Combinative Improving Effect of Increased Solubility and the Use of Absorption Enhancers on the Rectal Absorption of Uracil in Beagle Dogs

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An improvement of the rectal absorption of uracil was examined by the application of absorption enhancers in addition to the increased solubility of uracil.

Uracil was ground with additives such as MgO, sodium 2,6-dihydroxybenzoate, human serum albumin or hydroxypropylmethylcellulose acetate succinate. Aqueous, oily and powdery formulations, which consisted of the ground mixtures, nicotinamide, urea and absorption enhancers such as polyoxyethylene (23) cetylether (BC-23) or sodium caprate, were prepared.

Uracil solubility in the aqueous formulations was increased about 4—13 times that in the corresponding control formulations. When rectally administered to beagle dogs, marked increases in the plasma uracil level were observed in some of the cases of aqueous and oily formulations. In the powdery formulations and formulations containing macromolecular additives, however, absorption improvement was not observed.

The results indicated that an improvement in the absorption of uracil was caused by the combinative improving effect of the increased uracil solubility and the promoting effect of absorption enhancers.

Keywords uracil; solubility; grinding; absorption enhancer; rectal absorption

Recently, many investigations on the absorption enhancement of drugs from gastrointestinal tracts have been done using absorption enhancers.[1-5] However, successful results have been limited to the use of absorption enhancers on various water-soluble drugs. Studies on the application of absorption enhancers on drugs exhibiting poor water solubility have not been reported.

Most high-melting drugs, which are poorly soluble in both water and organic solvents, are confronted with the problem of low availability due to their low water-solubility and low lipophilicity. When solubility is too low at the absorption site after the administration of dosage forms, absorption would be less even if absorption enhancers are applied.

A combination therapy of uracil and tegafur has been performed on cancer patients in Japan,[6,7] and rectal administration is highly desirable. However, uracil, a typical high-melting compound, is sparingly soluble in water and is not adequate for rectal absorption. In fact, the solubility of uracil in water was low (4 mg/ml) as shown in this paper, while the used doses of uracil are relatively high, 224—448 mg, in clinical therapy. Solubility may be one of the most important factors in rectal therapy since 3 ml of rectal luminal fluid in adults is too little.[8]

In our previous study,[9] uracil solubility was increased 2.5—9 times by grinding it with additives, and a few times by the addition of nicotinamide, *etc.* In addition, other methods such as the use of absorption enhancers are needed in order to promote the rectal absorption of uracil. Therefore, we attempted to examine the improvement of uracil absorption following rectal administration in beagle dogs, using absorption enhancers in addition to uracil solubilized by the above methods.

Experimental

Materials Uracil (Kyowa Hakko Co.) was used as received. Not more than 0.2% (w/w) of uracil remained on 100 mesh sieve. Loss on drying (105 °C, 3 h) of uracil was less than 0.1% (w/w). Nicotinamide (Kawazu Sangyo Co.), urea (Wako Pure Chemicals Co.) and polyoxyethylene (23) cetylether (BC-23; Nikko Chemical Co.) were used as received. MgO (Wako Pure Chemicals Co.), human serum albumin (HSA, a crystallized and lyophilyzed type, Sigma Chemical Co., U.S.A.) and hydroxypropylmethylcellulose acetate succinate (HPMCAS, AS-MF type, Shin-Etsu Chemical Ind. Co.) were dried before grinding under the condition of 900 °C, 3 h; room temperature, reduced pressure, 8 h and 105°C, 1 h, respectively. Sodium 2,6-dihydroxybenzoate (Na DHB) was prepared from 2,6-dihydroxybenzoic acid (Lancaster Synthesis, England) and stoichiometrically equal NaOH, and dried (105 °C, 3 h). Sodium caprate (Tokyo Kasei Kogyo Co.) was pulverized and passed through a 100 mesh sieve. All other chemicals were of reagent grade quality and used as received.

Preparation of Ground Mixtures The ground mixtures were prepared in such mixing ratios of uracil and additives as indicated in Table I. The mixtures were ground for 30 h with an agate-made planetary ball mill (Model P-5; Fritsch Japan Co.), whose turning velocities of a supporting disk and bowls were about 270 and 570 rpm, respectively.

