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In vitro and in vivo evaluation of riboflavin-containing microballoons for a floating controlled drug delivery system in healthy humans

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Abstract

Microballoons (MB) possessing a spherical cavity enclosed within a hard polymer shell have been developed as a dosage form characterized by excellent buoyancy in the stomach. MB were prepared by the emulsion solvent diffusion method using enteric acrylic polymers dissolved in a mixture of dichloromethane and ethanol. Riboflavin-containing MB were administered orally to each of three healthy volunteers. The pharmacokinetics of riboflavin was investigated by analysis of the urinary excretion. Prolongation of the urinary excretion of riboflavin could be obtained by ingestion of water as well as "fed" conditions. This phenomenon was attributable to the buoyancy properties of MB in the stomach and an increase in the gastric residence time (GRT). The excretion half-life time ($t_{1/2}$) following administration of MB (particle size: 500–1000 μm) exhibiting high buoyancy was longer than that of MB (particle size: <500 μm) displaying low buoyancy. Therefore, the intragastric floating properties of MB are potentially beneficial as far as a sustained pharmacological action is concerned. MB prepared by mixing it with hydroxypropylmethylcellulose (HPMC) in different ratio, results in improved riboflavin-release properties. These MB were evaluated in vivo by analysis of the urinary excretion of riboflavin. As a result, strong correlations were observed between the buoyancy and excretion half-life ($t_{1/2}$) and between the riboflavin release from the MB and total urinary excretion.

Keywords: Floating controlled drug delivery system; Hollow microsphere (microballoon); Emulsion solvent diffusion method; Riboflavin; Gastric residence time (GRT); Urinary excretion

1. Introduction

Many oral controlled drug release systems have been developed to improve drug bioavailability. However, some of these systems do not work as planned with respect to release of the drug as, on occasion, they pass unexpectedly through the absorption window, e.g. the small intestine, before release of the loaded drug is complete. Therefore, the design of a sustained release preparation requires both prolongation of gastrointestinal transit of the dosage form as well as controlled drug release. Several gastrointestinal targeting dosage forms (Hwang et al., 1998; Moës, 1993; Deshpande et al., 1996), including intragastric flotation systems (Rouge et al., 1998; Yuasa et al., 1996; Lee et al., 1999), high density systems

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(Hwang et al., 1998), mucoadhesive systems, adhesion to the gastric mucosal surface in order to extend gastric residence time (GRT; Akiyama et al., 1995), magnetic systems (Gröning et al., 1998), unfoldable, extendible, or swellable systems (Fix et al., 1993) and superporous hydrogel systems (Park, 1988), have been developed.

As far as floating devices are concerned, air included within a multiple-unit compartment system resulted in excellent buoyancy in vitro and prolonged the GRT relative to controls in vivo in the fed state (Iannuccelli et al., 1998a,b, 2000). However, in the fasted state, the intragastric buoyancy of the devices did not affect the GRT. Hollow microspheres (microballoons) were developed in order to prolong the GRT of the dosage form (Kawashima et al., 1991). This gastrointestinal transit-controlled preparation is designed to float on the surface of gastric juice with a specific density of less than 1. When in vivo evaluation of microballoons (MB) was performed, extreme difficulty was encountered with respect to examination of the flotation behavior of MB in the stomach of animals such as rats and dogs. Therefore, oral administration of MB in humans is essential in order to assess intragastric buoyancy properties. The pharmacokinetic properties were examined by analysis of the urinary excretion of drugs following oral administration of MB.

Gastric retentive devices may be highly useful for the delivery of many different kind of drugs. Gastric retentive devices would provide the best results for drugs that act locally in the stomach or that are absorbed primarily in the stomach. For many drugs that are absorbed mainly from the proximal small intestine, controlled release in the stomach would result in improved bioavailability. Riboflavin absorption occurs mainly in the proximal small intestine; moreover, it undergoes very little metabolism. Riboflavin pharmacokinetics can be investigated by analysis of the urinary excretion following oral administration in humans, consequently, riboflavin has been adopted as a model drug.

In our previous study, the buoyancy properties and efficiency of drug entrapment within MB were evaluated. In addition, drug release from MB containing five drugs with different water solubilities was investigated. Moreover, MB were prepared by mixing polymers, such as hydroxypropylmethylcellulose (HPMC), in Eudragit® S100. Under these conditions, the amount of drug released from MB could be controlled.

The objective of the current investigation was to assess the usefulness of intragastric buoyancy properties in terms of sustained pharmacological action in humans. In addition, factors influencing the prolongation of urinary excretion and the amount of riboflavin in urine were examined.

2. Materials and methods

2.1. Materials

Riboflavin was employed as a model drug. Eudragit® S100, Preparation 4135F (Röhm Pharma GmbH, Germany) and hydroxypropylmethylcellulose (TC-5R, Shin-etsu Chemical, Japan) were used as polymers. Monostearin (Han-i Chemical, Japan) served as a wall membrane-reinforcing agent and polyvinyl alcohol (PVA-120, Kuraray, Japan) was used as a dispersing agent.

