Observational Study

Physicochemical Stability and Compatibility of Mixtures of Ropivacaine with Dexamethasone or Betamethasone for Epidural Steroid Injections: An In Vitro Study

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Background: Epidural steroid injections are widely used to treat spinal and radiating pain. However, crystal formation has recently been reported in mixtures of ropivacaine and nonparticulate steroids, commonly used in epidural steroid injections.

Objectives: Our study assessed the physicochemical stability of mixtures of different nonparticulate steroids and ropivacaine and aimed to propose a safe regimen for epidural steroid injections.

Study Design: An in vitro protocol was used to examine the physicochemical stability of epidural steroid injection mixtures most commonly used at our institution.

Setting: In vitro laboratory study.

Methods: Twelve solutions were prepared by mixing 0.75% or 0.2% ropivacaine with dexamethasone or betamethasone at volume ratios of 1:1, 2:1, and 3:1 in propylene syringes at 24°C. The physical properties of the mixtures were observed with the naked eye and under a microscope, and their pH was measured. The concentration of each drug in the mixture was evaluated using high-performance liquid chromatography.

Results: None of the ropivacaine and dexamethasone mixtures showed macroscopic or microscopic crystal formation after 2 hours, and there were no significant changes in pH. The concentrations of the 2 drugs remained stable for up to 2 hours. In contrast, at least 10 μm crystals were observed microscopically and macroscopically in all mixtures of ropivacaine and betamethasone; the ropivacaine concentration was reduced by > 10% after one hour.

Limitations: Confirming the stability of drugs in vitro does not ensure that their pharmacokinetics and pharmacodynamics remain unaltered in vivo.

Conclusion: The combination of ropivacaine and betamethasone should be avoided because of their physicochemical instability. Combinations of ropivacaine and dexamethasone should be administered cautiously because they are more physicochemically stable than combinations of ropivacaine and betamethasone.

Key words: Betamethasone, crystal formation, dexamethasone, drug stability, epidural injection, high-performance liquid chromatography, pH, physicochemical stability, spinal pain

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Epidural steroid injections are widely administered to treat spinal and radiating pain (1-3). The main goals of epidural steroid injections are targeted drug delivery and improvement of local inflammation. Accurate drug delivery to a target can ensure effective pain control with minimal complications. Approaches for epidural steroid injections can be classified as interlaminar or transforaminal, depending on the

final location of the needle tip. In particular, since the transforaminal approach directly advances the needle tip toward the trajectory of the ventrolateral space of the spinal foramen where the lesion exists, more precise drug delivery is possible, and better clinical effectiveness is obtained, compared with the interlaminar approach (4).

In the subpedicular method, most commonly used for transforaminal epidural steroid injections, the needle is introduced into the safety triangle on the superior and lateral sides of the spinal nerve. It is traditionally safe from nerve or disc damage (5,6). However, neuromuscular arteries pass through the safety triangle at the thoracolumbar level and needles can stimulate or penetrate blood vessels and nerve roots (7,8). Spinal cord infarction can occur when a transforaminal block is performed using particulate steroids (9-13).

Therefore, to prevent fatal complications such as spinal cord infarction, transient ischemic seizures, or severe cerebral infarction caused by the intra-arterial injection of particulate steroids, in 2014 the US Food and Drug Administration (FDA) recommended the use of nonparticulate steroids for epidural steroid injections rather than particulate steroids (14). Subsequently, following FDA recommendations, injectates of a local anesthetic and a nonparticulate steroid have been commonly used for epidural steroid injections. Ropivacaine, which has low heart and central nervous system toxicity, is often used as an anesthetic (15,16) in conjunction with nonparticulate steroids, such as dexamethasone or betamethasone (17-20).

However, recent reports have shown that macroscopic or microscopic crystals are formed when ropivacaine is mixed with dexamethasone or betamethasone, raising concerns over their combined use (14,21,22). However, these studies mixed 0.75 % ropivacaine and nonparticulate steroids at ratios of 1:1, 2:1, or 3:1 by volume of each undiluted solution (14,21,22).

In clinical practice, for epidural steroid injections to control spinal and radiating pain, diluting ropivacaine in normal saline to 0.2% is recommended rather than using it as an undiluted 0.75% solution (23-25). At our institution, ropivacaine is empirically diluted to 0.2% in normal saline using a propylene syringe and is then mixed with dexamethasone. No crystals were observed with the naked eye. In addition, a previous study reported that this mixture is stable when ropivacaine and dexamethasone are mixed in a 9:1 ratio (26). Therefore, we hypothesized that the ratio of ropivacaine to dexamethasone and its dilution with normal saline would affect crystal formation. We also compared a mixture of ropivacaine and betamethasone as a control.

Our study's first objective was to analyze the physicochemical stability of a clinically used mixture of ropivacaine and dexamethasone or betamethasone focusing on crystal formation and any decrease in drug concentration. The second objective was to determine safe combinations and ratios of nonparticulate steroids and ropivacaine to propose an appropriate regimen for epidural steroid injections.

Methods

Constituents of Drug Mixtures

Ropivacaine hydrochloride (0.75%; Nacain Injection®, 7.5 mg/mL; Huons Global), dexamethasone disodium phosphate (Dexamethasone Sodium Phosphate Injection®, 5 mg/mL; Yuhan Corp.), and betamethasone sodium phosphate (Betamethasone Sodium Phosphate Injectate®, 5.2 mg/mL; Huons Global) were obtained commercially (Table 1).

Normal saline (0.9%; Isotonic Sodium Chloride 20 mL/ampule; Dai Han Pharmaceutical Co., Ltd.), was prepared, and a commercially obtained polypropylene syringe was used to store the solution.

Drug Mixture Preparation

In this study, 6 drug mixtures were prepared by mixing 0.75% ropivacaine with either dexamethasone or betamethasone at ratios of 1:1, 2:1, and 3:1. Six additional solutions were prepared by diluting ropivacaine in normal saline to a clinical concentration of 0.2% and combining it with dexamethasone or betamethasone in 1:1, 2:1, and 3:1 ratios (volume ratio of 0.75% ropivacaine to dexamethasone or betamethasone) (Table 2).

Drug mixtures were stored in propylene syringes used in clinical settings. Propylene syringes containing each drug mixture were stored at a constant temperature of 24°C in the laboratory, without shading, to create an environment similar to that used clinically. The concentration range of each drug in the mixture was 2.00–5.69 mg/mL of ropivacaine, 0.44–2.50 mg/mL of dexamethasone, and 0.46–2.60 mg/mL of betamethasone.

For analytical accuracy, 5 replicates of each of the 12 combinations were prepared using this method.

Stability of Analgesic Mixtures

For each mixture, 1.5 mL samples were obtained from the propylene syringes immediately, at one hour,

Table 1. *Concentration, chemical formula, molecular weight, and pH of ropivacaine, dexamethasone, and betamethasone.*

Drug	Initial concentration (mg/mL)	Chemical formula	Molecular weight	pH
Ropivacaine (ropivacaine hydrochloride)	7.5	C17H26N2O (C17H27ClN2O)	274 (310)	6.20
Dexamethasone (dexamethasone disodium phosphate)	5	C22H29FO5 (C22H28FNa2O8P)	392 (516)	8.06
Betamethasone (betamethasone sodium phosphate)	5.2	C22H29FO5 (C22H28FNa2O8P)	392 (516)	8.26

Table 2. *Drug combinations evaluated in this study.*

Mixture	0.75% Ropivacaine (7.5 mg/mL)	Dexamethasone (5.0 mg/mL) or betamethasone (5.2 mg/mL)	Normal saline (mL)	Total amount (mL; mixing ratio of ropivacaine and steroids)
1	3 mL	Dexamethasone 3 mL	-	6 mL (1:1)
2	4 mL	Dexamethasone 2 mL	-	6 mL (2:1)
3	6 mL	Dexamethasone 2 mL	-	8 mL (3:1)
4	2 mL	Dexamethasone 2 mL	3.4 mL	7.4 mL (1:1)
5	2 mL	Dexamethasone 1 mL	4.5 mL	7.5 mL (2:1)
6	3 mL	Dexamethasone 1 mL	7.2 mL	11.2 mL (3:1)
7	3 mL	Betamethasone 3 mL	-	6 mL (1:1)
8	4 mL	Betamethasone 2 mL	-	6 mL (2:1)
9	6 mL	Betamethasone 2 mL	-	8 mL (3:1)
10	2 mL	Betamethasone 2 mL	3.4 mL	7.4 mL (1:1)
11	2 mL	Betamethasone 1 mL	4.5 mL	7.5 mL (2:1)
12	3 mL	Betamethasone 1 mL	7.2 mL	11.2 mL (3:1)

