Optimization study for the dilution of phenytoin sodium injection: safe administration in clinical practice

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Abstract

\textbf{Purpose:} The purpose of this study was to determine the optimum dilution of phenytoin sodium injection in isotonic sodium chloride that will prevent the precipitation of phenytoin. \textbf{Methods:} Solubility was predicted on the basis of pH measurements; the numbers of insoluble microparticles were measured using a light obscuration particle counter, and the phenytoin concentration was measured by HPLC. \textbf{Results:} Using the solubility based on pH measurement, it was predicted that precipitation would occur when the phenytoin sodium injection was diluted \textgeq 10.6-fold. The number of insoluble microparticles with a diameter of \textgeq 10 \textmu m in 20-fold diluted solutions was significantly greater than in solutions diluted 4- and 10-fold, which supported with the theoretical prediction of solubility based on pH measurement. The decrease of phenytoin content due to precipitation was evaluated by measuring the actual concentration of phenytoin in the diluted solutions. The concentration of phenytoin decreased to less than 95\% in 20-fold dilutions, while the concentration after 24 h was 95\% or more in 10-fold dilutions. The number of insoluble microparticles with a diameter of \textgeq 10 \textmu m in 20-fold dilutions was decreased significantly by passing through 5-\mu m or 0.2-\mu m in-line filters. There was no significant difference between the two pore sizes. \textbf{Conclusion:} These results suggest that it is clinically safe and economically beneficial to administer phenytoin sodium injection diluted 10-fold with isotonic sodium chloride solution and passed by drop infusion through a 5-\mu m in-line filter.

\textbf{Keywords:} Phenytoin; Optimum dilution; Solubility; Precipitation; In-line filter

1. Introduction

Phenytoin sodium injection is the only commercially available antiepileptic injection in Japan. The active ingredient, phenytoin, is an acidic drug with low water solubility, which is solubilized as a sodium salt by raising the pH and adding propylene glycol and ethanol [1]. However, incompatibility with other drug products is often a problem, as the pH of the solution is very high, usually about 12. Each 5-ml ampoule contains 250 mg phenytoin sodium. If the drug is administered too rapidly, circulatory and respiratory disturbances may result, ultimately leading to cardiac arrest and respiratory depression [1]. Therefore, it is recommended to keep the rate of intravenous administration at or under 1 ml/min.

In other words, when 2.5–5 ml (a normal adult dose) is administered, it is necessary to administer the dose over at least 2.5–5 min. Under normal clinical circumstances, the drug is administered as an intravenous drip, by diluting one ampoule in 100 ml isotonic sodium chloride solution.

There is one previous report that precipitation does not occur under these conditions, although precipitation does occur when the phenytoin sodium is diluted in 100 ml of a 5\% (w/v) glucose solution and in isotonic sodium chloride solutions of \textgeq 100 ml [2]. It has also been reported that incompatibility is not observed until 1 h after 4-fold dilution with isotonic sodium chloride solution or distilled water [1]. However, these reports have not been verified.

In addition, serious clinical problems have been reported when precipitates in the solution, caused by incompatibility, have caused venous blockages [3-6]. In-line filters with a pore size of 0.2 \textmu m are expensive, and usually only used to administer infusion solutions.
into central veins, although the use of an in-line filter is always recommended [7].

In this study, the optimum dilution of phenytoin sodium injection in isotonic sodium chloride to prevent the precipitation of phenytoin in infusion solutions was determined. Solubility was predicted on the basis of pH measurements. The method of administration was examined from the aspects of clinical safety and cost, by determining the phenytoin concentration in the infusion solution by HPLC and by measuring the number of insoluble microparticles in solutions gathered via drop infusion sets with different pore size filters using a light obscuration particle counter.

2. Materials and methods

2.1. Materials

Aleviatin® for injection containing 250 mg phenytoin sodium (Lot No. 1007C, Dainippon Sumitomo Pharma Co., Ltd, Oosaka, Japan), and isotonic sodium chloride solution 100ml (Lot No. 070625TA, Terumo Co., Ltd. Tokyo, Japan) were purchased for use in this study. All other reagents were of special grade.

An intravenous infusion set with no in-line filter (IAN-201E00, Lot No. 07F23A), an infusion set with an in-line filter of 5-μm pore size (Micro Check®, Lot No. 07D06B), and an infusion set with an in-line filter of 0.2-μm pore size (Lot No. 07G03) were kindly donated by Nipro Co. Ltd. (Osaka).

2.2. Preparation of sample

Sample solutions were prepared using a silicon-free syringe on a clean bench, and stored at 25°C. Sample solutions for the measurement of insoluble microparticles were made by putting 0.5 ml of phenytoin sodium injection (50 mg/ml) in a glass sample bottle and adding 1.5, 4.5, or 9.5 ml of isotonic sodium chloride solution, to make 4-, 10-, and 20-fold dilutions. Other samples were made by injecting 5 or 10 ml of phenytoin sodium injection (50 mg/ml) into an infusion bag containing 100 ml isotonic sodium chloride.

2.3. Measurement of insoluble microparticles using a light obscuration particle counter

The method employed was essentially same as the ‘Insoluble particulate matter test for injection by method 1. Light obscuration particle count test’ described in the 15th edition of the Japanese Pharmacopoeia (2006) [8].

All procedures were carried out on a clean bench. The number and size of microparticles were determined using a light obscuration particle counter KL-04 (Rion Co., Ltd). The thresholds of microparticle size were 1.3, 2.0, 5.0, 10.0, 20.0, 25.0, 40.0, 50.0, and 100.0 μm. The volume of each sample was 2 ml for the 4-fold dilution, 5 ml for the 10-fold dilution, and 10 ml for the 20-fold dilution. The first sample was discarded and the mean value of three samples was calculated. All instruments were washed with water for injection to eliminate insoluble microparticles derived from the devices. Gas bubbles in the sample solutions were eliminated by standing for 2 min.

The number of insoluble microparticles in the diluted solutions was measured immediately after dilution and 3, 6, or 24 h later; for the evaluation of the fluid infusion sets with different filters, the number of insoluble microparticles was measured immediately after dilution.

The results for the evaluation of the fluid infusion sets with different filters were evaluated according to the criteria for the maximum allowable number of insoluble microparticles in the 15th edition of the Japanese Pharmacopoeia. For injection preparations administered at a volume over 100 ml, the tolerated number of insoluble microparticles with a diameter 10 μm or greater is 25 or less, while that of microparticles with a diameter 25 μm or greater is 3 or less, per ml.

2.4. Phenytoin determination by HPLC analysis

The method employed was essentially same as that described in the 15th edition of the Japanese Pharmacopoeia (2006) [9]. A 0.5 ml sample solution was prepared by ultrafiltration, and concentrations of phenytoin in the filtered solution were determined using HPLC. For HPLC, 10 μl was injected onto a chromatograph
(Shimadzu LC-10AT, Kyoto, Japan) equipped with a UV detector (Shimadzu SPD-10A, Kyoto, Japan), an integrator (Shimadzu C-R7Ae plus, Kyoto, Japan) and reverse-phase column (Capcell Pak C18 UG120 S5: 4.6 mm × 250 mm, Shiseido, Tokyo, Japan). The column temperature was 40˚C. The mobile phase consisted of 50 mmol/l Na₂HPO₄ + 50 mmol/l KH₂PO₄, pH 6.8/CH₃CN (70/30), at a flow rate of 1 ml/min. The wavelength was set at 254 nm.

The concentrations of phenytoin in the sample solutions were determined immediately after dilution and 3, 6, and 24 h later.

2.5. Measurement of pH

Five ml of phenytoin sodium injection (50 mg/ml) was put into a glass beaker and isotonic sodium chloride solution was added ml by ml until the total volume was 105 ml. The sample solution was stirred constantly and the pH measured at intervals using a pH meter (Horiba, F-21, Kyoto, Japan). The volume of sodium chloride solution added was recorded each time the pH was measured.

2.6. Observation by stereomicroscope

Small samples were taken from an infusion bag containing 5 ml of phenytoin sodium injection (50 mg/ml) in 100 ml isotonic sodium chloride solution, 3, 6, and 24 h after preparation. The samples were observed under a stereomicroscope (Olympus Ltd. SZX10).