2.2. Preparation of microballoons (MB) and non-floating microspheres (NF)

MB and NF were prepared employing identical equipment using the emulsion solvent diffusion method (Kawashima et al., 1992) as follows. Riboflavin (0.1 g), polymers (1.0 g) and monostearin (0.5 g) were dissolved in a mixture of dichloromethane (8 ml) and ethanol (8 ml) at room temperature in order to generate MB. In the case of NF, riboflavin (0.1 g) and polymers (1.5 g) were dissolved in a mixture of dichloromethane (8 ml) and ethanol (8 ml) at room temperature. Each solution was introduced into an aqueous solution of polyvinyl alcohol (0.75 w/v%, 200 ml) at 40 °C. Resultant emulsions were stirred at 300 rpm with a propeller type agitator for 1 h. Subsequently, the resulting polymeric spheres were sieved $<$500 and 500–1000 μm and dried overnight at 40 °C, which led to MB or NF.

2.3. Observation of the cross-sections of MB and NF

The cross-sections of MB and NF were examined by scanning electron microphotography (SEM)

(JSM-T330A, Nihon Densi, Japan). To investigate the internal morphology, MB and NF were cut into two pieces with a knife.

2.4. Measurement of physicochemical properties of MB and NF

2.4.1. Buoyancy

MB (100 mg) and NF (100 mg) were dispersed in JP XIII No. 1 solution (300 ml, pH 1.2, 37 °C) containing Tween 20 (0.02 w/v%) to simulate gastric fluid. Each mixture was stirred with a paddle at 100 rpm. After 4 h, the layer of buoyant particles was removed by pipette and the floating particles were separated by filtration. Particles in the sinking particulate layer were separated by filtration. Both particle types were dried at 40 °C overnight. Weights were measured and buoyancy was determined by the weight ratio of the floating particles to the sum of floating and sinking particles.

2.4.2. Riboflavin release

The level of riboflavin release from MB or NF with diameters of between 500 and 1000 μm was measured by the paddle method at 100 rpm, specified in JP XIII as follows. MB (100 mg) and NF (100 mg) were dispersed in JP XIII No. 1 solution (300 ml, pH 1.2, 37 °C) containing Tween 20 (0.02 w/v%). After 2 h, all particles were separated by filtration. The particles were dispersed in JP XIII No. 2 solution (300 ml, pH 6.8, 37 °C) containing Tween 80 (0.5 w/v%) for 2 h. Similarly, filtered particles were dispersed in phosphate buffer (300 ml, pH 7.2, 37 °C) containing Tween 80 (0.5 w/v%) for 3 h. Tween 80 was introduced into JP XIII No. 2 solution or phosphate buffer to solubilize drugs. The level of riboflavin release was determined spectrophotometrically using a UV-detector (UV-160A, Shimadzu, Japan).

2.5. Measurement of riboflavin concentration in urine

The level of riboflavin in urine was measured by the lumiflavin fluorescence technique (Ohishi, 1983). Fluorescence intensity was measured in a fluorescence spectrophotometer (650-60, Hitachi, Japan) (excitation wavelength: 445 nm; emission wavelength: 530 nm). The concentration of riboflavin in test solutions was calculated from Eq. (1):

Table 1
Volunteer demographics

Volunteer number	Sex	Height (cm)	Weight (kg)	Age (years)
1	Male	175	68	30
2	Male	165	55	29
3	Male	183	93	28
4	Male	171	72	26
5	Male	165	65	26
6	Male	172	64	25

$$C_{\text{test}} = C_{\text{std}} \times \frac{f_A - f_C}{f_B - f_A} \quad (1)$$

where C_{test} is the concentration of riboflavin in test solution (μg/ml), C_{std} is the concentration of riboflavin in standard solution (μg/ml), f_A is the fluorescence intensity in test solution (illuminated for 60 min), f_B is the fluorescence intensity in standard solution (illuminated for 60 min), and f_C is the fluorescence intensity in blank solution (illuminated for 0 min).

2.6. Evaluation of pharmacokinetics of urinary excretion

2.6.1. In vivo study design

This was a single, blind, randomized study in six healthy male volunteers (Table 1). The clinical protocol was approved by Kyoto University Graduate School and Faculty of Medicine, Ethics Committee. Each volunteer provided informed consent to participate in the study. There was at least 2 weeks washout between each administration.

Riboflavin-containing MB (200 mg) and riboflavin-containing NF (200 mg) (amount of riboflavin 10 mg) were filled in hard gelatin capsules. After an overnight fast for 12 h, each of the three healthy volunteers swallowed the capsules with 150 ml water on an empty stomach. No other food or drink was allowed during the 12-h period immediately following administration (fasted conditions). The remaining three healthy volunteers received a breakfast consisting of two rice balls and green tea (350 kcal) 30 min prior to administration and a lunch (800 kcal) 2 h after administration (fed conditions). Also, one healthy volunteer consumed 100 ml water every hour during the 12-h period immediately following administration. The volunteer did not receive a breakfast and a lunch during the 12-h period (water ingestion conditions). All

volunteers received a standard meal 12 h following administration. Urine samples were obtained pre-dose and at scheduled times following administration: 2, 4, 6, 8, 10, 12, 14 and 22 h. Subsequently, the amount of riboflavin in urine was measured.

