistically significant.

**RESULTS AND DISCUSSION**

**Gamma oryzanol absorption from triolein solution**

Gamma oryzanol in triolein was given as a single oral dose (150 mg/kg body weight) to the rabbits to measure the amount that can be absorbed and circulated. Plasma level of γ-oryzanol was quantified using HPLC. Figure 1 shows that four peaks were dominant at retention times of 16.720, 18.294, 19.568 and 22.390 min, respectively. Plasma concentrations for γ-oryzanol were calculated using the standard curve equation. As shown in Figure 2, a good linearity was obtained upon plotting the graph of peaks’ area under the curve versus standards concentrations. Figure 3 shows γ-oryzanol level in the plasma during 72 h. Results show that 0.48 ± 0.10 µg γ-oryzanol/ml plasma was detected after 0.5 h of administration. However, plasma level increased gradually to reach 6.37 ± 1.48 µg/ml after 4 h (Figure 3), and this concentration was the maximum plasma level of γ-oryzanol that was detected from γ-oryzanol triolein solution. This result supports Fujiwara et al. (1983) who found that maximum level of γ-oryzanol and its metabolites reached after 4 h of oral ingestion. Plasma level of γ-oryzanol was decreased after that to reach 0.09 ± 0.01 µg/ml after 48 h, and no amount was detected after 72 h as shown in Figure 3, indicating that it was completely cleared from the blood after that time. Fujiwara et al. (1983) had found that γ-oryzanol and its metabolites were cleared from rats after 48 h. However, Fujiwara et al. (1983) had used rats not rabbits, and
Figure 2. Calibration curve for γ-oryzanol standards.

Figure 3. Plasma level of γ-oryzanol given orally in triolein solution during 72 h in rabbits (n = 3) at 150 mg/kg body weight. Each value represents the mean of three replicates ± SD.

different animals’ species could result in different metabolism rate. No recent studies are available about amount of γ-oryzanol absorbed in human or animals. However, Lubinus et al. (2013) had found in a clinical study that 80% of orally administered γ-oryzanol was excreted in human feces.
Gamma oryzanol absorption from microspheres

Among biodegradable polymers, poly(lactic-co-glycolic acid) (PLGA) copolymers have suitable biodegradability and biocompatibility properties (Sonam et al., 2013), which make them the best choice for this study. They are polyesters with properties, which depend on polymer composition, molecular weight, hydrophobicity, crystallinity, surface charge and the nature of coating material (Sonam et al., 2013). For this work, copolymer with 50:50 molar composition of lactic acid and glycolic acid was used since the mixture degrades by 1 week, while other ratios had been found to have longer degradation time (Jain, 2000). The method that was used to load γ-oryzanol in microspheres was oil in water emulsion/solvent evaporation process, which is the primary method that had been used to encapsulate lipophilic drugs into PLGA microspheres (Mirakabad et al., 2014). Loading percentage of γ-oryzanol in PLGA microspheres was calculated using HPLC. Results showed that γ-oryzanol was well loaded into PLGA with a percentage of 63.3 ± 5.9%. Solubility of drugs both in polymer and water as well as their molecular weight are the main factors that affect loading in PLGA (Perugini et al., 2003). Gamma oryzanol is a water insoluble compound and this could be a reason for its high loading percentage.

Amount containing 150 mg γ-oryzanol/kg body weight was given orally to rabbits, and plasma γ-oryzanol level was quantified using HPLC. Results in Figure 4 shows that after 0.5 h, plasma concentration of γ-oryzanol was 19.33 ± 5.15 μg/ml. This concentration was significantly higher (p < 0.01) than plasma γ-oryzanol concentration from triolein solution at the same time, which was 0.48 ± 0.10 μg/ml (Figure 3). Figure 4 shows that plasma level of γ-oryzanol increased gradually until 4 h after ingestion, when the maximum concentration of γ-oryzanol reached 130.30 ± 30.40 μg/ml. Plasma level of γ-oryzanol decreased gradually to reach 6.20 ± 0.60 μg/ml at 72 h (Figure 4), indicating that microspheres also sustained the level of γ-oryzanol in plasma. PLGA polymers have been used as a good delivery system for many drugs to sustain and control their release (Yihan et al., 2016). The maximum concentration of γ-oryzanol from triolein was only 6.37 ± 1.48 μg/ml as shown in Figure 3, which was significantly lower (p < 0.01) than the level from γ-oryzanol loaded microspheres.

Conclusion

In this study, it was found that upon loading γ-oryzanol in microspheres, its bioavailability was significantly improved in comparison with its triolein solution. In addition, polymeric materials used in microspheres preparation have the capability to keep plasma concentration for γ-oryzanol for longer time, since it is released slowly by the decomposition of the polymers. Improvement of γ-oryzanol bioavailability leads to improving its therapeutic efficacy. Loading γ-oryzanol in microspheres will also contribute in improved patient’s compliance since it possess a sustained release pattern,

Figure 4. Plasma level of γ-oryzanol given orally in microspheres during 72 h in rabbits (n = 3) at 150 mg γ-oryzanol/kg body weight. Each value represents the mean of three replicates ± SD.
as such, low doses frequency is needed.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES


