Contribution of Glucose to Crystallization of Phenytoin in Injectable Dosage Form by Dilution with Infusion Fluids

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The crystallization of phenytoin occurring after its dilution with infusion fluid is a major concern in the clinical use of injectable phenytoin. To gain further understanding of the crystallization, this study assessed details of the involvement of glucose in this action. For sample preparation, phenytoin crystals were created by diluting the injectable phenytoin with infusion fluids with different glucose concentrations at different temperature, and then the characteristics of the crystallization (*e.g.*, crystal size in the long direction, accumulated amount over 24 h, and crystallization rate constant) were measured. Results of the analysis of variance indicated that the glucose concentration and temperature had significant impacts on the crystallization. The mode of action of the glucose concentration was suggested to be different from that of the incubation temperature. This study also examined the molecular mobility of components (*i.e.*, glucose, propylene glycol, phenytoin) in the admixtures using diffusion NMR techniques. The findings will provide valuable information for the clinical use of injectable phenytoin.

Key words phenytoin; crystallization characteristic; glucose infusion fluid; Johnson–Mehl–Avrami model; diffusion coefficient

Phenytoin sodium injection is indicated for the control of status epilepticus of the grand mal type and for the prevention and treatment of seizures that occur during neurosurgery. As phenytoin is a weak-acid drug (pK_a=8.3)[1] with low solubility at biological pH values,[2,3] phenytoin sodium injection is prepared using an organic cosolvent consisting of 40% (v/v) propylene glycol and 10% (v/v) ethanol, with the pH adjusted to 12.

In clinical practice, it is well known that the intravenous (i.v.) administration of phenytoin sodium injection should be conducted with care. In this regard, the following two points are important. First, the administration rate of the injection is strictly limited; the recommended rate of delivery of the injection should not exceed 50 mg/min,[4] as too rapid an i.v. push carries the risk of serious adverse effects on the cardiorespiratory system.[5–7] Second, although the continuous infusion is currently in great demand, diluting the phenytoin sodium injection with i.v. infusion fluid should be avoided. That is because there is a chance that crystallization of phenytoin is triggered by diluting the injection with infusion fluid. To resolve the crystallization problem, fosphenytoin sodium injection was recently released on the market.[8] Fosphenytoin is a phenytoin prodrug; it has higher water solubility than phenytoin, and its injection can be diluted with injection fluids for i.v. drip.

Concerning the cause of the crystallization in phenytoin sodium injection admixtures, it is widely accepted to be mostly due to the reduction in pH.[9,10] The pH of the admixture is reduced once the phenytoin sodium injection is diluted with an excessive amount of infusion fluids. Hence, the solubility threshold of phenytoin might be exceeded, resulting in the crystallization.

In addition, it is well known that the reduction in pH is not always the sole determinant of the crystallization. The influence of various factors other than the final pH of the admixture has been investigated. These factors include dilution of the phenytoin solubilizing agent by infusion fluids,[11–13] particulate matter present in infusion fluids,[13] time delay from the dilution,[12,14] and solvent evaporation and absorption of carbon dioxide when the solution is exposed to air.[15,16] Despite the best endeavors, there are still some unknown factors associated with the crystallization from phenytoin sodium injection occurring after mixing with infusion fluids. On the other hand, there are many studies indicating that the admixture diluted with saline is stable for many hours.[11,12,14,17,18] We also observed no crystallization from the admixtures as long as the injection was diluted with saline or water.[19]

In the previous study, we have investigated the crystallization that occurs upon mixing with glucose-containing infusion fluid.[19] The findings showed that the crystallization provoked by glucose-containing infusion fluid was obviously different from that caused by simply a reduction in pH value. If we can elucidate the mechanism of significant crystallization in a glucose-containing infusion fluid admixture, the findings might provide a major breakthrough for the establishment of the dilution regime of phenytoin infusion injection, without the crystallization problem. Notwithstanding the presence of fosphenytoin sodium injection on the market, a better understanding of the mechanism of crystallization will be advantageous for the continuous infusion of phenytoin. This is mainly because the phenytoin sodium injection is less expensive than fosphenytoin sodium injection, and it can be stored at room temperature. The aim of this study is to gain further understanding the contribution of glucose on the crystallization. First, the kinetics of the crystallization of phenytoin in the admixtures was investigated, for effects of glucose concentration and incubation temperature were identified. We also examined the molecular mobility of components in the admixtures by

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diffusion NMR technique.

