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SHORT COMMUNICATION

Improvement of bioavailability of water insoluble drugs: Estimation of intrinsic bioavailability

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Abstract−A series of attempts to enhance the bioavailability of water insoluble drugs have been made by the fine grinding technique using a planetary ball mill in wet milling process. Here, the possibility of improving the dissolution properties of water insoluble drugs such as ursodeoxycholic acid (UDCA), diphenyl hydrantoin (phenytoin) and biphenyl dimethyl dicarboxylate (DDB) based on ultra-fine grinding process has been discussed with comparison to experimental data. Also examined was the intrinsic bioavailability estimated based on a molecular modeling approach and thermophysical data.

Key words: Water Insoluble Drug, Bioavailability, Top-down Process, UDCA, DDB, Phenytoin

INTRODUCTION

The bioavailability of low solubility drugs is often intrinsically related to the primary particle size of raw drug based on the followings: the solubility of a crystal is a function of the heat of melting and the melting temperature of the solid and the activity coefficient of the solution and the speed of dissolution depends on the particle size. Particle size reduction by top-down processing, milling, inclusion of active drug in additive drugs, solid dispersion formulations, salt formation, and appropriate polymorph selection is one of various strategies for improving solubility or increasing the dissolution rate of poorly water-soluble active pharmaceutical ingredients. Stricter regulatory requirements limiting the use of organic solvents, and the need for preparing more cost-effective formulations projects have created the need in the pharmaceutical industry to explore alternative technologies.

Many attempts have been conducted to obtain a good bioavailability achieved by creating an amorphous product [1,2]. The amorphization of drug in the co-grinding process has been recognized as one of the effective ways to improve the dissolution behavior [3,4]. It is well known that the pharmaceutical processing of a solid causes defects in the crystal lattice, which contribute to the disorder [4]. A grinding process could induce defects in the crystalline network: these defects would improve the compression and dissolution [5]. The amorphous and crystalline states are two extreme states of solid substances. In an amorphous product, molecules are in an irregular arrangement within the particles. The lack of long-range order that characterizes crystalline state related with the particles on a good compression ability due to the plasticity and isotropy of force transmission through such a structure. However, amorphous states have been known as a high energy state, and their physical and chemical stability is poor [6].

The most reliable and commonly used experimental method for determining intrinsic aqueous drug solubility is the shake flask method [7]. However, this method's usefulness is limited due to being time consuming for several days to weeks. Moreover, accurate determination of lipophilic, insoluble substances may be troublesome because of the loss of substance in the filtration step. In addition, the experiments are traditionally performed on a large scale, and large amounts (grams) of substances are required [8].

A series of attempts to enhance the bioavailability of insoluble drugs such as ursodeoxycholic acid (UDCA), diphenyl hydrantoin (phenytoin) and biphenyl dimethyl dicarboxylate (DDB) have been made by the fine grinding technique using a planetary ball mill [9-11]. Here, theoretical calculations in conjunction with a quantitative structure property relationship approach for intrinsic aqueous solubility have been performed for the investigation of molecular bioavailability without a consideration of morphology and discussed with comparison to experimental data.

EXPERIMENTAL

1. Materials

Ursodeoxycholic acid (UDCA), dipheny hydrantoin (phenytoin) and biphenyl dimethyl dicarboxylate (DDB) were supplied from Daewoog Pharmaceutical Co. Ltd. (Seoul, Korea), from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan), and from Daewoo Pharmaceutical Co. Ltd. (Busan, Korea), respectively. All the other chemicals were of analytical reagent grade and used without further purification.

2. Preparation Method of Ground Products

A vertical type planetary ball mill (KVP-03, power 2.2 kW) was mainly used [9,10,12]. The revolution speed of the turntable was kept constant at 112 rpm in anti-clock direction in all runs. The inner volume and diameter of the pot of cylindrical shape were 372 ml and 74 mm, respectively. The pot was made of wear-resistant zirconia and the grinding balls of 1 mm, 2 mm, and 5 mm were also made of the same material. Both the pot and grinding balls were made by Nikkato Co., Ltd. (Osaka, Japan). The stainless ball and nylon-coated stainless ball were partly used as grinding media. As

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the standard operating conditions, the previously described procedures were used in this study [12].

