Compatibility of Heparin Sodium in Intravenous Line with Nicardipine Injected from a Side Tube via T-shape Stopcock

Takafumi Hayashi, Koji Uwai, Eri Sasaki, Keisuke Sasaki, Yasushi Iwabuchi, Hitoshi Agatsuma, and Tsuneyoshi Suzuki

Laboratory of Pharmaceutical Sciences, Tohoku Pharmaceutical University, Department of Applied Chemistry, Muroran Institute of Technology, Friend Pharmacy Ishinomaki, Department of Pharmacy, Ishinomaki Red Cross Hospital

received February 28, 2012 accepted September 2, 2012

The compatibility caused by injection of Nicarpine® 10 mg / 10 mL (Nicarpine®) from a side tube into an intravenous line infused with Replas® 1 Injection (Replas® 1) and Novo-Heparin® 10,000 units / 10 mL for Injection (Novo-Heparin®) was investigated. We observed a mixed solution of each constituent of Replas® 1 and Novo-Heparin® with Nicarpine®, and measured the IR spectrum of the precipitate and the 1H-NMR spectrum of a saturated solution of the mixture of heparin and nicardipine. Our findings suggested that the compatibility was caused by an intermolecular interaction between heparin and nicardipine when in a 1:2 ratio via the formation of a salt between the sulfate group and/or the carboxylic group of heparin and the amino group of nicardipine.

Key words — heparin sodium, nicardipine, compatibility, injection via T-shape stopcock, infusion

Introduction

Injectable drugs are prepared on the premise of administration as a single item. However, in therapeutically needed cases, to reduce pain or for efficient practice, several injections are routinely mixed, especially with infusion solution or fluid replacement, and then administered in a realistic medical setting. Mixed injections could, in theory, be more physically or chemically unstable than individual components. Thus, drug compatibilities associated with the coloration, precipitation or decrease of a titer, or increase of side effects may occur. Such drug compatibilities would not only be of great disadvantage for patients, such as the direct injection of unknown substances, but could also cause economic losses to medical institutions through waste of compatible injections. Therefore, it is very important to prevent drug compatibilities.

Medical staff are required to understand the features of individual injectable drugs and their compatibilities. A manual on the operating procedure for the safe use of pharmaceutical preparations by the Research of Health, Welfare and Labor Sciences in 2006 includes a statement that indicates that the stability, incompatibility and compatibility of a drug should be confirmed, in the section related to the dispensing of injectable drugs, and that pharmacists should actively provide medical staff with information about such incompatibility, how to mix, the procedure for mixing, and procedures for administration in the section related to passing the prescription onto a

* 27-1 Mizumoto-cho, Muroran-shi, Hokkaido, 050-8585 Japan
medical ward. Thus, for proper use of pharmaceutical preparations, pharmacists, who have knowledge of physics and chemistry, are required to be actively involved with issues related to drug compatibility.

The injection of Nicarpine® 10 mg / 10 mL (Nicarpine®) from a side tube to an intravenous line infused with Replas® 1 Injection (Replas® 1) and Novo-Heparin® 10,000 units / 10 mL for Injection (Novo-Heparin®) is frequently performed. However, it has recently been found that white turbidity in the intravenous line and syringe is occasionally observed at the bedside. In the Interview Form of Nicarpine®, the combination of heparin sodium and nicardipine is absolutely incompatible because the prescription is formed shortly after mixing, but in this case, it is formed in a mixture of undiluted solution of Nicarpine® and heparin sodium (5,000 U / 5 mL). On the other hand, cases have been reported in which heparin sodium is diluted with an infusion solution. Consequently, it is insufficient to consider this case within the information contained in the Interview Form.

In this study, to provide information to better evaluate whether this intravenous system is suitable for operation from the perspective of a pharmacist, the scientific basis of this compatibility and the structure of the precipitate were chemically investigated.