Preparation of Formulations The constituents of aqueous formulations are listed in Table I. Nicotinamide, urea, sodium caprate or BC-23 were previously dissolved in water or JP XI 2nd Fluid (JP disintegration medium pH 6.8), and aqueous formulations were prepared by adding the ground mixtures (codes A-1—A-4) or fine powder of uracil (codes A-5—A-8) to these solutions at 37 °C, prior to dissolution studies, or 1 min before rectal administration to the beagle dogs. The amount of water in each formulations A-1—A-4 was adjusted to a concentration which would keep the viscosity in a range that would allow its passage through membrane filters used in the disolution studies. The concentration of an absorption enhancer (BC-23 or sodium caprate) in each aqueous formulation was 2% (w/v). Formulations A-5, A-6, A-7 and A-8 do not contain ground mixtures, urea nor nicotinamide, to make them as control formulations corresponding to A-1, A-2, A-3 and A-4, respectively. Formulation A-9 is the supersaturated solution of uracil, which was prepared by boiling the mixture of water, uracil and sodium caprate, and then cooling it to 37 °C just before rectal administration.

The constituents of oily formulations and powdery formulations are listed in Tables II and III, respectively.

Dissolution Studies for Aqueous Formulations Dissolved amounts of uracil in aqueous formulations were measured in the beakers maintained at 37 °C and stirred with magnetic stirrers. Ten ml of water equivalent to each aqueous formulation was prepared in the beaker as described above, and simultaneously, the dissolution study was started. A suitable aliquot was removed at a specified time and filtered (0.45 μm pore size, Millipore Co.).

***In Vivo* Absorption Studies** Male beagle dogs weighing about 10 kg

TABLE I. Constituents of Aqueous Formulations (mg)

Constituents	Code								
	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9
Uracil	224	224	224	224	224	224	224	224	224
MgO[a]	896	—	—	—	—	—	—	—	—
Na DHB[a]	—	616	—	—	—	—	—	—	—
HSA[a]	—	—	896	—	—	—	—	—	—
HPMCAS[a]	—	—	—	896	—	—	—	—	—
Nicotinamide	300	—	1120	840	—	—	—	—	—
Urea	—	300	—	840	—	—	—	—	—
Na Caprate	—	60	224	168	—	60	224	168	400
BC-23	60	—	—	—	60	—	—	—	—
Water[b]	3.0	3.0	11.2	—	3.0	3.0	11.2	—	20.0
JP XI 2nd Fluid[b]	—	—	—	8.4	—	—	—	8.4	—

a) Ground-mixed with uracil. *b*) Expressed as ml.

TABLE II. Constituents of Oily Formulations (mg)

Constituents	Code					
	O-1	O-2	O-3	O-4	O-5	O-6
Uracil	224	224	224	224	224	224
MgO[a]	896	—	—	—	—	—
Na DHB[a]	—	616	—	—	—	—
HSA[a]	—	—	896	—	—	—
HPMCAS[a]	—	—	—	896	—	—
Nicotinamide	300	—	300	300	—	—
Na caprate	—	60	100	100	—	60
BC-23	100	—	—	—	100	—
Miglyol 812	3540	2100	3540	3540	4676	2716
Total	5060	3000	5060	5060	5000	3000

a) Ground-mixed with uracil.

TABLE III. Constituents of Powdery Formulations (mg)

Constituents	Code			
	P-1	P-2	P-3	P-4
Uracil	224	224	224	224
MgO[a]	896	—	—	—
Na DHB[a]	—	616	—	—
HSA[a]	—	—	896	—
HPMCAS[a]	—	—	—	896
Nicotinamide	300	—	300	300
Na caprate	—	60	60	60
BC-23	60	—	—	—
Total	1480	900	1480	1480

a) Ground-mixed with uracil.

were fasted for 16 h prior to the experiments. Aqueous formulations and oily formulations were rectally administered to a 5 cm depth from the anus with a disposable syringe (Terumo Co.) equipped with a silicon made tube. Powdery formulations were administered with the device illustrated in Fig. 1. The powder mixture was put between the stem and pipe, and the pipe was pulled out immediately after insertion of the device into the rectum. The stem had been left inserted in the rectum during the absorption study. The blood samples, collected from foreleg veins at specified intervals in heparinized test tubes, were centrifuged (3000 rpm, 10 min), and the plasma was stored in a refrigerator at −20 °C until assayed.