2-1. Particle Size Distribution (PSD)

The particle size distribution of the single ground sample and co-ground sample was measured with Mastersizer microplus of Malvern Instruments Ltd. (Spring Lane South, UK) on the basis of particle size analysis by the laser diffraction and scattering method. Prior to measurement, the sample was externally dispersed for 2 min. with an ultrasonic homogenizer, US-300T (Nihonseiki Co., Ltd. Osaka, Japan). The optimum value of the refractive index for the three sample drugs was experimentally determined to be 1.680.

2-2. Powder X-Ray Diffraction (PXRD)

A Miniflex diffractometer (Rigaku, Tokyo, Japan) was employed. The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 30 kV; current, 15 mA; and scanning speed; 4°/min.

2-3. *In Vitro* Apparent Solubility and Dissolution Rate Test

The solubility of UDCA was determined by the extrapolation method using Model Multisizer II of Coulter Electronic Ltd. (Luton, UK) [13]. The solubility of phenytoin and DDB was determined by the chemical analysis described by the general testing method of Korean Pharmacopoeia.

Table 1 shows a summary of the physico-chemical data of the drugs as water insoluble drugs, e.g. phenytoin and biphenyl dimethyl dicarboxylate (DDB) including ursodeoxycholic acid (UDCA) and their chemical structure together with the therapeutic category.

RESULTS AND DISCUSSION

1. Experimental Results by Planetary Ball Mill

Fig. 1 shows the effect of experimental conditions on particle size distribution of ground DDB. The grinding conditions were as shown in the figure. The broad particle size distribution of intact DDB with a median diameter of 22.7 μm was changed to the sharp one with a median diameter of 297 nm after 24 h of grinding time.

Fig. 2 shows a typical example of the correlation observed between crystallinity and apparent solubility of DDB. The diagram indicates that these variables are correlated fairly well. Here, the degree of crystallinity of ground products was calculated according to Herman's method. In the symbols of the sample ID, P indicates the planetary ball mill, A, S, and N for ball materials for, Alumina, SUS, and Nylon coated SUS, respectively, and subscripts of P and A, S, N for rpm of mill and ball diameter of grinding medium, re-

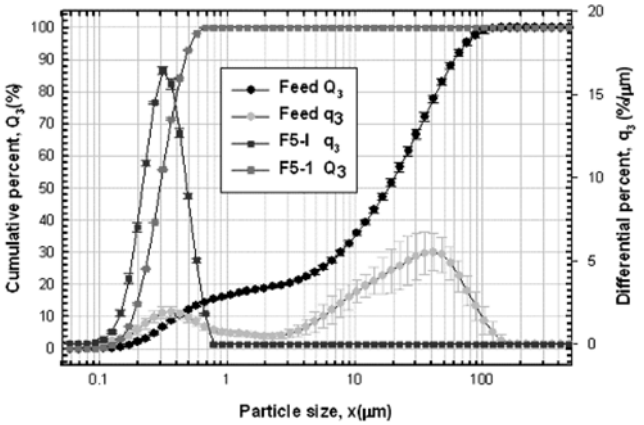

Fig. 1. Particle size distribution of intact DDB and ground DDB. Experimental conditions: Additives: PCNa : 0.5 g, Polysorbate 80 : 2.75 g, PVP10 : 3.0 g, d_B=φ1.0 mm, Grinding time: 24 h.

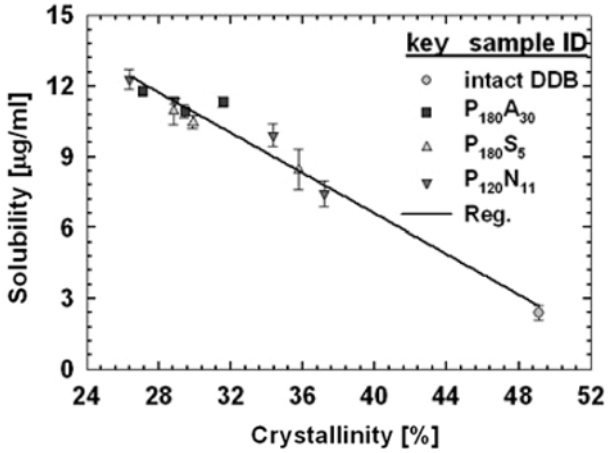

Fig. 2. Correlation between crystallinity and solubility of DDB.