2.7. Evaluation of fluid infusion sets with different filters

Five ml of phenytoin sodium injection (50 mg/ml) was injected into 100 ml of isotonic sodium chloride solution in an infusion bag using a silicon-free syringe on a clean bench. The infusion fluid was allowed to drip by gravity at a flow rate of 15 ml/min through three different infusion sets (one with no in-line filter, one with an in-line filter of 5 μm pore size, and one with an in-line filter of 0.2 μm pore size). The infusion fluid was collected through the outlets in particle-free glass beakers (10 ml) immediately after preparation, and the number of insoluble microparticles in each fluid measured according to the method described in section 2.3. above. The flow rate was assumed to be 15 ml/min when the 0.2-μm filter set was completely open; phenytoin would be likely to be precipitated immediately if the pH was reduced [1] by carbon dioxide in the air on sampling. The first 20 ml was discarded to remove the influence of insoluble microparticles derived from the infusion set [10].

2.8. Statistical analysis

The numbers of insoluble microparticles and the concentrations of phenytoin are given as the mean of three values, plus or minus the standard deviation. The numbers of insoluble microparticles in the dilute solutions were analyzed by two-way repeated ANOVA followed by Tukey’s HSD test. The numbers of insoluble microparticles obtained in the evaluation of the three fluid infusion sets were analyzed by the Tukey–Kramer test; statistical significance was accepted at the P<0.05 or P<0.01 level.

3. Results and discussion

3.1. The theoretical prediction of solubility based on pH measurement

Fig. 1 shows the results of pH measurement and the theoretical prediction value of solubility based on pH measurement when the phenytoin sodium injection was slowly diluted with isotonic sodium chloride solution. The theoretical prediction value of the solubility was calculated from the development equation (Equation 1) of the theoretical equation of Asahara and Uno’s pH characteristic curve (Equation 2) [11, 12], using the approximate analytical Newton Rapson method:

\[
\begin{align*}
[H^+]^3 - (C_i-K_s)[H^+]^2 - (C_iK_s + C_iK_a + C_s)[H^+] - K_aK_sC_s &= 0 \quad (1) \\
[H^+] &= \frac{C_iK_s}{[H^+] + K_s} + \frac{K_s}{[H^+]} - C_s \quad (2)
\end{align*}
\]

where \( C_s \) is the concentration of a weakly acidic drug, \( K_s \) is the dissociation constant of a weakly acidic
drug, $K_a$ is the ionic product of water, and $C_i$ is the concentration of a strong base, respectively.

The solid curve (A) represents the experimental value and the broken curve (B) represents the predicted value when the commercial phenytoin sodium added sodium hydroxide as a pH adjustment was diluted with isotonic sodium chloride solution. A good correlation was observed between the measured and predicted values, but there were some discrepancies, thought to be due to the pKa shift [13] which accompanied the addition of cosolvents in the first stage of dilution, and the carbon dioxide produced in the sample, as well as the ion strength, in the later stages of dilution. The solubility of each solution was then predicted.

The solubility of a weakly acidic drug at a particular pH is given by:

$$S = S_w (1 + 10^{pH-pKa})$$

where $S$ is the solubility of a weakly acidic drug, and $S_w$ is the solubility of the drug in water.

Cosolvents, such as ethanol and propylene glycol, are used to enhance the solubility of the phenytoin sodium injection. Rubino, Blanchard et al. [14] reported that the solubility of phenytoin in ternary mixtures of cosolvent and water is described mathematically by:

$$\log \left( \frac{S}{S_w} \right) = \sum_{i=1}^{n} (\sigma f_i)$$

where, $S_w$ is the solubility of drug in the solvent mixture, $S$ is the solubility of drug in water, $\sigma_i$ is the solubilization power of the cosolvent for the drug, and $f_i$ is the volume fraction of cosolvent. In an earlier study, Millard et al. [15] combined experimental and literature values to derive regression equations of the solubilization power ($\sigma$) for each cosolvent vs. the log partition coefficient ($\log K_{ow}$). The log partition coefficient of phenytoin was applied to these regressions, and the calculated $\sigma$ for ethanol was 2.70, and for propylene glycol 2.48. The solubility was predicted from these values and the experimental pH value. $S_w$ is 0.0152 mg/ml (from the Merck Index), and pKa is 8.33 [13]. Fig. 2 shows the concentration of phenytoin in the solution and the predicted solubility result. The X-axis shows the formation fractions (ff) and the Y-axis shows the concentration or the solubility of phenytoin in the solution. The broken curve (A) represents the theoretical concentration of phenytoin in the solution, while the solid curve (B) represents the predicted value of the solubility including the cosolvent effects of ethanol and propylene glycol. Considering the cosolvent effects of ethanol and propylene glycol, the concentration of phenytoin in the solution exceeded the solubility by ff=0.09 (corresponding with a 10.6-fold dilution). In other words, the possibility of precipitation is present at a dilution of ≥10.6-fold.
3.2. The time-dependent change of insoluble microparticles in the diluted solution