Analytical Methods Uracil concentrations in dissolution studies were determined by a high performance liquid chromatography (LC-6A: Shimazu Co.) equiped with a ultraviolet (UV) detector and a reverse phase column (μ Bondapak C18, Waters Co.). The mobile phase was water containing 20 mM Na acetate and 5 mM PIC-A (Waters Co.). The flow rate was maintained at 1.0 ml/min. The wavelength of the UV detector was

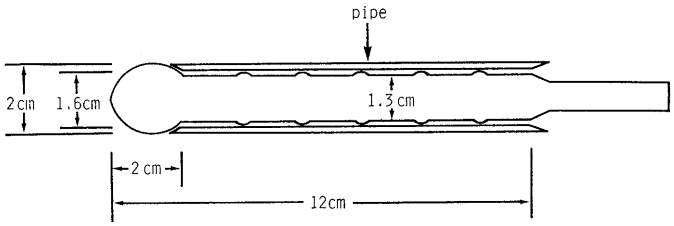

Fig. 1. The Instrument for Rectal Administration of Powdery Formulations

254 nm. Uracil concentrations in plasma were determined following the method of Marunaka *et al.*[10]

Results

Dissolution Studies for Aqueous Formulations The dissolution profiles of uracil in aqueous formulations A-1, A-2, A-3 and A-4 at 37 °C are shown in Fig. 2, together with the respective control formulations A-5, A-6, A-7 and A-8, which contained no ground mixture, urea nor nicotinamide.

In formulations A-1—A-4, a marked increase in uracil solubilities was observed. At 1 min, formulations A-1, A-2, A-3 and A-4 yielded uracil solutions approximately 12, 13, 4 and 5 times more concentrated than their control formulations, respectively. In this earlier stage, about 60—80% of uracil in each formulations A-1—A-4 was dissolved, and later, a decline was observed in the amount of uracil dissolved.

***In Vivo* Absorption Studies** Figure 3 shows the plasma concentrations of uracil after the rectal administration of aqueous formulations A-1, A-2, and their control formulations to the beagle dogs. Table IV summarizes the results of the absorption studies in aqueous formulations.

The administration of the A-1 formulation, which contains uracil–MgO ground mixture, nicotinamide and BC-23, caused a rapid increase in the plasma uracil level. The rise in the plasma uracil level (ΔC) from a normal level after rectal administration of formulation A-1 was 10.9 times that of its control formulation A-5. Similarly, the ΔC of formulation A-2, which contains uracil–Na DHB ground mixture, urea and Na caprate, increased 14.5 times that of its control formulation A-6. The ΔC of formulation code A-3 was only 1.3 times higher than its control formulation A-7. In the case of formulation A-4, only a little uracil

TABLE IV. Plasma Concentrations of Uracil after Rectal Administration of Aqueous Formulations to Beagle Dogs

Code	Plasma concentrations (μg/ml)			$R^{b)}$	(Ref.)[c)]
	C_0	C_{max}	$\Delta C^{a)}$		
A-1	0.139±0.006	1.622±1.042	1.482±1.045	10.9±7.7	(A-5)
A-2	0.192±0.017	1.858±0.389	1.666±0.322	14.5±3.2	(A-6)
A-3	0.149±0.003	1.357±0.445	1.207±0.444	1.3±0.6	(A-7)
A-4	0.193±0.001	0.891±0.372	0.698±0.390	—	
A-5	0.158±0.011	0.294±0.039	0.136±0.040		
A-6	0.179±0.008	0.294±0.022	0.115±0.035		
A-7	0.183±0.017	1.115±0.179	0.932±0.195		
A-9	0.132±0.021	3.170±0.705	3.038±0.720		

Each value represents the mean±S.E. of three dogs. *a*) $C_{max}-C_0$. *b*) ΔC(sample)/ΔC(control). *c*) Controls.