and 2 hours after mixing. The physicochemical properties of these mixtures were also evaluated.

Physical Characteristics

Appearance, Clarity, and Color

Each sample was placed in a colorless silicate glass test tube to evaluate their physical properties, and visually inspected for color, turbidity, and crystallization using white and black backgrounds. The formation of fine crystals was confirmed at ×200 using a BX51 optical microscope (Olympus Corporation). The physical stability of the mixture was defined as the retention of the original transparent, colorless, and particle-free solution (27).

Chemical Characteristics

pH

The pH of each aliquot was measured using a digital PHS-3C pH meter (Thermo Scientific). The mean ± SD of the pH values was calculated using 5 pH readings for each mixture; this was used to confirm whether the chemical properties of each mixture changed over time.

Compound Concentrations

The drug concentration in each mixture was measured by high-performance liquid chromatography (HPLC). Before analyzing the samples by HPLC, the HPLC peaks of the 3 drugs were confirmed. Subsequently, HPLC runs were performed on 100 µL samples of each drug mixture taken at the noted time points. This experiment assessed whether drug concentrations remained constant over time. In addition, using the chromatogram obtained from the solution immediately after mixing as a standard, we determined whether any other degradation peaks that interfered with the quantification of each drug in the mixture at 1 – 2 hours after mixing were generated.

For each mixture, the drug concentration was set to 100 immediately after mixing and the ratio of change in concentration of each drug over time was calculated. The mean ± SD of the rate of change in concentration over time was calculated using 5 replicates of each mixture. According to the US Pharmacopeial Convention, drug stability is defined as maintaining 90 – 110% of the initial drug concentration (28).

HPLC Equipment and Chromatography Conditions

A YL9100 HPLC system was used for the reverse-phase HPLC. The system comprised a YL9110 quaternary pump, YL9101 vacuum degasser, and YL9120 UV/Vis detector integrated with the YL Clarity software. HPLC separation was performed using a Vydac C18 column (250.0 × 7.6 mm ID). The eluent for the analysis was injected in a gradient of 0.05% trifluoroacetic acid (TFA)-H_2O and 0.05% TFA-acetonitrile–acetonitrile at a flow rate of 1.5 mL/min (flow conditions: 0 – 40 minutes, increase in the concentration of acetonitrile from 20% to 80%; 40 – 50 minutes, 10% water, and 90% acetonitrile). The UV-Vis detector wavelength was set from 245 nm to 281 nm.

Analytic Validation

The guidelines laid down at the International Conference on Harmonization were referenced to validate the analytical techniques (29).

Calibration

The relationship between the peak area for each drug and the amount of drug applied was determined using linear regression above the previously defined range. For calibration, each drug standard was analyzed 4 times at 4 concentrations.

Accuracy

Accuracy was calculated using the relative standard deviation (RSD) of the experimental concentration obtained from the mixture, with the theoretical concentration calculated from 4 concentrations measured in quadruplicate for each drug. This was expressed as the coefficient of variation of the accuracy (CVa). CVa was calculated for each drug in each combination.

Repeatability

HPLC analysis was performed by the same researcher in the same laboratory using the same equipment by following the same analytical procedures. Repeatability was calculated using the RSD of the mean ± SD of values from 5 replicates. Repeatability is expressed as the coefficient of variation of repeatability (CVr). CVr was calculated for each drug in each combination.

Results

Physical Stability

The mixtures of ropivacaine and dexamethasone were colorless and transparent; no precipitates were observed during visual or microscopic examinations. All mixtures of ropivacaine and dexamethasone were considered physically compatible, as no evidence of incompatibility (precipitation, turbidity, or color change) was observed.