2.6.2. Analysis of urinary excretion data

The amount of riboflavin in urine for 2 h was calculated from Eq. (2):

$$\text{Amount of riboflavin for 2 h (mg)} = C_{\text{test}} \times V \times \frac{1}{1000} \quad (2)$$

where C_{test} is the concentration of riboflavin in test solution (μg/ml) and V is the volume of urine for 2 h (ml).

An equation describing the relationship between post-dose time and the accumulated urinary excretion (Xu) was introduced from Xu-time profiles following oral administration. The accumulated urinary excretion at 48 h post-dose was calculated; in addition, Xu ($t = 48$ h) was denoted by Xu^{∞}. The urinary excretion process was described by first-order kinetics plotting $\log(Xu^{\infty} - Xu)$ versus time. The elimination rate constant (K) and the excretion half-life ($t_{1/2}$) were calculated from Eqs. (3) and (4), respectively:

$$K = -2.303 \times k \quad (3)$$

$$t_{1/2} = \frac{0.693}{K} \quad (4)$$

where k is the slope of the first-order urinary excretion plots. Moreover, the total urinary excretion (%) of riboflavin was calculated from Eq. (5):

$$\text{Total urinary excretion (\%)} = \frac{\text{amount of total excretion (0–22 h) (mg)}}{\text{amount of riboflavin administered (mg)}} \times 100 \quad (5)$$

3. Results and discussion

3.1. In vitro floating and drug releasing properties of MB and NF

In order to assess the usefulness of MB intragastric buoyancy properties in terms of the sustained pharmacological action, NF exhibiting 0% buoyancy were prepared. The formulation and buoyancy of MB and NF are given in Table 2. Scanning electron microphotographs (SEMs) of cross-sections of MB-1 and NF-1 are shown in Fig. 1, while the riboflavin release from MB and NF is presented in Fig. 2.

Table 2
Formulation and buoyancy of microballoons (MB) and non-floating microspheres (NF)

Sample	MB-1	MB-2	NF-1	NF-2
Riboflavin (g)	0.1	0.1	0.1	0.1
Eudragit® S100 (g)	1.0	0.9	–	0.6
Preparation 4135F (g)	–	–	1.5	0.8
HPMC[a] (g)	–	0.1	–	0.1
Monostearin (g)	0.5	0.5	–	–
CH_2Cl_2 (ml)	8	8	8	8
EtOH (ml)	8	8	8	8
Buoyancy at 4 h (%)	97.2	93.6	0	9.2

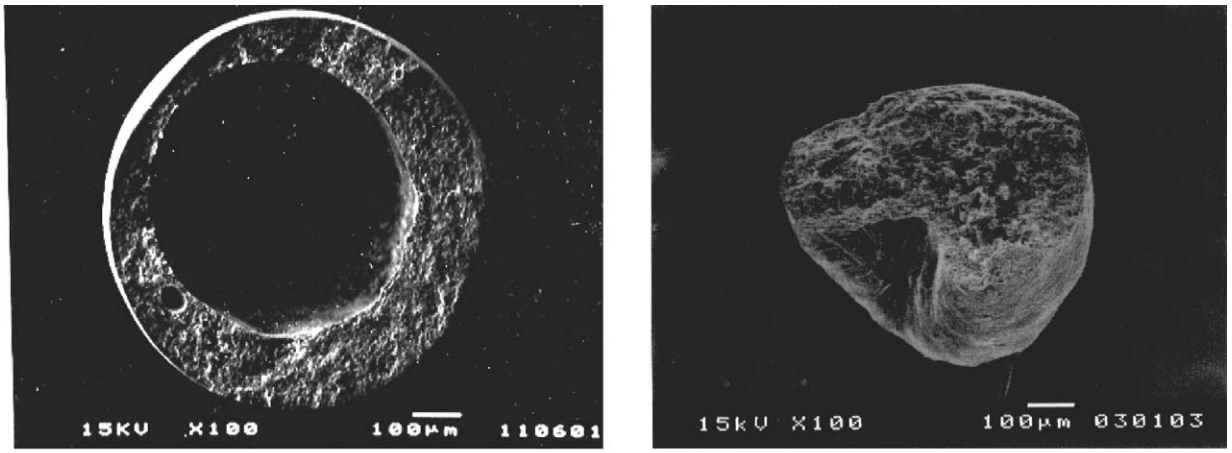
[a] HPMC: hydroxypropylmethylcellulose.

MB-1 with diameters of between 500 and 1000 μm are characterized by a spherical cavity enclosed within a hard polymer shell with excellent buoyancy properties (Fig. 1a). Non-floating microspheres (NF-1) lacking an internal cavity were prepared using Preparation 4135F, which consists of methacrylic acid copolymer (Fig. 1b).