Materials and Methods

Novo-Heparin® 10,000 units / 10 mL for Injection (Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) was used as the heparin sodium preparation. Nicarpine® 10 mg / 10 mL (Sawai Pharmaceutical Co., Ltd., Osaka, Japan) was used as a nicardipine hydrochloride preparation. Other reagents used include Replas® 1 Injection (Fuso Pharmaceutical Industries, Ltd., Osaka, Japan), nicardipine hydrochloride, hydrochloric acid, sodium lactate solution (about 70%), D-glucose, heparin sodium ( > 130 U/mg), deuterium oxide, potassium bromide, sodium 3-(trimethylsilyl)-1-propanesulfonate, 25% ammonia solution, chloroform, methanol (the last 11 reagents, Wako Pure Chemical Industries, Ltd., Osaka, Japan), heparin sodium 204 U/mg (Nacalai Tesque, Inc., Kyoto, Japan), chondroitin sulfate sodium salt (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), and chloroform-d (Cambridge Isotope Laboratories, Inc., MA, USA).

1. Identification of the constituents causing the compatibility in the infusion solution

1.1 Reproducing the compatibility in the same manner by putting in practice in a medical setting

In accordance with common procedures, the infusion system currently in place was replicated (Fig. 1). A mixed solution of Replas® 1 (500 mL) and Novo-Heparin® (10 mL), as an infusion solution, was passed into an intravenous line by an in-
fusion pump (Terufusion® Infusion Pump TE-161SA, Terumo Corporation, Tokyo, Japan) at 20 mL/h, and Nicarpine® (50 mL) was introduced into the line via a T-shape stopcock by a syringe pump (Terufusion® Syringe Pump TE-331S0N, Terumo Corporation, Tokyo, Japan) at 8 mL/h. Then, the appearance of the solution in syringe and line was observed. Alternatively, the line was forcefully obstructed downstream of the T-shape stopcock.

1.2 Observed changes by mixing Nicarpine® with individual constituent (Replas® 1 and Novo-Heparin®)

Individual constituent of the mixed solution of Replas® 1 (d-glucose: 13,000 g, NaCl: 2.070 g, sodium lactate: 1.120 g in 500 mL) and Novo-Heparin® (the concentrations of the prepared constituents in aqueous solution were adjusted with the mixed solution) was dripped continuously as 20 µL doses into Nicarpine® (50 mL), and the appearance was observed. As a pH adjuster, hydrochloric acid was diluted to pH 5.15 using a pH meter (D-51, Horiba Ltd., Kyoto, Japan). Novo-Heparin® was used to the diluted solution and made up to 500 mL with distilled water.

1.3 Observed and pH changes by the drip volume of Novo-Heparin® into Nicarpine®

Novo-Heparin® was added to a stirred Nicarpine® (2 mL) by 20 µL. The appearance of the solution was observed and pH of the solution was measured every addition of 20 µL of Novo-Heparin®.

2. Characterization of the precipitates formed by the compatibility

2.1 IR spectra of the precipitate formed by the compatibility

An aqueous solution of heparin sodium (10 mL, 1,000 U/mL) was added to an aqueous solution of nicardipine hydrochloride (10 mL, 5 mg/mL). The resulting suspension was centrifuged at 1,870 g (10 min., 4 °C). The supernatant was decanted off. IR spectra of the collected precipitate (59.0 mg), nicardipine hydrochloride and heparin sodium were measured with a Fourier transform infrared spectrophotometer (1725X, Perkin Elmer Inc., MA, USA) in KBr plates.

2.2 1H-NMR spectra of the saturated solution of the mixture of heparin sodium and nicardipine hydrochloride

A 1:1 (v/v) mixture of a solution heparin sodium (2 mL, 100 mg/mL) and a solution of nicardipine hydrochloride in D2O (2 mL, 5 mg/mL) was centrifuged at 1,870 g (10 min., 4 °C), and the supernatant was filtered through a membrane filter (0.45 µm, Millex®-HA, Millipore Co., MA, USA). 1H-NMR spectra of the filtrate, nicardipine hydrochloride, and heparin sodium were measured with JNM-LA600 (JEOL Ltd., Tokyo, Japan) with DSS as the external standard.

2.3 Compatibility between chondroitin sulfate and nicardipine

An aqueous solution of heparin sodium (500 mL, 20 U/mL) was dripped into a stirred aqueous solution of nicardipine (2 mL, 1 mg/mL) with a syringe pump at a rate of 40 µL/min. The absorbance value of the solution at 600 nm was measured by the addition of each 20 µL of heparin solution. Alternatively, an aqueous solution of chondroitin sulfate (13 mL, 77 µg/mL) was dripped into a stirred aqueous solution of nicardipine hydrochloride (2 mL, 1 mg/mL) with a syringe pump at a rate of 40 µL/min. The absorbance value of the solution at 600 nm was measured by the addition of each 20 µL of chondroitin sulfate solution.