Precipitation in the mixtures of ropivacaine and betamethasone was observed with the naked eye. In addition, microscopic analysis revealed crystals of 100 µm or larger in the mixture of 0.75% ropivacaine and betamethasone, whereas crystals of 10 µm – 50 µm were observed in the mixture of 0.20% ropivacaine and betamethasone.

Chemical Stability

pH

The pH values of the mixtures did not change remarkably during the study period for any of the combinations analyzed. The pH values of the mixtures of ropivacaine and dexamethasone or betamethasone varied by less than 2.86% and 3.77%, respectively, at all time intervals compared with those immediately after mixing (Table 3).

Concentration

The drug concentration of each mixture was calculated by integrating the surface areas of the chromatographic peaks. The retention times of ropivacaine, dexamethasone, and betamethasone were approximately 17.9, 25.7, and 24.8 minutes, respectively (Fig. 1).

Figures 2 and 3 show the trends of the average ratio over time, beginning immediately after mixing. Ropivacaine and dexamethasone concentrations in their mixture remained stable between 90% to 110% of the initial concentration up to 2 hours after mixing (Fig. 2). Conversely, in all mixtures of ropivacaine and betamethasone, the ropivacaine concentration decreased by more than 10% after the first hour of mixing compared to that obtained immediately after mixing (Fig. 3). In addition, as the mixing ratio of ropivacaine increased, the rate of decrease in ropivacaine concentration tended to decrease. However, the concentration of betamethasone in all mixtures remained between 90% and 110% of the initial concentration up to 2 hours after mixing. No new degradation peaks were detected in the mixture of ropivacaine and betamethasone.

Analytic Validation

Calibration

The linear regression equations were as follows:

Table 3. *pH values of the mixtures of ropivacaine and dexamethasone or betamethasone at different time intervals.*

Time after mixing	Mixture composition	Immediately	After 1 h	After 2 h
Mixture 1	0.75% Ropivacaine + dexamethasone (1:1)	6.94 ± 0.05 (100)	6.98 ± 0.03 (100.55 ± 0.01)	7.07 ± 0.03 (101.94 ± 0.01)
Mixture 2	0.75% Ropivacaine + dexamethasone (2:1)	6.73 ± 0.08 (100)	6.87 ± 0.04 (102.07 ± 0.01)	6.88 ± 0.04 (102.19 ± 0.02)
Mixture 3	0.75% Ropivacaine + dexamethasone (3:1)	6.63 ± 0.09 (100)	6.74 ± 0.02 (101.68 ± 0.02)	6.72 ± 0.07 (101.41 ± 0.02)
Mixture 4	0.20% Ropivacaine + dexamethasone (1:1)	6.72 ± 0.15 (100)	6.89 ± 0.07 (102.49 ± 0.01)	6.91 ± 0.07 (102.86 ± 0.02)
Mixture 5	0.20% Ropivacaine + dexamethasone (2:1)	6.77 ± 0.07 (100)	6.77 ± 0.08 (99.96 ± 0.02)	6.79 ± 0.06 (100.22 ± 0.02)
Mixture 6	0.20% Ropivacaine + dexamethasone (3:1)	6.63 ± 0.01 (100)	6.75 ± 0.08 (101.78 ± 0.01)	6.70 ± 0.04 (101.06 ± 0.01)
Mixture 7	0.75% Ropivacaine + betamethasone (1:1)	7.43 ± 0.07 (100)	7.21 ± 0.10 (97.06 ± 0.01)	7.20 ± 0.09 (96.91 ± 0.01)
Mixture 8	0.75% Ropivacaine + betamethasone (2:1)	7.13 ± 0.08 (100)	6.93 ± 0.07 (97.13 ± 0.02)	6.94 ± 0.07 (97.24 ± 0.02)
Mixture 9	0.75% Ropivacaine + betamethasone (3:1)	6.90 ± 0.15 (100)	6.90 ± 0.04 (99.98 ± 0.02)	6.82 ± 0.07 (98.84 ± 0.03)
Mixture 10	0.20% Ropivacaine + betamethasone (1:1)	7.16 ± 0.15 (100)	7.39 ± 0.21 (103.25 ± 0.02)	7.36 ± 0.17 (102.83 ± 0.02)
Mixture 11	0.20% Ropivacaine + betamethasone (2:1)	7.05 ± 0.08 (100)	7.26 ± 0.12 (102.89 ± 0.01)	7.32 ± 0.12 (103.77 ± 0.01)
Mixture 12	0.20% Ropivacaine + betamethasone (3:1)	7.08 ± 0.07 (100)	7.27 ± 0.11 (102.66 ± 0.01)	7.33 ± 0.11 (103.51 ± 0.01)