Table 2 illustrates the clear differences in buoyancy between MB-1 and NF-1. Although the objective NF exhibiting 0% buoyancy could be prepared, the differences in riboflavin release profiles of MB-1 and NF-1 are displayed in Fig. 2. As a result, formulation of the organic phase was modified in order to prepare NF-2 with equivalent riboflavin release profiles to MB-2 in JP XIII No. 1 solution (pH 1.2) and No. 2 solution (pH 6.8) as riboflavin was absorbed mainly in the proximal small intestine (pH 4–6.5).

3.2. Effect of drinking water

In our previous study, riboflavin-containing MB were administered orally to each of three healthy volunteers under fed conditions; subsequently, riboflavin pharmacokinetics was investigated by an analysis of the urinary excretion of riboflavin. As a result, the excretion half-life ($t_{1/2}$) of MB was prolonged significantly by feeding. This phenomenon could be



(a) (b)

Fig. 1. Scanning electron microphotographs of cross-sections: (a) microballoons (MB-1), (b) non-floating microspheres (NF-1). Formulation of MB-1: riboflavin, 0.1 g; Eudragit® S100, 1.0 g; monostearin, 0.5 g. Formulation of NF-1: riboflavin, 0.1 g; Preparation 4135F, 1.5 g.

attributed to the prolonged GRT following pylorus closure due to feeding.

In order to determine whether the prolonged excretion half-life ($t_{1/2}$) was attributable to the buoyancy properties of MB in the stomach following pylorus closure due to feeding, one healthy volunteer consumed 100 ml water every hour during the 12-h period immediately following administration of MB-1; subsequently, the amount of riboflavin in urine was measured. Volumes of liquids affect gastric emptying of liquids. Gastric emptying of small volumes like 100 ml or less is governed by the cyclic activity referred to as the interdigestive migrating motor complex (IMMC) whereas large volumes of liquids like 200 ml or more are emptied out immediately after administration (Deshpande et al., 1996). The urinary excretion of riboflavin under fasted, fed and water ingestion conditions following administration of MB-1 are displayed in Fig. 3.

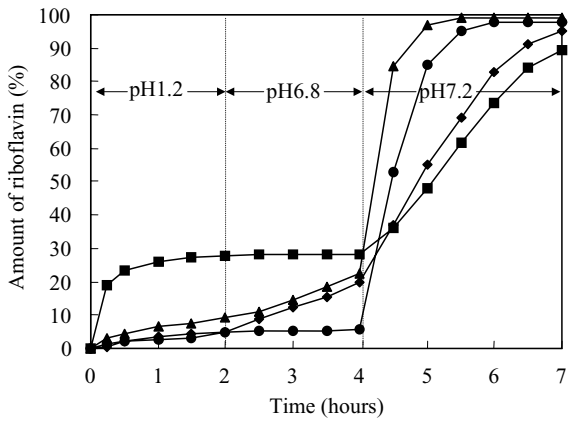


Fig. 2. Riboflavin release from microballoons (MB) and non-floating microspheres (NF): (●) microballoons (MB-1), (▲) microballoons (MB-2), (■) non-floating microspheres (NF-1), (◆) non-floating microspheres (NF-2).

Fig. 3 indicates that the urinary excretion of riboflavin in the 12–22 h period under fed and water ingestion conditions following administration of MB-1 was higher compared with fasted conditions. This phenomenon could be attributable to the buoyancy properties of MB-1 in the stomach with respect to the presence of ingested water; consequently, the urinary excretion of riboflavin was prolonged. The excretion half-life ($t_{1/2}$) and total urinary excretion of riboflavin are summarized in Table 3.

Table 3
Effect of food and water on the excretion half-life ($t_{1/2}$) and total urinary excretion

Volunteer number	Conditions	$t_{1/2}$ (h) (mean ± S.D.)	Total urinary excretion (%) (mean ± S.D.)
1–3	Fasted ($n = 3$)	4.87 ± 0.51	13.9 ± 3.7
4–6	Fed ($n = 3$)	5.43 ± 0.04	12.0 ± 3.8
1	Water ($n = 1$)	5.30	9.1

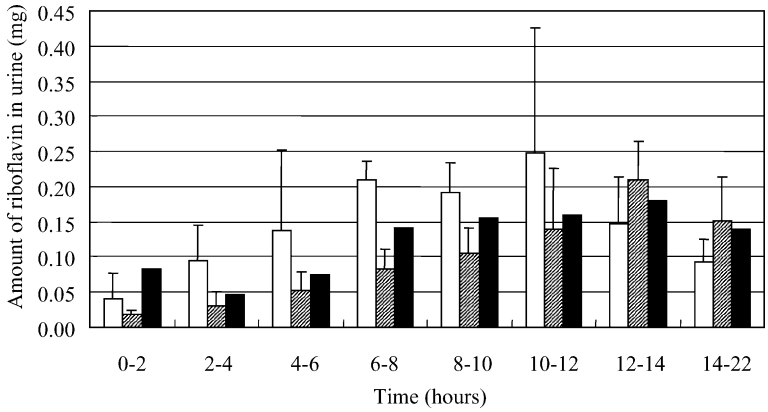


Fig. 3. Amount of riboflavin in urine following administration of microballoons (MB-1): (□) fasted condition ($n = 3$), (▨) fed condition ($n = 3$), (■) ingestion of water condition ($n = 1$).