Materials and Methods

Materials Phenytoin sodium injection (Aleviatin®) was purchased from Dainippon Sumitomo Pharma (Osaka, Japan). Saline (Otsuka normal saline) and 5% glucose-containing infusion fluid (Otsuka glucose injection 5%) were purchased from Otsuka Pharmaceutical Co. (Tokyo, Japan). Deuterium oxide (D, 99.9%), D-glucose, acetonitrile, hydrochloric acid (HCl), and sodium hydroxide (NaOH) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Propylene glycol (PG) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). All other reagents were of chemical grade.

Evaluation of Crystallization Characteristics of the Infusion Fluid Admixtures According to L4 orthogonal experimental design, phenytoin sodium injection was diluted with glucose-containing infusion fluids (Table 1). The test glucose-containing fluid comprised 4% or 20% glucose in purified water. The dilution rate was fixed at 4×. The glucose concentration of infusion fluid and the incubation temperature were selected as crucial factors. Afterwards, at designated intervals, phenytoin crystals in the admixtures were collected by vacuum filtration with a water aspirator. Quantitative determination of phenytoin crystals was performed using HPLC according to the protocol of Li *et al.*, with minor modifications.[20] Phenytoin crystals on the filtration paper were extracted with a designated volume of the mobile phase. The sample solution was injected into a Shimadzu LC-20AT HPLC pump equipped with a C18 reverse-phase column (YMC-pack A-302 S-5 A, 150×4.6 mm i.d.; Yamamura Chemical Laboratories, Kyoto, Japan). A Shimadzu SPA-20 A UV/VIS detector was set at 254 nm. An acetonitrile–0.1% phosphoric acid solution in water (50 : 50, v/v) was used as the mobile phase. The flow rate was 1.0 mL/min. HPLC analysis was performed at room temperature. LC Solution version 1.24 SP1 (Shimadzu, Kyoto, Japan) was used as the acquisition and analysis software. With regard to the samples incubated for 24 h, photographs of phenytoin crystals on the filter paper were taken using a polarizing microscope (CX41; Olympus, Tokyo, Japan). The size of phenytoin crystals in the long direction was measured.

Data Analysis Measurements of the characteristics of the crystallization (*e.g.*, crystal size in the long direction, accumulated amount over 24 h, and crystallization rate constant) were repeated three times. Analysis of the effects of glucose concentration and incubation temperature on each of the characteristics was carried out *via* multi-way ANOVA using JMP 9 (SAS Institute, Cary, NC, U.S.A.).

Diffusion-Ordered NMR Spectroscopy (DOSY) For sample preparation, the phenytoin sodium injection was diluted with 5% glucose in deuterium oxide (D_2O) at 4× dilution. The DOSY NMR spectra were recorded on a JNM-ECS 400 spectrometer (Jeol Resonance, Japan). Data were acquired and processed using Delta software, v5.0, with bipolar pulse pairs (BPP)–stimulated echo (STE)–longitudinal eddy current delay (LED) pulse sequence. A diffusion time Δ of 0.1 s, field gradient pulse δ of 3.5 ms, and relaxation time of 25 s were used at 25°C. The gradient strength g was varied in 16 steps, from 10 to 300 mT/m.

Results and Discussion

We first investigated the time-dependent crystallization of phenytoin sodium injection accompanied by dilution. In this study, we used aqueous HCl solution admixture to observe the crystallization caused by simple pH reduction. In a previous study, we observed no crystallization of phenytoin sodium injection after dilution with purified water at 4× dilution.[19] Four types of glucose-containing infusion fluid admixtures were prepared according to L4 orthogonal experimental design and then the effects of crucial factors on crystallization characteristics were evaluated (Table 1). The glucose concentration in the diluents and the incubation temperature were selected as crucial factors.