Measured pH (percentage of pH immediately after mixing). Data are expressed as mean ± standard deviation.

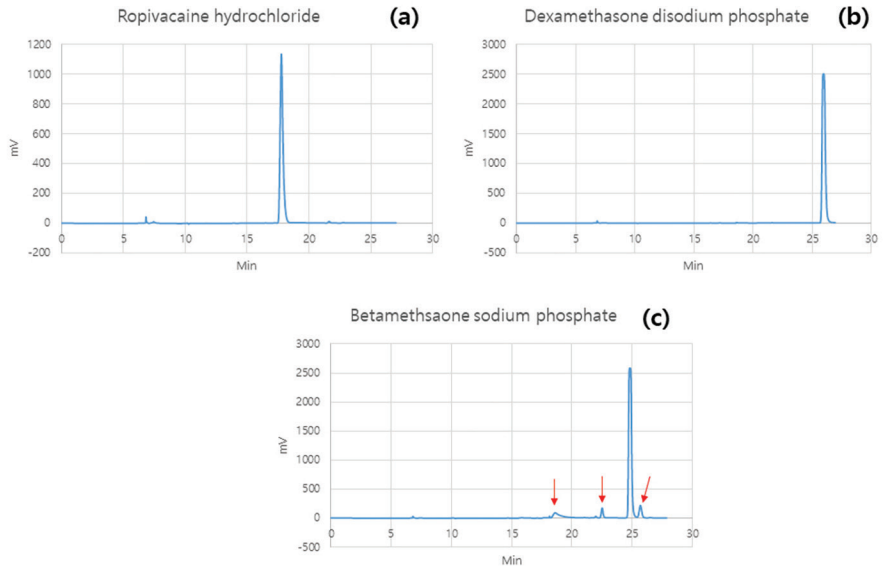

Fig. 1. *High-performance liquid chromatography peaks with 50 µL injection of ropivacaine (A), dexamethasone (B), and betamethasone (C) stock solutions. (C) In addition to the betamethasone peak, another small peak (red arrow), suspected to be a preservative peak, is observed.*