Table 1. Summary on physico-chemical data of drugs used in this research

Drug	Molecular formula	Molecular weight	Physical data		Particle size (μm)	Therapeutic category
			m.p. (°C)	Solubility in water		
UDCA[a]	$C_{24}H_{40}O_4$	392.56	203	Practically insoluble	25.3	Anticholelithogenic
Phenytoin[b]	$C_{15}H_{12}N_2O_2$	252.46	295-298	Practically insoluble	12.5	Anticonvulsant
DDB[c]	$C_{20}H_{18}O_{10}$	418.36		Practically insoluble	66.6	Hepatoprotectors

Structure

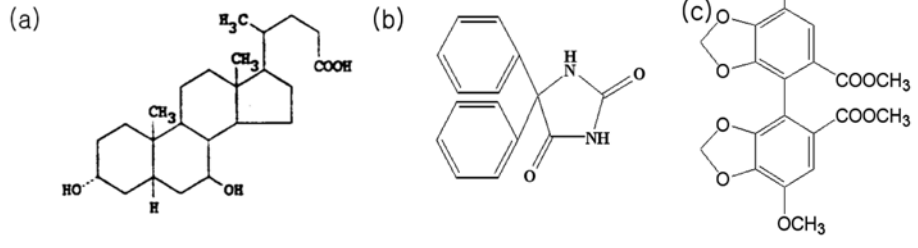

Table 2. Summary of experimental data of poorly water-soluble drugs for particle size distribution of intact and ground products and their solubility

Drug	Particle size distribution (μm)		Solubility (μg/ml)	
	Intact	Ground products	Intact	Ground products
UDCA	x_{10}=10.8	x_{10}=0.27[a]	160±8	336±15
	x_{50}=42.1	x_{50}=0.48		
	x_{90}=106.7	x_{90}=1.68		
Phenytoin	x_{10}=17.5	x_{10}=1.3[b]	58.6±0.7	106.2±5.4
	x_{50}=21.9	x_{50}=3.3		
	x_{90}=45.2	x_{90}=26.0		
DDB	x_{10}=0.42	x_{10}=0.13[c]	1.96±0.09	4.20±0.17
	x_{50}=22.7	x_{50}=0.30		
	x_{90}=62.0	x_{90}=4.8		

[a]Exp. conditions for grinding : grinding time : 16 h with no additives, D_B=φ1.0 mm.

[b]Exp. conditions for grinding : PVA10: 20 wt%, Grinding time: 30 min.

[c]Exp. conditions for grinding : Additives : PCNa: 0.5 g, Polysorbate80: 2.75 g, PVP10: 3.0 g, d_B=φ1.0 mm, Grinding time: 24 h.

spectively.

The straight line indicates the regression line of all the data. This figure shows that the amorphization of insoluble drugs is very important for enhancing the bioavailability. It should be noted that we cannot discuss only the crystallinity effect without considering the size effect separately. It was confirmed that the amorphous state is important for enhancing bioavailability of insoluble drugs.

Table 2 shows a summary of typical experimental data for particle size distribution and apparent solubility of intact and ground products for poorly water-soluble drugs used in this work under experimental conditions as shown at the bottom. The increase of solubility of ground products is considered to be attributed to the morphological change of drug solid state by mechano-chemical effects during grinding process including the effect of particle size reduction.

2. Consideration of Basic Equations on Bioavailability

We have examined three basic equations to improve the bioavailability (BA) of insoluble drugs. One is for dissolution rate of the Noyes-Whitney equation; another is for the solubility of the Kelvin equation, and the last is for a fundamental thermodynamic equation of thermodynamic law. These equations indicate that various physical factors influencing BA have interactions with each other; the particle size of intact drugs is involved in these equations as the term that should be considered.