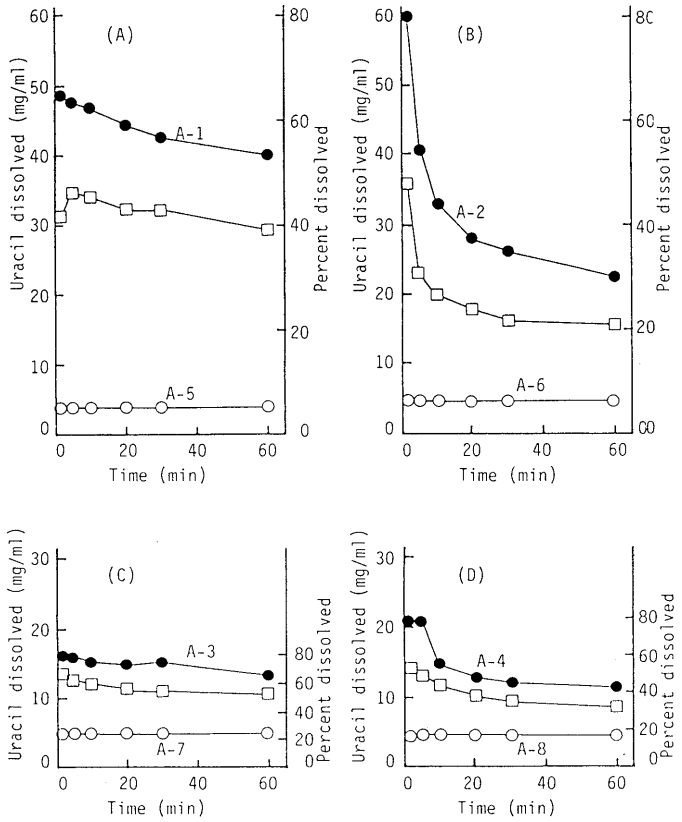

Fig. 2. Uracil Dissolution Profiles in Aqueous Formulations

The symbols indicate formulations with ground mixture, nicotinamide and/or urea (●), formulations without nicotinamide and/or urea (□) and formulations as controls which do not contain ground mixture, nicotinamide or urea (○).

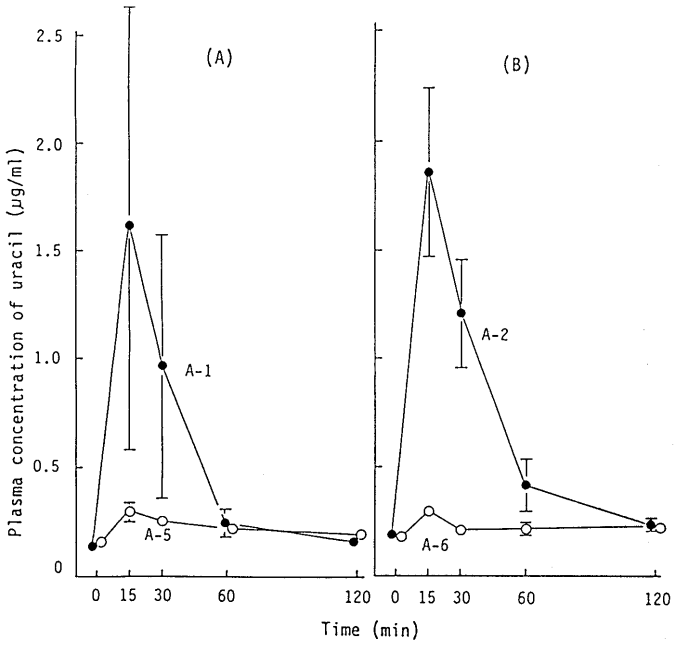

Fig. 3. Plasma Concentrations of Uracil after Rectal Administration of Aqueous Formulations

Each point represents the mean±S.E. of three dogs.

absorption was observed.

Tables V and VI summarize the results of absorption studies in oily formulations and powdery formulations, respectively.