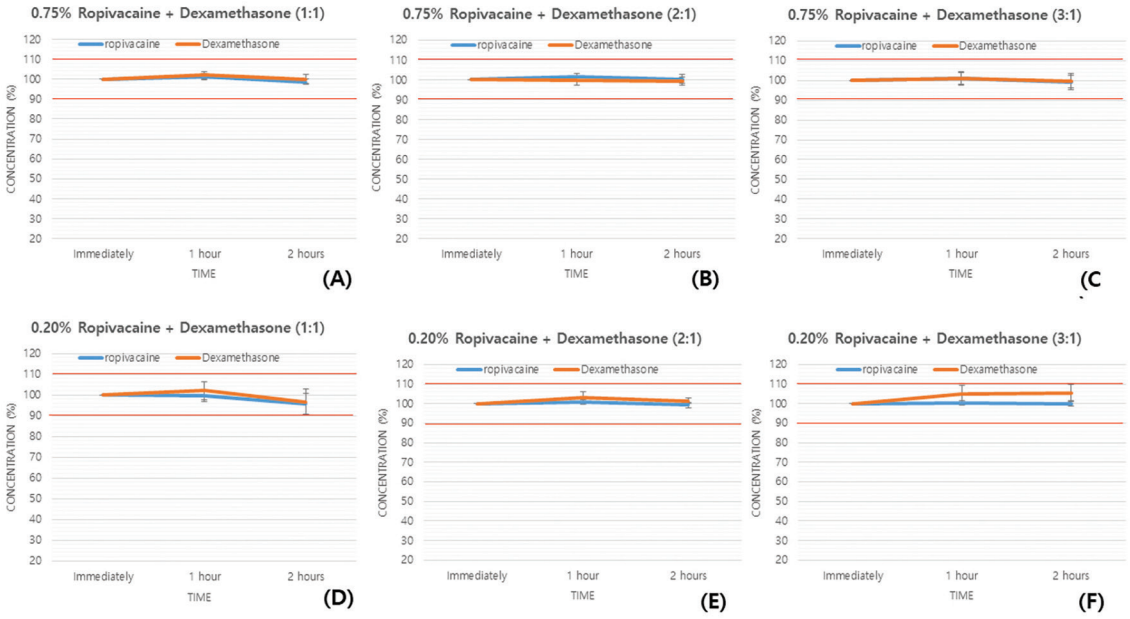

Fig. 2. *Rate of change in the concentration of each drug over time in ropivacaine and dexamethasone mixtures. (A) Mixture 1, (B) mixture 2, (C) mixture 3, (D) mixture 4, (E) mixture 5, and (F) mixture 6.*

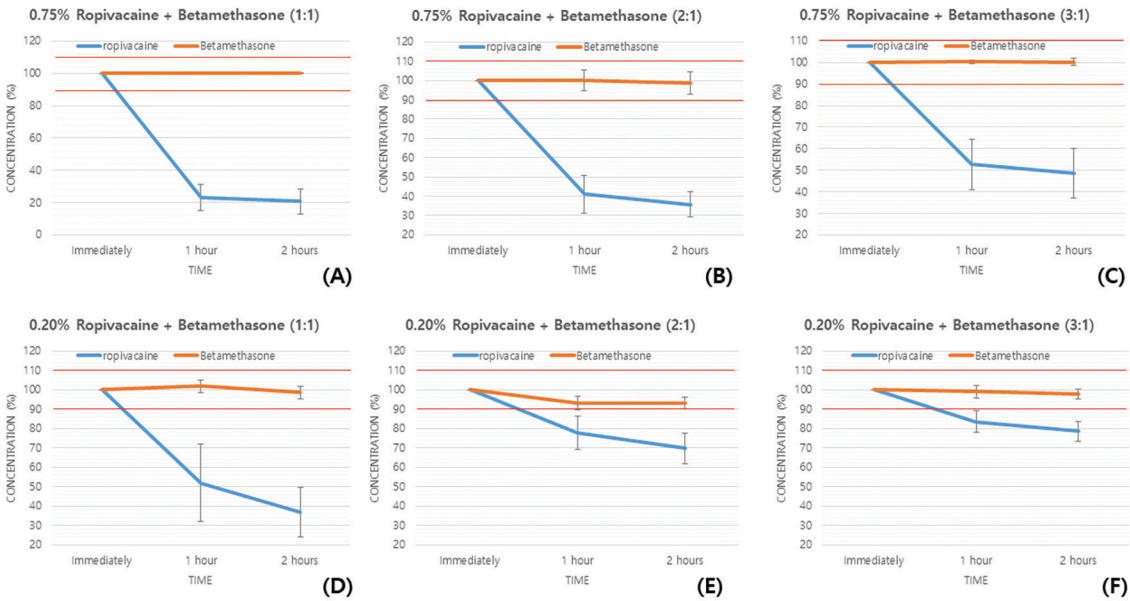

Fig. 3. *Rate of change in the concentration of each drug over time in ropivacaine and betamethasone mixtures. (A) Mixture 7, (B) mixture 8, (C) mixture 9, (D) mixture 10, (E) mixture 11, and (F) mixture 12.*

ropivacaine, y = 1768.7 (x) - 6021.4, mean r^2 = 0.9929; dexamethasone, y = 915.3 (x) - 1126.2, mean r^2 = 0.9934; and betamethasone, y = 961.9 (x) - 1336.1, mean r^2 = 0.9934. All drugs exhibited adequate linear responses and correlation coefficients (r^2) between the peak area and concentration.