The excretion half-life ($t_{1/2}$) under fed and water ingestion conditions following administration of MB-1 was longer than that under fasted conditions; in addition, water ingestion conditions resulted in sustained urinary excretion of riboflavin like that under fed conditions. This finding could be attributable to the buoyancy properties of MB-1 in the stomach as well as the prolonged GRT following pylorus closure due to feeding.

3.3. Effect of particle size of MB on urinary excretion

The effect of the buoyancy (<500 μm: 33.6%; 500–1000 μm: 93.6%) of MB-2 in terms of different particle size (<500 and 500–1000 μm) on urinary excretion was investigated. The riboflavin release from MB-2 in JP XII No. 1 solution containing 0.02 w/v% Tween 20 (pH 1.2), JP XIII No. 2 solution containing 0.5 w/v% Tween 80 (pH 6.8) and phosphate buffer containing 0.5 w/v% Tween 80 (pH 7.2) is illustrated in Fig. 4.

The buoyancy of MB-2 was closely correlated with particle size; the buoyancy of MB-2 decreased, accompanied by a decrease in particle size due to the reduced cavity volume. In addition, the amount of riboflavin released in the initial burst from both MB-2 particles types (particle size: <500 and 500–1000 μm) was minor in JP XIII No. 1 solution (pH 1.2). The amount of riboflavin released from smaller MB-2 (particle size: <500 μm) in each solution was slightly higher than that from larger MB-2 (particle size: 500–1000 μm). This observation could be due to the increased contact area with the solution associated with the reduced particle size and solubility of polymers in phosphate buffer containing 0.5 w/v% Tween 80 (pH 7.2). The change in the urinary excretion of riboflavin under fed conditions is shown in Fig. 5.

The urinary excretion of riboflavin for 2–10 h following administration of MB-2 (particle size: <500 μm) was high. This phenomenon was a result of the rapid release in the proximal small intestine where riboflavin is mainly absorbed. Moreover, the urinary excretion of the 12–22 h period post-dosing of MB-2 (particle size: 500–1000 μm) was higher than that of MB-2 (particle size: <500 μm). This sustained

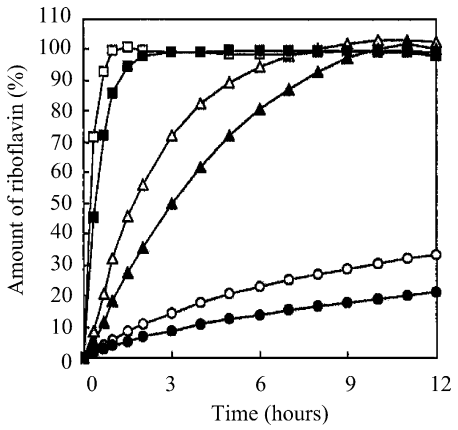


Fig. 4. Riboflavin release from microballoons (MB-2): (●) pH 1.2, 500–1000 μm; (○) pH 1.2, <500 μm; (▲) pH 6.8, 500–1000 μm; (△) pH 6.8, <500 μm; (■) pH 7.2, 500–1000 μm; (□) pH 7.2, <500 μm.

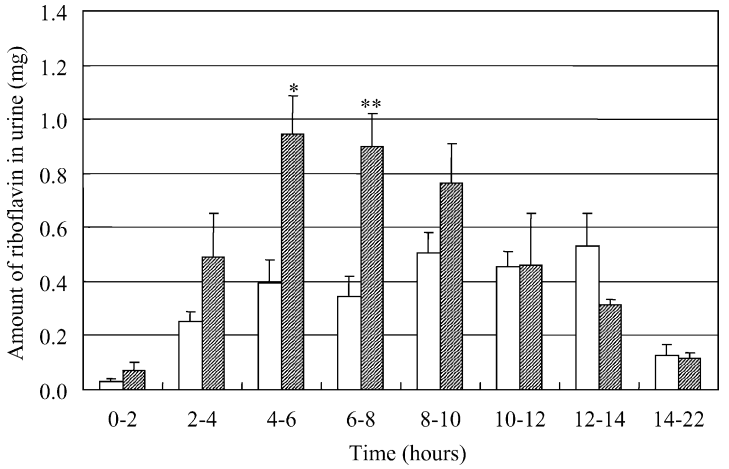


Fig. 5. Amount of riboflavin in urine under fed conditions. The values are represented as mean ± S.D. ($n = 3$). Statistical significance: $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with microballoons (MB-2, 500–1000 μm) using Student's unpaired *t*-test. (□) Microballoons (MB-2: 500–1000 μm), (▨) microballoons (MB-2: <500 μm).

urinary excretion of riboflavin was attributed to the prolonged GRT due to high buoyancy. The excretion half-life ($t_{1/2}$) and total urinary excretion of riboflavin are summarized in Table 4.