The dissolution rate of Noyes-Whitney is defined as follows:

$$\frac{dC}{dt} = \frac{DA}{hV}(C_s - C) \tag{1}$$

where D is diffusion coefficient, h thickness of diffusion layer, A surface area, V volume of media, Cs concentration of a saturated solution, and C drug concentration.

The solubility equation of Kelvin is expressed as follows:

$$\ln\frac{S}{S_o} = \frac{2\gamma V_m}{rRT} \tag{2}$$

where S_0 is the solubility of infinitely large particle, S is the solubility of small particle of radius r, γ is the surface tension, and V_m is the molar volume of the solid.

3. Theoretical Prediction of Intrinsic Bioavailability

In order to estimate the intrinsic bioavailability without a consideration of the particle size and the crystallinity of drug, we predicted the theoretical values of aqueous solubility, human intestinal absorption and blood-brain barrier (BBB) penetration based on the quantitative structure property relationship (QSPR) approach [14]. QSPR is one kind of computational methods that formulates the functional relationship between the target property and the molecular descriptors. Herein, the molecular descriptor quantitatively represents the molecular structure and the correlation is generally characterized by regression analysis, genetic algorithm [15] and artificial neural network [16].

All the calculations in this study were performed by ADME module in Cerius 2 Ver. 4.10 (Accelrys Inc., San Diego CA) [17]. Polar surface area and AlogP98 were used as the molecular descriptors for the calculation of human intestinal absorption and BBB penetration. For solubility prediction, hydrogen bonding donor, hydrogen bonding acceptor, rotatable bonds, AlogP98, Wiener index and so on were used as the descriptors. Multiple linear regression analysis and genetic algorithm were adapted to investigate the correlation of the target property with the descriptors.

The theoretical solubility of the two methodologies was calculated and compared to minimize the computational uncertainty. The one is developed by Accelrys Inc. [17] and the other is based on a Syracuse function [18]. In case of Syracuse solubility, the following regression equation was used [19]:

$$\log S\ (mol/L) = 0.796 - 0.854\ K_{ow} - 0.00728\ MW + Corrections \tag{3}$$

$$\log S\ (mol/L) = 0.693 - 0.96\ K_{ow} - 0.0092\ (T_m - 25) - 0.00314\ MW \tag{4}$$

where MW is molecular weight, T_m is melting point in °C, K_{ow} is log octanol-water partition coefficient and corrections are applied to 15 structure type descriptors. Eq. (4) was used when the measured melting point was available; otherwise Eq. (3) was used. The database of experimental log K_{ow} was searched and this value was used if a match was found; otherwise an estimated value was used. The solubility developed by Accelrys Inc. is based on the following equation [20]

$$\begin{aligned} \text{Log } S = &-0.7325\ \text{AlogP98} - 0.4985\ (\text{HBD}\times\text{HBA}) \\ &- 0.5172\ \text{Zagreb} - 0.0780\ \text{S\_aaaC} \\ &+ 0.1596\ \text{Rotlbonds} + 0.2057\ \text{HBD} \\ &+ 0.1834\ \text{S\_sOH} + 0.2539\ \text{Wiener} \end{aligned} \tag{5}$$

where AlogP98 is octanol-water partition coefficient, HBD is hydrogen bonding donor, HBA is hydrogen bonding acceptor, Zagreb is Zagreb Index, S_aaaC and S_sOH are electronic state key, Rotlbonds is the number of rotatable bonds and Wiener is Wiener Index. This model has a coefficient of determination, R^2=0.84 for the training set of 775 compounds. We also predicted the theoretical values of human intestinal absorption and blood-brain barrier (BBB) penetration based on quantitative structure property relationship (QSPR) approach; these are assessed in terms of where the compounds lie

Table 3. Predicted bioavailability: water solubility, human intestinal absorption and blood brain barrier penetration