Table 1. L4 Orthogonal Experimental Design for the Preparation of Glucose-Containing Infusion Fluid Admixtures

	Glucose concentration (%)	Incubation temperature (°C)
Rp. 1	5	37
Rp. 2	5	4
Rp. 3	20	37
Rp. 4	20	4

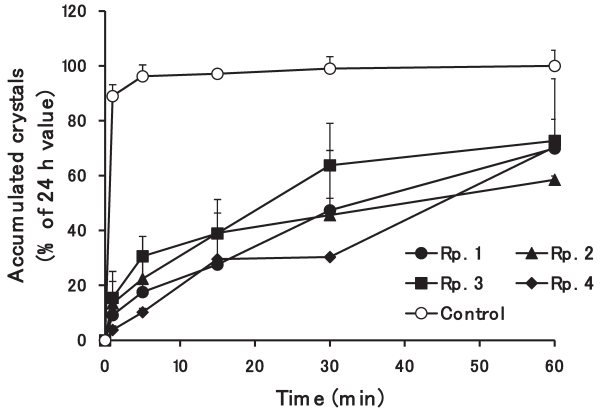

Fig. 1. Accumulation Curves of Phenytoin Crystals after Mixing with Infusion Fluids

Values are expressed as relative percentages to the values at 24 h. The dilution ratio of admixtures used was 4× (1 : 3 ratio of injectable phenytoin to dilution fluids). Phenytoin sodium injection diluted with aqueous HCl solution (11 mM) was used as control. We regarded the crystallization as being caused by simple pH reduction. Each value represents the mean±S.D. of three determinations.

Figure 1 shows the accumulation curves of phenytoin crystals after mixing with infusion fluids. The crystallization caused by simple pH reduction rapidly reached saturation point. By contrast, the crystals in a glucose-containing infusion fluid admixture formed and then grew gradually. Visible observation revealed a similar picture: the phenytoin sodium injection turned cloudy just after addition of the aqueous HCl solution, while it took about 30 min to perceive cloudiness after adding glucose-containing infusion fluids. This indicates that the effect of glucose addition was more moderate than the effect of pH reduction.

We next investigated the crystallization kinetics due to glucose-containing infusion fluid. The crystallization kinetic model of phenytoin can be quantified by application of the Johnson–Mehl–Avrami (JMA) model.[21–24] The JMA model can be used to describe the development of the relative degree of crystallization as a function of time according to the equation

$$\alpha(t) = 1 - \exp(-kt^n)$$

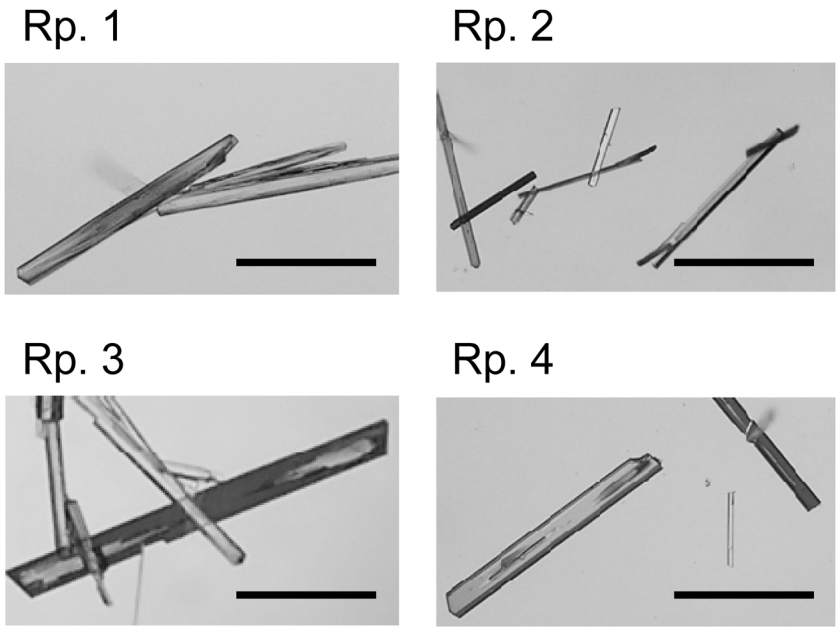

Fig. 2. Photographs of Phenytoin Crystals Taken Using a Polarizing Microscope

The phenytoin crystals were created according to the conditions listed in Table 1, and then were collected after 24 h incubation The scale bar shown represents 500 μm.

Table 2. Crystallization Characteristics of Glucose-Containing Infusion Admixtures Prepared According to L4 Orthogonal Experimental Design

	Crystal size in the long direction (μm)[a)]	Accumulated amount of phenytoin (mg)[b, c)]	k[c)]
Rp. 1	718.8±320.5	9.80±0.99	0.0191±0.0045
Rp. 2	299.4±132.7	17.54±0.61	0.0083±0.0036
Rp. 3	1414.5±540.0	27.59±0.17	0.0114±0.0234
Rp. 4	656.4±308.1	49.21±0.32	0.0130±0.0054

a) Twenty crystals were randomly selected from the measured and then the mean and S.D. were calculated. *b*) Test admixtures contained 88.43 mg of phenytoin. *c*) For the accumulated amount and k values, each value represents the mean±S.D. of three determinations.