The excretion half-life ($t_{1/2}$) following administration of smaller MB-2 (particle size: <500 μm) was shorter than that of larger MB-2 (particle size: 500–1000 μm). On the other hand, the total urinary excretion of riboflavin following administration of smaller MB-2 (particle size: <500 μm) was 1.5 times as high as that of larger MB-2 (particle size: 500–1000 μm). This finding was attributed to the increasing release in the proximal small intestine where riboflavin is mainly absorbed due to the rapid dissolution of polymers, consisting of MB-2 (particle size: <500 μm). Therefore, the finding the urinary excretion of riboflavin over the period 12–22 h after administration of MB-2 (particle size: 500–1000 μm) reading a peak was consistent with the high buoyancy. Thus, the intragastric floating properties of MB are likely to be beneficial with respect to the sustained pharmacological action.

3.4. Improvement of total urinary excretion by mixing HPMC

As previously noted, the total urinary excretion of riboflavin following administration of riboflavin-containing MB was clearly lower than that of riboflavin powder, as very little riboflavin was released from MB in JP XIII No. 1 solution (pH 1.2), this was problematic. Thus, MB were prepared by mixing with a water-soluble polymer, HPMC in Eudragit® S100. The objective of these MB is to increase the amount of riboflavin released from MB in JP XIII No. 1 and No. 2 solutions and to increase the total urinary excretion of riboflavin. The formulation and buoyancy of MB are given in Table 5.

The buoyancy of MB decreased on increasing the HPMC ratio. These results were attributable

Table 4
Effect of particle size on excretion half-life ($t_{1/2}$) and total urinary excretion under fed conditions ($n = 3$)

Volunteer number	Sample	Particle size (μm)	Buoyancy at 4 h (%)	$t_{1/2}$ (h) (mean ± S.D.)	Total urinary excretion (%) (mean ± S.D.)
1–3	MB-2	500–1000	93.6	5.62 ± 0.51	29.7 ± 6.4
4–6	MB-2	<500	33.6	4.44 ± 0.01	42.3 ± 5.6

Table 5
Formulation and buoyancy of microballoons (MB)

Sample	MB-1	MB-2	MB-3	MB-4	MB-5
Riboflavin (g)	0.1	0.1	0.1	0.1	0.1
Eudragit® S100 (g)	1.0	0.9	0.8	0.7	0.6
HPMC[a] (g)	–	0.1	0.2	0.3	0.4
Monostearin (g)	0.5	0.5	0.5	0.5	0.5
CH_2Cl_2 (ml)	8	8	8	8	8
EtOH (ml)	8	8	8	8	8
Buoyancy at 4 h (%)	97.2	93.6	62.7	38.0	38.2

[a] HPMC: hydroxypropylmethylcellulose.

to conversion from the spherical form of MB to needle-like particles possessing no hollow structure on increasing the HPMC ratio. In addition, the JP XIII No. 1 solution can readily penetrate MB due to the dissolution of HPMC in solution. The riboflavin release from MB in JP XIII No. 1 and No. 2 solutions is shown in Fig. 6.

The amount of riboflavin released from MB in JP XIII No. 1 solution containing 0.02 w/v% Tween 20 (pH 1.2) increased on increasing the HPMC ratio (Fig. 6a). This observation could be attributable to the increased contact area with the solution due to the poor buoyancy associated with the increased HPMC ratio. The amount of riboflavin released from MB in JP XIII No. 2 solution containing 0.5 w/v% Tween 80 (pH 6.8) increased markedly in parallel with the increased HPMC ratio (Fig. 6b). The phenomenon appeared to afford high dissolution rate of Eudragit® S100 in JP XIII No. 2 solution. In particular, MB (Eudragit® S100:HPMC = 7:3 and 6:4) could provide increased bioavailability as riboflavin was absorbed mainly from the proximal small intestine (pH 4–6.5). The change in the urinary excretion of riboflavin under fed conditions is shown in Fig. 7.

Fig. 7 illustrates that the increased urinary excretion and increased amount of riboflavin in urine in association with the increased HPMC ratio. The physicochemical properties and pharmacokinetic parameters of MB prepared by mixing various HPMC ratios and NF-1 are summarized in Table 6.

The buoyancy of MB decreased on increasing the HPMC ratio. On the other hand, the amount of riboflavin released from MB in JP XIII No. 2 solution containing 0.5 w/v% Tween 80 (pH 6.8) increased on increasing the HPMC ratio. This phenomenon could provide increased the urinary excretion of riboflavin for the 4–6 and 6–8 h period post-dosing. Although the buoyancy of the MB (Eudragit® S100:HPMC = 10:0 and 9:1) were high, the urinary excretion of riboflavin for the 4–6 and 6–8 h period post-dosing attained low value, because riboflavin was scarcely released from MB in JP XIII No. 1 solution (pH 1.2). In the case of these MB, the urinary excretion of riboflavin for the 12–14 and 14–22 h period post-dosing were high due to sustained release of riboflavin from MB.