Drug	Water solubility 1[a]	Water solubility 2[b]	Human intestinal absorption[c]	Blood brain barrier penetration[d]
UDCA	2.41	10^{-6}-10^{-4}	Good	0.3 : 1-1 : 1
Phenytoin	178.62	10^{-4}-10^{-2}	Good	0.3 : 1-1 : 1
DDB	1.59	10^{-6}-10^{-4}	Good	Almost 0 : 1

[a]This solubility was calculated by Syracuse function (at 25 deg. C in mg/L).
[b]This solubility was represented by the aqueous solubility, S_w, of Cerius 2 (at 25 deg. C in mol/L, pH=7.0).
[c]"Good" means that more than 90% of drug could be absorbed with 95% confidence level.
[d]BBB penetration was represented by the relative ratio of brain concentration vs. blood concentration.

in (the polar surface area)-(AlogP98) model space [14]. In this work, all the calculations were performed by ADME module in Cerius2 4.10 (Accelrys Inc., San Diego CA) [18].

Although these predicted properties in Table 3 do not consider the morphology and particle size of compounds, we could compare the potential bioavailability of our drugs. The aqueous solubility of phenytoin was estimated to be higher than that of UDCA and DDB, while UDCA and DDB might have comparable very low solubility from a comparison of the experimental solubility in Table 2. These differences might originate from computational uncertainty and no consideration of morphology and particle size. If the experimental solubility for these compounds was accumulated under various morphologies and particle sizes, a more reliable model for the prediction of the solubility could be formulated with a consideration of the accumulated data. In case of human intestinal absorption, all three compounds could be considered to be absorbed easily from intestinal lumen to the blood stream. It was also found that the BBB penetration of UDCA and phenytoin could not be easy and that of DDB seems to be difficult.

Fig. 3 shows a comparison between the calculated solubility based on Syracuse function and the experimental solubility of an intact drug. Though we did not present the comparison between the solubility 2 by Cerius 2 and the experimental solubility, this prediction also seriously underestimated the UDCA solubility. One of the reasons for this underestimation might originate from the overestimation of AlogP in equation of 5, because the calculated AlogP was 4.014 by Cerius 2 and the experimental logP was 3.0 [17,18].

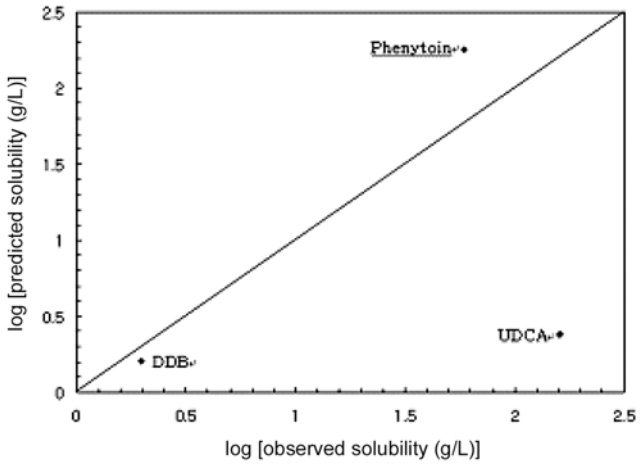

Fig. 3. Scatter plot for the comparison of the observed solubility and the calculated solubility.

It was also confirmed that the water solubility for DDB at 25 deg. C was also predicted to be 1.29 mg/L, e.g. 5.58e-8 mole fraction by the COSMO-RS calculation results [21]. In the calculation process, the morphology of the DDB in the crystal state is represented by the following data: specific heat of fusion of 85.3 J/g and melting temperature of 182-188 deg. C, which was measured with Perkinelmer PYRIS1 DSC (Norwalk, USA).

CONCLUSIONS

From a series of grinding experiments of poorly water-soluble drugs, UDCA, phenytoin and DDB, the following results were mainly obtained.

It was confirmed that the solubility data of ground samples could be improved by decreasing the particle size and the crystallinity of ground samples.

The prediction of the theoretical values of aqueous solubility, human intestinal absorption and blood-brain barrier (BBB) penetration based on quantitative structure property relationship (QSPR) approach was tried and examined in terms of usefulness on the particulate design of pharmaceutical powder.

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