The ΔC of oily formulation O-1 was almost the same value as that observed in the aqueous formulation A-1, and about 5.5 times that of its control formulation O-5. In formulation O-2, uracil absorption was about two times better than that of its control formulation O-6. Only a little ΔC was observed in formulations O-3 and O-4.

No marked absorption of uracil was observed in the powdery formulations.

Discussion

The increase of uracil solubility in dissolution studies of aqueous formulations may be attributed partly to mix-grinding (□ in Fig. 2), and the following decline in dissolusion profiles may be due to recrystalization from the transiently supersaturated solution caused by mix-grinding. Morevoer, the other factors of increased uracil solubility may be due to the further addition of uracil solubilizing agents, nicotinamide and/or urea (● in Fig. 2), due to alkalifying with the addition of MgO in formulation A-1 (pH, about 10; pK_a of uracil, 9.4), or due to the uracil solubilizing property of Na DHB[9)] in formulation A-2.

In addition to the increased uracil solubility, the use of an absorption enhancer is supposed to be needed to improve the rectal absorption of uracil because lipophilicity of uracil is very poor. In fact, uracil was hardly absorbed after the rectal administration of the supersaturated solution which was prepared in the same way as formulation A-9 without the addition of sodium caprate (data not shown). Sodium caprate[2)] and BC-23[3)] used in this study were previously known absorption enhancers.

Some plasma uracil concentration profiles from *in vivo* absorption studies were correlated as to the effect of increased solubility in dissolution studies. The increase in

TABLE V. Plasma Concentrations of Uracil after Rectal Administration of Oily Formulations to Beagle Dogs

Code	Plasma concentrations (μg/ml)			$R^{b)}$	(Ref.)[c)]
	C_0	C_{max}	$\Delta C^{a)}$		
O-1	0.187±0.009	1.534±0.577	1.347±0.582	5.5±2.4	(O-5)
O-2	0.237±0.010	0.484±0.076	0.247±0.081	1.9±1.1	(O-6)
O-3	0.176±0.002	0.312±0.026	0.136±0.027	—	
O-4	0.181±0.008	0.384±0.030	0.203±0.026	—	
O-5	0.162±0.003	0.405±0.133	0.243±0.136		
O-6	0.242±0.039	0.370±0.048	0.127±0.067		

Each value represents the mean±S.E. of three dogs. *a)* $C_{max}-C_0$. *b)* ΔC(sample)/ΔC(control). *c)* Controls.

TABLE VI. Plasma Concentrations of Uracil after Rectal Administration of Powdery Formulations to Beagle Dogs

Code	Plasma concentrations (μg/ml)		
	C_0	C_{max}	$\Delta C^{a)}$
P-1	0.148±0.005	0.258±0.044	0.110±0.042
P-2	0.182±0.029	0.423±0.157	0.242±0.128
P-3	0.147±0.002	0.224±0.028	0.078±0.027

Each value represents the mean±S.E. of three dogs. *a)* $C_{max}-C_0$.

uracil absorption after the rectal administration of aqueous formulations A-1 and A-2 (Fig. 3, Table IV) was consistent with the solubility increase in the dissolution studies. On the other hand, Na DHB and urea did not enhance the absorption of uracil from the supersaturated solution (data not shown). Thus, increased absorption in formulation A-2, compared with A-6 formulation, may be directly explained by the increase in uracil solubility.

Although formulations A-1 and A-2 contain 60 mg (2% in water) of BC-23 or sodium caprate, the same as formulation A-5 or A-6 (Table I), a greater improvement of uracil absorption was obtained. Furthermore, the increase of plasma uracil in formulation A-2 was 1.8 times higher than in formulation A-7 (Table IV), although the sodium caprate content in A-2 was much less than in formulation A-7 (Table I). It has been observed in general that the more absorption enhancer that is applied, the stronger is the absorption promotion effect.[1)] However, it was demonstrated in this case that the increased solubility of a poorly soluble compound caused a marked increase in plasma levels by the application of a small amount of absorption enhancers. The applicable amount of absorption enhancer is limited because an over dose causes mucosal damage or the feeling of defecation.[5,11)] Therefore, these findings may be useful for improving the absorption of poorly lipophilic and water-insoluble drugs. Their poor absorbability may be improved with the application of absorption enhancers under the conditions of increased solubility.