Furthermore, the relationships between buoyancy and excretion half-life ($t_{1/2}$) and between ri-

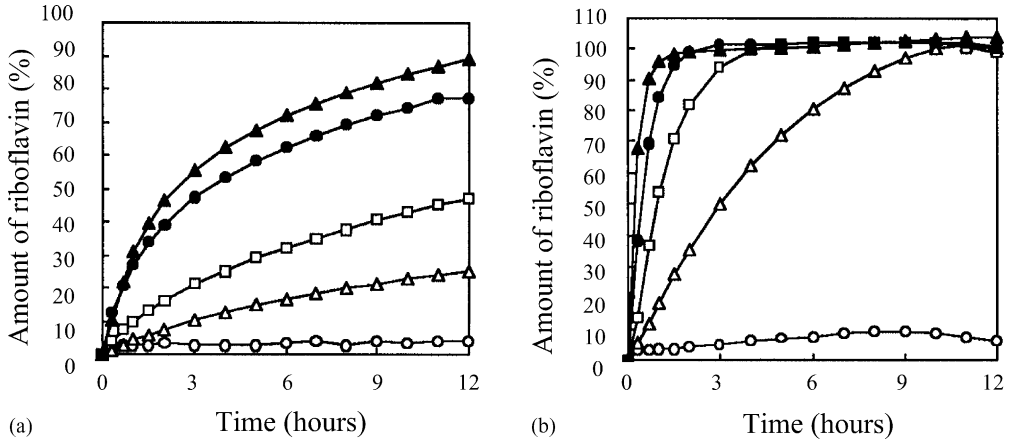


Fig. 6. Riboflavin release from microballoons (MB): (a) JP XIII No. 1 solution (pH 1.2); (b) JP XIII No. 2 solution (pH 6.8). (○) MB-1 (Eudragit® S100:HPMC = 10:0), (△) MB-2 (Eudragit® S100:HPMC = 9:1), (□) MB-3 (Eudragit® S100:HPMC = 8:2), (●) MB-4 (Eudragit® S100:HPMC = 7:3), (▲) MB-5 (Eudragit® S100:HPMC = 6:4).

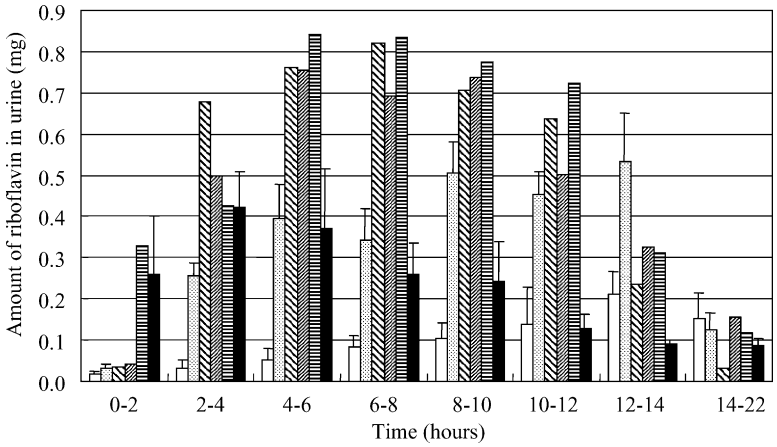


Fig. 7. Amount of riboflavin in urine under fed conditions: (□) MB-1 (Eudragit® S100:HPMC = 10:0), (▨) MB-2 (Eudragit® S100:HPMC = 9:1), (▧), MB-3 (Eudragit® S100:HPMC = 8:2), (▨), MB-4 (Eudragit® S100:HPMC = 7:3), (▤), MB-5 (Eudragit® S100:HPMC = 6:4), (■) NF-1.

boflavin released and total urinary excretion are shown as a function of HPMC ratio in Figs. 8 and 9, respectively.

The buoyancy of MB and the excretion half-life ($t_{1/2}$) decreased on increasing the HPMC ratio (Fig. 8). Furthermore, the amount of riboflavin released from MB in JP XIII No. 2 solution (pH 6.8) at 20 min increased; subsequently, the total urinary excretion of riboflavin increased on increasing the HPMC ratio (Fig. 9). This percentage released was adopted as the amount of riboflavin released from MB in JP XIII No. 2 solution (pH 6.8) at 20 min as the absorption of riboflavin was closely related to the rapid release in the proximal small intestine. Because the buoyancy of the MB (Eudragit® S100:HPMC = 9:1 and 8:2) were high in spite of the poor amount of riboflavin released

Table 6
Physicochemical properties and pharmacokinetic parameters of microballoons (MB) and non-floating microspheres (NF)