2.4 Quantitative determination of the nicardipine content in the precipitate formed by the compatibility
An aqueous solution of heparin sodium (2 mL, 100 mg/mL) was added to an aqueous solution of nicardipine hydrochloride (10 mL, 5 mg/mL). The resulting suspension was centrifuged at 1,870 g (10 min., 4 °C), and the precipitate was dried under vacuum. The resulting white solid was dissolved in a 5% aqueous solution of ammonia (10 mL), and successively, extracted with CHCl₃ (10 mL). The organic layer was desiccated over Na₂SO₄ and concentrated in vacuo to give nicardipine.

## Results

### 1. Identification of the constituents causing the compatibility in the infusion solution

1.1 Reproducing the compatibility in the same manner by putting into practice in a medical setting

No changes were observed in the control injection system. However, when the line was forcefully obstructed downstream of the T-shape stopcock, white turbidity was observed in the upper part of the syringe (Table 1).

Furthermore, by repeating the obstruction, subsequent alarm activation, and successive relief of obstruction, white turbidity was observed in the whole of the inner side of the syringe, and gradually the precipitates were observed. Finally, precipitates were also observed in the intravenous line.

1.2 Observation of changes after mixing Nicarpine® with individual constituent (Replas® 1 and Novo-Heparin®)

When the individual constituents of Replas® 1 were added to Nicarpine®, no white turbidity or precipitate was observed. On the other hand, when Novo-Heparin® was added, white turbidity was observed (Table 2).

1.3 Observed and pH changes by the drip volume of Novo-Heparin® into Nicarpine®

Considering the relationship between the drip volume of Novo-Heparin®, the pH of the solution, and the formation of precipitates, minute precipitates were observed after 80 µL was dripped (pH 3.80). In addition, precipitates were observed in the whole drip solution at 100 µL (pH 3.82) (Table 3).

### 2. Characterization of the precipitates formed by the compatibility

2.1 IR spectra of the precipitate formed by the compatibility

Comparing the IR spectrum of the precipitate (Fig. 2 solid line) with that of nicardipine hydrochloride (Fig. 2 dotted line) and heparin sodium (Fig. 2 dashed line), spectral changes were observed in the area between 1220, 1440, 1600 and 2200-2700 cm⁻¹.

### Table 1

<table>
<thead>
<tr>
<th>Flow volume (mL)</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>front side</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>lateral side</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
</tbody>
</table>
2.2 $^1$H-NMR spectra of the supernatant of the solution that formed a precipitate

Comparing the $^1$H-NMR spectrum of the supernatant of the centrifuged liquid of the suspension (Fig. 3A) with that of nicardipine (Fig. 3B) and heparin sodium (Fig. 3C), signals in the supernatant derived from nicardipine (the encircled part in Fig. 3A) shifted to a lower field than those of nicardipine (Fig. 3B).

2.3 Compatibility between chondroitin sulfate and nicardipine

No change in the appearance of a precipitate was observed after the addition of chondroitin sulfate to nicardipine even if the addition of equivalent moles of heparin sodium of precipitate formation.

2.4 Quantitative determination of nicardipine hydrochloride content in the precipitate formed by the compatibility

$^1$H-NMR spectra of the CHCl$_3$-extracted compounds from the alkalified aqueous solution of the precipitate (Fig. 4) were completely consistent with those of nicardipine.

Considering the extraction ratio of the precipitate to the residue of the CHCl$_3$-extracted layer (Table 4), it was assumed that the precipitate was composed of heparin and nicardipine in the 1:2 ratio.

### Discussion

Recently, it was found at the bed side that white turbidity formed in the intravenous line and syringe – a rare observation – in a system in which Nicarpine® was injected from a side tube by a syringe pump at 8 mL/min to an intravenous line of 500 mL of Replas® 1 and 10 mL of Novo-Heparin® flowing by an infusion at 20 mL/min. It was inferred that these cases were caused by compatibility. No turbidity was observed in the line and syringe in bedside-replicated situation (Fig. 1). However, white turbidity was observed in the upper part of the syringe constructing the line force downstream of the T-shape stopcock (Table 1). Actually, when compatibility was detected as a white precipitate at the bedside, it was reported through the activation of an alarm. Furthermore, by repeating the obstruction, subsequent alarm activation, and successive relief of obstruction,
the entire inner side of the syringe became cloudy and precipitates were also observed in the intravenous line. These results suggest that refluxing the infusion composed of Replas® 1 and Novo-Heparin® into the side tube filled with Nicarpine® resulted in compatibility. Furthermore, a study in which individual constituents, namely Replas® 1 and Novo-Heparin®, were mixed with Nicarpine®, resulted in precipitates forming only when Novo-Heparin® was used (Table 2). This result suggests that the formation of precipitates in this case was caused by the compatibility between heparin and nicardipine.