Solutions of phenytoin sodium injection (50 mg/ml), diluted 4-, 10-, and 20-fold by isotonic sodium chloride solution, were stored at 25°C. The number of insoluble microparticles in each solution was measured immediately after mixing and 3, 6 and 24 h later. Table 1 shows the number of insoluble microparticles with a diameter of ≥10 μm and ≥25 μm per 2 ml in the 4-fold dilution, per 5 ml in the 10-fold dilution, and per 10 ml in the 20-fold dilution. The number of insoluble microparticles with a diameter ≥10 μm in the 20-fold dilution was significantly greater than the number in the other dilutions. There was no significant difference between the numbers of insoluble microparticles in the 4- and 10-fold dilutions. The number of insoluble microparticles in the 4-fold dilution had decreased by 6 h, but increased again after 24 h, while the number in the 10- and 20-fold dilutions had decreased by 3 h, but increased again by 6 h. The number of insoluble microparticles with a diameter ≥25 μm was not significantly affected by the degree of dilution. The pHs of the 4-, 10- and 20-fold dilutions were 10.72, 10.24, and 9.97, respectively. The pH hardly changed over the 24-h period. It should be noted that the pHs of the 10-, and 20-fold dilutions were both below 10.71, the pH at which the precipitation of phenytoin occurs when 0.65 ml of 0.1 M hydrochloric acid is added to 5 ml of phenytoin sodium injection [1], while no precipitation could be seen with the naked eye.

The most insoluble microparticles were found in the 20-fold dilution because the precipitation of phenytoin occurs predominantly at lower pHs. This corresponded with the theoretical prediction of solubility based on pH measurements which showed that the precipitation of phenytoin occurred when the dilution was ≥10.6-fold. These results suggest that incompatibility in the injection medicine, which is difficult to determine with the naked eye, may be evaluated using a light obscuration particle counter. The number of insoluble microparticles immediately after dilution was greater than the number 3 h after sample preparation. The main reason for this seems to be gas bubbles remaining in the sample solution. While an attempt was made to eliminate gas bubbles in the sample solutions by standing for 2 min, this would not eliminate all gas bubbles, which would require standing for 30–60 min [16]. Ethanol and propylene glycol disturb the movement of the film at the interface between gas and liquid due to an increase in viscosity on the surface of the bubble caused by the hydrophobic group (–CH₂), thus increasing the stability of the gas bubbles [17]. Therefore, the greater number of insoluble microparticles immediately after dilution might be due to the presence of gas bubbles.

These insoluble microparticle measurements suggested that the use of 4- or 10-fold dilutions, which had a lower number of insoluble microparticles than the 20-fold dilution, was safer, and that it is preferable to administer the solution in the first 3 h after dilution.


**Table 1**

Number of insoluble microparticles with a diameter of ≥10 μm and ≥25 μm per 2 ml in the 4-fold dilution, per 5 ml in the 10-fold dilution, and per 10 ml in the 20-fold dilution, which phenytoin sodium injection diluted with isotonic sodium chloride as measured in a light obscuration particle counter.