Volunteer number	Sample	S100:HPMC[a]	Buoyancy at 4 h (%)	Riboflavin released (%)			$t_{1/2}$[b] (h)	Urinary excretion (%)
				Time	pH 1.2	pH 6.8		
1–3	MB-1	10:0	99.2	20 min	2.4	3.0	5.43 ($n = 3$)	12.0 ($n = 3$)
				12 h	4.2	5.9		
1–3	MB-2	9:1	93.6	20 min	1.3	5.3	5.63 ($n = 3$)	27.1 ($n = 3$)
				12 h	25.0	100.1		
4	MB-3	8:2	62.7	20 min	4.2	13.6	4.68 ($n = 1$)	39.5 ($n = 1$)
				12 h	46.8	97.1		
5	MB-4	7:3	38.0	20 min	12.7	38.4	3.85 ($n = 1$)	40.4 ($n = 1$)
				12 h	77.1	100.6		
6	MB-5	6:4	38.2	20 min	10.3	68.1	4.25 ($n = 1$)	47.5 ($n = 1$)
				12 h	89.3	103.6		
4–6	NF-1	–	0	20 min	22.7	28.5	4.35 ($n = 3$)	20.7 ($n = 3$)
				12 h	29.7	38.8		

[a] S100: Eudragit® S100; HPMC: hydroxypropylmethylcellulose.
[b] $t_{1/2}$: excretion half-life time.

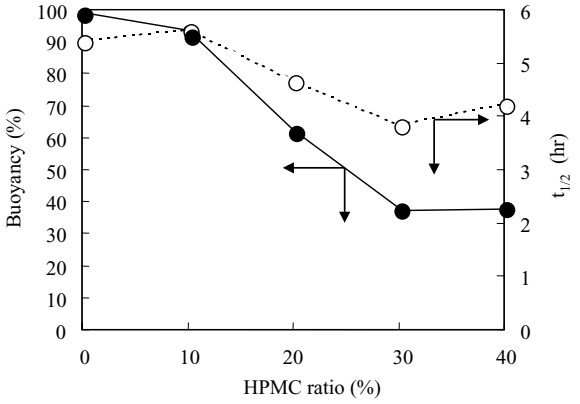


Fig. 8. Relationship between buoyancy and excretion half-life ($t_{1/2}$) as a function of HPMC ratio: (●) buoyancy (%) and (○) $t_{1/2}$ (h).

from the MB at 20 min, the total urinary excretion of riboflavin increase relatively.

In summary, sustained urinary excretion was greatly influenced by the intragastric buoyancy properties of MB. In addition, it is necessary to increase the level of riboflavin release in proximal small intestine in order to increase its total urinary excretion. The relationship between the buoyancy of MB and the level of riboflavin release from MB is inversely proportional. When developing an intragastric flotation system employing these MB, it is necessary to select an appropriate balance between the buoyancy and the percentage released.

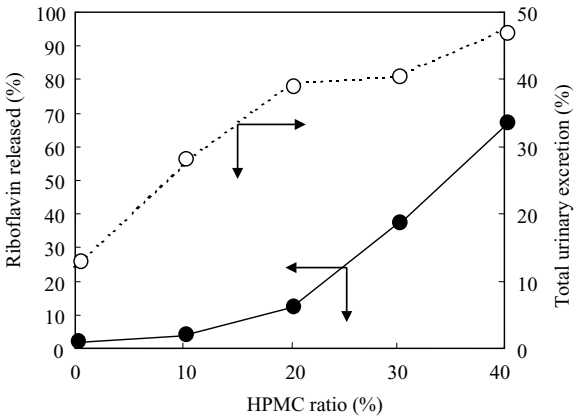


Fig. 9. Relationship between riboflavin released and total urinary excretion as a function of HPMC ratio: (●) riboflavin released (%) and (○) total urinary excretion (%).

4. Conclusion

Riboflavin-containing MB were administered orally to one healthy volunteer under water ingestion conditions; subsequently, the pharmacokinetics of riboflavin was investigated by analysis of its urinary excretion. As a result, water ingestion conditions, as well as fed conditions, lead to sustained urinary excretion of riboflavin. This phenomenon could be attributable to the buoyancy properties of MB in the stomach as well as a prolonged GRT following pylorus closure due to feeding. When an in vivo evaluation of MB of different particle sizes (<500 and 500–1000 μm) was carried out, the excretion half-life ($t_{1/2}$) following administration of larger MB (particle size: 500–1000 μm) exhibiting high buoyancy was longer than that of smaller MB (particle size: <500 μm) displaying low buoyancy. Thus, the intragastric floating properties of MB are likely to be beneficial as far as a sustained pharmacological action is concerned.

In order to increase the total urinary excretion of riboflavin, MB were prepared by mixing various HPMC ratios. When in vivo evaluation of the MB was conducted, the buoyancy of the MB correlated closely with the excretion half-life ($t_{1/2}$). In addition, a strong correlation was observed between riboflavin release from the MB and total urinary excretion. So, the buoyancy of MB is an essential factor in terms of the sustained urinary excretion. Factors influencing the buoyancy of MB were the particle size and HPMC ratio in the formulation of MB. Furthermore, total urinary excretion was greatly influenced by riboflavin released from MB, while riboflavin release was affected by the HPMC ratio. Therefore, the ideal properties of MB are a high buoyancy and sufficient release of drug in JP XIII No. 2 solution (pH 6.8). There was an inverse relationships between the buoyancy of MB and the level of riboflavin release from MB. In developing a desired intragastric flotation system employing these MB, it is necessary to select an appropriate balance between buoyancy and drug releasing rate.

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