There are no descriptions about the compatibility between Novo-Heparin® and Nicarpine® in each Drug Package Insert and Interview Form. However, because nicardipine hydrochloride has a pH-dependent compatibility over pH 6.0, turbidity was observed over pH 5.08-5.11. Perdipine® Injection 10 mg, an original nicardipine drug preparation, showed compatibility with heparin sodium i.e., there exists a description about their incompatibility. On the other hand, when the features and appearance of some drugs change with variations in pH, the pH-safety area is extended and the reversion of such changes may be possible by diluting with a large volume such as infusions (i.e., the diluting effect). Non-diluted drug preparations (Perdipine® Injection 10 mg: 1 mg/mL and heparin sodium: 10,000 U / 10mL, respectively) were employed in the drug incompatibility test of the Interview Form of Perdipine® Injection 10 mg. However, the infusion system in this study did not reveal any turbidity when used conventionally, not causing a diluting effect when Novo-Heparin® was in the main route and Nicarpine® was injected from a side tube to the intravenous line when diluted with Replas® 1.

![Fig. 3](image-url) $^\text{1}$H-NMR spectra of the A) supernatant, B) nicardipine and C) heparin

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Weights of the CHCl$_3$-extracted compounds from the alkalified aqueous solution of the precipitate and extracts (Fig. 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precipitates (mg)</td>
<td>Extracts (mg)</td>
</tr>
<tr>
<td>Sample</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>39.2</td>
</tr>
<tr>
<td>B</td>
<td>36.2</td>
</tr>
<tr>
<td>C</td>
<td>39.4</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>38.3 ± 1.8</td>
</tr>
</tbody>
</table>
other hand, although there are many reports that variation in pH causes a compatibility involving a change in appearance, in this case compatibility was not derived from a variation in pH (Table 3). Specifically, pH variation at pH 0.13 in this study resulted in a change in appearance although this pH value was much lower than the pH point of variation (i.e., the pH value at which a change in appearance is observed following the addition of HCl or NaOH solution to a substrate) of nicardipine (http://www3.osaka-ohtani.ac.jp/ph/pre_jitu-mu/pdf/1-5.pdf). It is suggested that the formation of precipitates in this case was caused by the compatibility between heparin sodium and nicardipine.

Characterizing the precipitate formed by compatibility might provide valuable information not only to determine its cause but also to consider a way to avoid it. Thus, to obtain structural information of the precipitate, IR spectra and 1H-NMR spectra were measured and their structural features were considered by comparing with spectra of nicardipine hydrochloride and heparin sodium. Comparing the IR spectrum of the precipitate with those of nicardipine hydrochloride and heparin sodium (Fig. 2), the absorbance based on the sulfate salt around 1220 and 1440 cm\(^{-1}\) (stretching vibration) in heparin sodium shifted and the absorbance based on the saturated tertiary amine around 2200-2700 cm\(^{-1}\) (stretching vibration) in nicardipine hydrochloride disappeared. Furthermore, when comparing the 1H-NMR spectrum of the precipitate with that of nicardipine hydrochloride and heparin sodium (Fig. 3), signals around 7.4 and 8.1 ppm derived from dihydropyridine and signals around 2.2 ppm derived from methyl at the aryl position and N-methyl of the precipitate shifted to a lower field compared to those of nicardipine. Since each of these signals positioned around the amino group in nicardipine, it was supposed that the compatibility occurred by a molecular interaction between the amino group of nicardipine hydrochloride and heparin sodium.