<table>
<thead>
<tr>
<th>Particle size</th>
<th>Dilution ratio</th>
<th>0 h</th>
<th>3 h</th>
<th>6 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥10 μm</td>
<td>× 4</td>
<td>49.33 ± 13.58</td>
<td>17.00 ± 2.00</td>
<td>13.67 ± 6.51</td>
<td>56.00 ± 15.13</td>
</tr>
<tr>
<td></td>
<td>× 10</td>
<td>46.00 ± 9.54</td>
<td>22.33 ± 5.13</td>
<td>28.67 ± 7.23</td>
<td>150.00 ± 63.64</td>
</tr>
<tr>
<td></td>
<td>× 20</td>
<td>106.67 ± 29.14</td>
<td>63.00 ± 28.48</td>
<td>76.67 ± 29.96</td>
<td>204.50 ± 84.15</td>
</tr>
<tr>
<td>≥25 μm</td>
<td>× 4</td>
<td>6.00 ± 6.24</td>
<td>0.67 ± 1.15</td>
<td>0.33 ± 0.58</td>
<td>4.33 ± 2.08</td>
</tr>
<tr>
<td></td>
<td>× 10</td>
<td>5.33 ± 3.06</td>
<td>0.67 ± 0.58</td>
<td>0.67 ± 0.58</td>
<td>3.00 ± 1.36</td>
</tr>
<tr>
<td></td>
<td>× 20</td>
<td>5.00 ± 2.00</td>
<td>1.00 ± 1.00</td>
<td>2.33 ± 1.53</td>
<td>2.00 ± 1.00</td>
</tr>
</tbody>
</table>

* * P<0.05; ** P<0.01
3.3. Visual observation using a stereomicroscope

Precipitation in the solution for injection was just visible under a stereomicroscope 3 h after mixing, although no precipitation could be seen in the infusion bag with the naked eye. The precipitation increased in a time-dependent manner (Fig. 3). These results suggest that dilute solutions should be administered immediately after dilution.

3.4. Phenytoin determination by HPLC analysis

Table 2 shows the results of phenytoin determinations using HPLC. The concentrations of phenytoin in the 10-fold dilutions were still 95% or more 24 h after dilution, while those of the 20-fold dilutions were still 95% or more 6 h after dilution. In the more concentrated solutions, the decrease in phenytoin concentrations was less, as the degree of precipitation remained under 5% for longer. These results suggest that more dilute solutions may still be used if precipitated microparticles are removed using a filter in advance.

3.5. Evaluation of fluid infusion sets with in-line filters

The results for measuring insoluble microparticles suggest that so many microparticles exist in diluted solution. And from the results of phenytoin determination using HPLC, the decrease in phenytoin concentrations was less, as the degree of precipitation remained under 5% for longer. These results suggest that more dilute solutions may still be used if precipitated microparticles are removed using a filter in advance. However, the infusion set with a 0.2-μm filter is very expensive, therefore the number of insoluble microparticles with a diameter of ≥10 μm and/or ≥25 μm after passing through each filter was measured to evaluate the infusion set with a 5-μm filter which was developed for peripheral intravenous drip.

Five ml of phenytoin sodium injection (50 mg/ml) was injected into 100 ml of isotonic sodium chloride solution in an infusion bag using a silicon-free syringe on a clean bench. The infusion fluid was allowed to drip through three different infusion sets: one with no in-line filter, one with an in-line filter of 5-μm pore size, and one with an in-line filter of 0.2-μm pore size. The infusion fluid was collected into a particle-free 10-ml glass tubes immediately after preparation, and the number of insoluble microparticles was measured according to the method described in the materials and methods, section 2.3. Fig. 4 shows the results of the measurement of insoluble microparticles with a diameter of ≥10 μm in each solution. The number of insoluble microparticles
with a diameter of ≥10 μm in the 20-fold dilution was decreased significantly by passing through a 5- or 0.2-μm filter. There was no significant difference between the two pore sizes. Insoluble microparticles with a diameter of ≥25 μm were not detected in this experiment.

The cost of the infusion set with a 5-μm filter in Japan is relatively low, being 1.7 times the price of the no-filter infusion set and 0.2 times that of the infusion set with a 0.2-μm filter. The 5-μm filter removes the insoluble microparticles while keeping the desired flow rate. Therefore, the use of this piece of medical equipment is both safe and practical.

4. Conclusion

There is no significant difference between the number of insoluble microparticles in the 4-fold dilution (written in the Drug Information Sheet) and the 10-fold dilution. Administration of a 10-fold dilution for 5 min or more is the best practical way to avoid circulatory and respiratory disturbances caused by rapid intravenous infusion.

As precipitation of phenytoin may occur when the pH of the phenytoin sodium injection is lower, the use of an infusion set with a 5-μm filter is recommended for administration of dilute solutions of phenytoin sodium injection.

References