Table 3. ANOVA Table for k

	Degrees of freedom	Mean square	Observed F value	p Value
A. Glucose concentration	1	6.90×10^{-6}	0.047	0.834
B. Incubation temperature	1	6.49×10^{-5}	0.438	0.525
A×B	1	1.16×10^{-4}	0.763	0.408

where $\alpha(t)$ is the relative crystallinity at the incubation time t, k is the crystallization rate constant, and n is the Avrami exponent. The Avrami exponent depends on the nucleation and growth mechanism and the number of dimensions, m, in which crystal growth occurs.[25)] According to a related article by Sinclair *et al.*, the equation describes a random nucleation and growth model in which transformations start at a particular site and continue by progressive propagation of the reaction interface.[25)] Various nucleation mechanisms are known, such as sporadic (random) nucleation and instantaneous nucleation. In sporadic nucleation (thermal), the nuclei are formed continuously (linearly) with first-order time dependence. In instantaneous nucleation (athermal), all the nuclei will appear at one time with zero-order time dependence. Crystal growth proceeds on the nuclei as soon as they are formed. The crystal growth dimension, m, is either one, two, or three, corresponding to rod-like (unidimensional), disk-like (bidimensional), or spherulitic (tridimensional) crystal habits, respectively.[25,26)] The reaction order, n, is equal to m for instantaneous nucleation or to m+1 for sporadic nucleation. Figure 2 shows the microscopic aspects of phenytoin crystals. There seemed to be no difference in terms of crystal shape; rod-like crystals were observed in all samples. Therefore, this study fixed n at "1" then calculated the crystallization rate constant, k.

The crystallization characteristics (*e.g.*, crystal size in the long direction, accumulated amount over 24 h, and k) are summarized in Table 2. ANOVA was performed on these variables to clarify the contributions of crucial factors to the characteristics (Tables 3–5). With regard to k, all values were similar (Table 2) and no significant effect of factors was observed (Table 3). In contrast, change in the factors significantly affected the crystal size and accumulated amount (Tables 4, 5). Because a higher F_o value represents a more potent effect of the factor, the relative magnitude of the contributions of the factors can be compared. As shown in Table 4, the F_o value for glucose concentration was higher than that for incubation

Table 4. ANOVA Table for Accumulated Amount of Crystals after 24 h Incubation

	Degrees of freedom	Mean square	Observed F value	p Value
A. Glucose concentration	1	1.83×10^3	4.96×10^3	$*p<0.001$
B. Incubation temperature	1	6.46×10^2	1.75×10^3	$*p<0.001$
A×B	1	1.44×10^2	3.91×10^2	$*p<0.001$

$*p<0.001$.

Table 5. ANOVA Table for Crystal Size

	Degrees of freedom	Mean square	Observed F value	p Value
A. Glucose concentration	1	5.54×10^6	43.7	$*p<0.001$
B. Incubation temperature	1	6.93×10^6	54.7	$*p<0.001$
A×B	1	5.74×10^5	4.53	0.04

$*p<0.001$.