Heparin sodium is known to cause compatibility easily and reportedly forms a precipitate by binding to basic drugs in near neutral solution. It is a heterogeneous mixture of acidic mucopolysaccharides and is composed of alternately-\(\alpha_1,4\) linked uronic acid and glucosamine. Heparin sodium has a carboxyl group and a sulfate group in its structure both of which possibly interact with amino groups in nicardipine. For example, the compatibility involving a carboxyl group is the incompatibility of gabexate mesilate (brand name: FOY\textsuperscript{®}) and heparin sodium. This compatibility was reportedly caused by a salt exchange by binding the basic nitrogen atom of gabexate mesilate and the carboxyl group of heparin sodium. In our research, in contrast to heparin sodium and nicardipine, turbidity was not observed when chondroitin sulfate and nicardipine were employed. This result suggests that the sulfate group of heparin sodium did not play a part in the molecular interaction of heparin sodium and nicardipine.

The structural unit of heparin sodium (a disaccharide composed of uronic acid and glucosamine) was derived with an average of 2.5-3 sulfate groups. Thus, the molecular weight of the unit was presumed to be 573-613. In contrast to this, the molecular weight of nicardipine hydrochloride is 515.99. Considering the results shown in Fig. 4 and Table 4 (the precipitate consisted of heparin sodium and nicardipine hydrochloride at 1:2), it was assumed that the number of bound heparin sodium to nicardipine hydrochloride molecules was at least two per structural unit. Addi-
tionally, because glucosamine does not have a carboxylic group, it turns out that the structural unit has only one carboxyl group, and thus the sulfate group possibly bound to the basic nitrogen of nicardipine. On the other hand, chondroitin sulfate employed in this research was sulfated at one site per disaccharide in contrast to heparin sodium which was 2.5-3 sites per disaccharide. Thus, it is suggested that the sulfate group may be related with compatibility.

The results shown in this paper suggest that the compatibility in the prescription might seriously influence drug therapy. Heparin sodium shows an anticoagulant effect by stimulating the inhibition of thrombin in antithrombin III and activated factor X by the specific formation of a complex with antithrombin III. Around one third of the heparin binds to antithrombin III, and in this part, there is an antithrombin high affinity pentasaccharide that assumes the anti coagulant effect of heparin sodium \(^{13,14}\) (Guidelines for management of anticoagulant and antiplatelet therapy in cardiovascular disease (JCS 2009), http://www.j-circ.or.jp/guideline/index.html). This pentasaccharide is composed of GlcNS6S-GlcA-GlcNS3, 6S-IdoA2S-GlcNS6S (S indicates the sulfated part) and the sulfate group of heparin sodium contributes to the stability of the heparin sodium and antithrombin III complex by trapping the amino acid of antithrombin, followed by a conformation change to antithrombin III, resulting in the inactivation of activated factor X. \(^{14,15}\) Thus, since both the carboxyl group and sulfate are related to the compatibility, there is a possibility that the titer of not only nicardipine but also heparin sodium might be reduced by such as compatibility.

If compatibility of the injection should occur, normally an operation is suspended. However, for a pharmacist, it is important to understand how to approach the compatibility so as to execute the operation without changing the purpose of the prescription. As shown in Table 1, the compati-
bility only occurred by obstructing the infusion line. Thus, preventing the reflux of heparin from the main route to nicardipine in the syringe by this obstruction would be one of the effective methods to avoid this compatibility. There is a suspicion of the infusion line was stressed by a reposition of the patient as a cause of the reflux of infusion solution containing heparin into the side line. To prevent such an incident, it would be effective to device an installation position of the infusion line and to provide the information to patients and nurse from pharmacists.

It was suggested that the compatibility was resulted in the refluxing the infusion composed of Replas® 1 and Novo-Heparin® into the side tube filled with Nicarpine®. Thus, as a possible method to avoid the adverse effect of the compatibility, the line filter might be connected at the last part of the side tube just before the intravenous line. This method can avoid the influx of the precipitate into the human body, but cannot be fundamental solution for the compatibility.

The current research, from a point of view of the cause of formation and of structural information about the precipitation provides valuable information for the care of patients and an infusion preparation method without significantly changing the prescription so as to avoid compatibility between heparin sodium and nicardipine hydrochloride in the infusion line. This reveals the possibility of exerting the occupational ability of pharmacists in the field to administer drugs more than ever.

References


6) Interview Form of Novo-Heparin® 10,000 units / 10 mL for injection, revised 2nd ed., ed. by Mochida Pharmaceutical Co., Ltd., Tokyo, October 2009.