It remains unclear whether or not the transient supersaturation itself contributed to the absorption improvement. This question remains of interest, considering the following factors. Absorption enhancers are much more effective on small molecular weight materials than on large ones. Meanwhile, the mechanism of transient supersaturation from non-crystalline drugs has sometimes been proposed to be coacervated droplets[12)] or colloidal dispersions,[13)] and an apparent molecular weight of the aggregates formed from the coprecipitate of griseofulvin with phospholipids in causing supersaturation has been reported to be larger than 3500.[14)] It is impossible to discuss precisely about this point from the presented data. However, comparing the results from dissolution and absorption studies in formulations A-2 and A-7, the following interpretations may be made. In the dissolution study of formulation A-2, at 60 min, a decline in uracil solubility still continued. Therefore, in a steady state, the percentage dissolved in this formulation is considered to be far below 30 (right vertical axis in Fig. 2B) and not to be so different from the percentage dissolved in fromulation A-7 which is about 24.5 (right vertical axis in Fig. 2C). As for the absorption enhancer, formulation A-2 is considered to be less effective than formulation A-7 (60 and 224 mg, respectively) or, at most, equivalent to formulation A-7 (either was 2% in water, Table I). In this condition, the rise in the plasma uracil level after the rectal administration of formulation A-2 was 1.8 times higher than that of formulation A-7 (Table IV). This superior rise in the plasma uracil level may be attributed to the transient supersaturation. Therefore, not only the improvment of uracil solubility under a steady state but also such improvement under a transiently supersaturated state are supposed to be effective for the absorption of uracil.

Contrary to the results from formulations A-1 and A-2, the ΔC of formulation A-3 (Table IV) was only slightly higher than its control formulation A-7. In the case of formulation A-4, uracil absorption was less than A-7, though the dissolution behavior of formulation A-4 was better than that of formulation A-7. It is most likely due to the difference in constituents between formulations A-1, A-2 group and formulations A-3, A-4 group. The former contains no high molecular weight adjuvant and the latter is mainly composed of high molecular weight ajuvants (HSA or HPMCAS) as mix-grinding additives. Rim *et al.* have reported that the rate of transcellular diffusion of solutes across the monolayers of the *in vitro* brain microvessel endothelial cell model was reduced by the addition of bovine serum albumin (BSA) and that the interaction of BSA and other macromolecules with fibrous surface glycoproteins may cause such inhibition of solute diffusion.[15)] High molecular weight compounds as mix-grinding additives may not be appropriate for the improvement of the rectal absorption of uracil. Consequently, little improvement in uracil absorption was observed in both oily formulations (O-3 and O-4) and powdery formulations (P-3 and P-4), which are the mimic formulations of aqueous formulations

A-3 and A-4, containing macromolecules without water, respectively, as shown in Tables V and VI.

A marked rise in the plasma uracil level after administration of oily formulation O-1 (Table V), which is the mimic formulation of aqueous formulation A-1, was observed. This result was consistent with the absorption studies in aqueous formulations and indicated the usefulness of the combinative application of solubility improvement and absorption enhancers to oily formulations, too. However, only a little absorption improvement in formulation O-2 was observed (Table V). Though oily formulation O-2 was prepared also as the mimic of aqueous formulation A-2 (Tables I, II), it had some restrictions. First, urea was removed because of its property causing temperature descent of water. Second, viscosity of the oily formulation was very high (not measured). Therefore, only a little (about 2 times, Table V) improvement of uracil absorption is considered to have been caused by these factors in oily formulation O-2.

Powdery formulations were expected to exert a close adherance of the ground mixture to the luminal membrane on the absorption of uracil. However, absorption improvement was not observed (Table VI).

In conclusion, the present investigation showed that increased solubility brought out the efficiency of absorption enhancers to the compound which is poorly soluble in both water and organic solvents. It should be of interest to explore the combinative applications of solubility improvement and absorption enhancers to high melting point drugs.

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