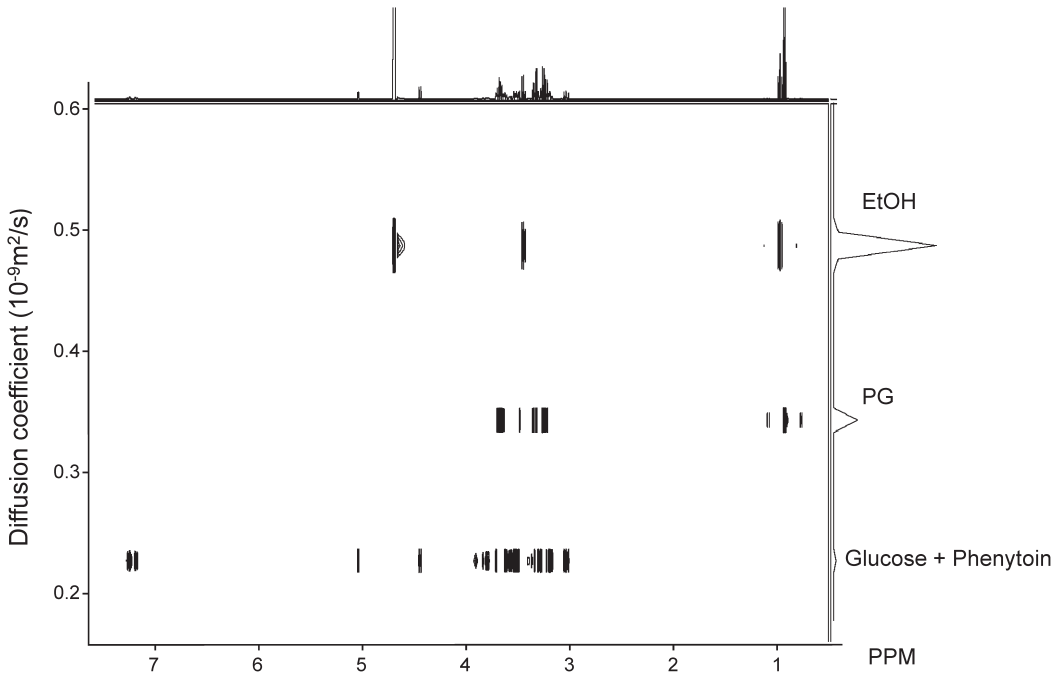

Fig. 3. 2D-DOSY Spectrum of Phenytoin Sodium Injection Diluted with 5% Glucose/Deuterium Oxide

The dilution ratio of admixtures used was 4× (1 : 3 ratio of injectable phenytoin to 5% glucose/deuterium oxide).

temperature; thus, glucose concentration had a greater impact on the accumulated amount. As for crystal size, the difference in the F_o values was not large, indicating that their contributions were comparable. Significant interaction between glucose concentration and incubation temperature was observed from the accumulated amount, while no significant interaction was observed from crystal size. Results indicated that the mode of action of glucose concentration on the crystal characteristics differed from that of the incubation temperature. For example, a higher glucose concentration and lower temperature resulted in a larger amount of crystals. A higher glucose concentration led to a larger crystal size, while a lower incubation temperature was associated with shorter crystals. One possible mechanism that could explain these findings involves a difference in the mode of nuclei generation. A reduction in incubation temperature enhances not only crystallization but also nuclei organization, while the glucose concentration hardly affects the nuclei organization. If the accumulated amount of crystals is the same, larger crystals should be created from a smaller number of nuclei; thus, longer crystals would be obtained from the glucose-containing infusion fluid admixtures.

The above experiments proved the significant effect of glucose on the crystallization of phenytoin. We next investigated the states of the components in the admixture using diffusion NMR. Figure 3 shows the two dimensional (2D)-DOSY spectrum of phenytoin sodium injection diluted with 5% glucose in D_2O (the net concentration of glucose in the admixture was 1.25%). The spectral data provides information on the diffusion coefficients of the compounds in the admixture as well as chemical shifts of their protons. It is evident that the diffusion coefficient of glucose was clearly different from the coefficients of the components of injectable phenytoin, including phenytoin, ethanol and PG. The rank order of diffusion coefficients was as follows: ethanol>PG>glucose and phenytoin. The order was in good agreement with the lower molecular weights (MWs); the MWs of ethanol, PG, glucose and pheny-

toin are 46.07, 76.10, 180.16 and 252.27. Furthermore, these three compounds showed a single peak, indicating that these molecules existed as a single state in the admixture.

From the nuclear Overhauser effect spectroscopy data obtained in our previous study, we suggested the possibility that glucose interacts with PG in the admixture.[13] However, the present diffusion NMR study did not provide positive results for such interaction.

This study clarified the unique and significant effect of glucose on the crystallization of phenytoin in infusion fluid admixtures. However, the detailed mechanism still requires elucidation. Further investigations are required to clarify this issue. Although, at present, it is still uncertain whether there is a dilution regime of phenytoin sodium injection without the crystallization problem, it is obvious that diluting the injection with glucose-containing infusion fluids should be avoided in the clinical practice.

Conclusion

This study investigated the kinetics of crystallization of phenytoin occurring after mixing its injection with infusion fluids. The results proved that the effect of glucose on the crystallization was obviously different from the other factors such as reduction in pH and temperature. This study will offer profound insight into the clinical practice of the phenytoin sodium injection.

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