Accuracy

The CVa values between the estimated theoretical and observed experimental concentrations for each drug were as follows: ropivacaine, 0.1% – 6.3% (accuracy, ≥ 93.7%); dexamethasone, 0.2% – 3.1% (accuracy, ≥ 96.9%); and betamethasone, 0.2% – 8.9% (accuracy, ≥ 91.1%). The CVa for all 3 drugs in all combinations was < 8.9%.

Repeatability

The CVr for each drug was estimated using the results obtained from 5 replicates of each mixture. The CVr values were as follows: ropivacaine, 0.1% – 8.5% (accuracy, ≥ 91.5%); dexamethasone, 2.9% – 6.5% (accuracy, ≥ 93.5%); and betamethasone, 2.6% – 7.9% (accuracy, ≥ 92.1%). The CVr for all 3 drugs in all combinations was < 8.5%.

Discussion

Our study aimed to evaluate the physicochemical stability of ropivacaine combined with dexamethasone or betamethasone. We observed that all mixtures of ropivacaine and dexamethasone were physicochemically stable for up to 2 hours. In contrast, in the mixture of ropivacaine and betamethasone, precipitation was observed visually and microscopically, and the HPLC analysis showed that the ropivacaine concentration decreased by over 10% in one hour and 2 hours after mixing compared to that obtained immediately after mixing, indicating that it was physiochemically unstable.

Our initial observation was that previous studies reporting crystal formation (14,21,22) had been conducted using 0.75% ropivacaine rather than 0.2% ropivacaine, which is a clinically used concentration. Therefore, we speculated that crystal formation would be different at clinically used concentrations. However, in our study, precipitation was neither observed in the mixture of 0.2% ropivacaine and dexamethasone nor the mixture of their stock solutions, visually or microscopically. In addition, the concentration of each drug in the ropivacaine and dexamethasone combination remained stable for 2 hours after mixing. In contrast, a significant decrease in the concentration of ropivacaine was observed in the mixture of 0.2% ropivacaine and betamethasone and the mixture of their stock solutions. This result differs from our initial speculations and from the results of previous studies (14,21,22), which reported that a ropivacaine and dexamethasone combination is unstable.

Physicochemical instability in drug mixtures is often explained by differences in pH between the drugs (30,31). In previous studies describing the instability of mixtures of ropivacaine and dexamethasone or betamethasone, crystal formation was primarily explained by pH, including the alkalinization of ropivacaine (14,21,22,32). Although local anesthetics are weak bases, they are usually formulated at acidic pH to maximize water solubility (33,34). In contrast, many commercial corticosteroid solutions contain weak bases. Therefore, when mixing a local anesthetic and a steroid, the local anesthetic is alkalized, and crystals may form because of nonionization of the drug (33).

If crystal formation occurs in mixtures of ropivacaine and dexamethasone or betamethasone owing to pH differences, precipitation should be uniform. However, the crystal sizes were heterogeneous in previous studies on the stability of ropivacaine and dexamethasone mixtures.

According to Watkins et al (22), crystals were observed with the naked eye, and crystals ≥ 100 µm were observed under a microscope (ropivacaine, pH 5.3; dexamethasone, pH 8.4) following the mixture of one mL of 0.75% ropivacaine and dexamethasone (4 mg/mL or 10 mg/mL). Hwang et al (21) reported that when 2 mL of 0.75% ropivacaine and dexamethasone (5 mg/mL) were mixed, crystals were not observed with the naked eye; however, linear crystals of 10 µm – 100 µm were observed under a microscope (ropivacaine, pH 6.2; dexamethasone, pH 7.7). Choi et al (14) reported that when 0.75% ropivacaine and dexamethasone (5 mg/mL) were mixed at 1:1, 2:1, and 3:1 ratios by volume, the size of the crystals as seen under a microscope was < 10 µm (ropivacaine, pH 6.1; dexamethasone, pH 7.6). In addition, a study by Hoerner et al (35) evaluated the stability of a mixture of ropivacaine and dexamethasone and reported crystals > 100 µm (ropivacaine, pH 4.1; dexamethasone, pH 8.5).

Overall, these studies showed that greater pH differences between ropivacaine and dexamethasone increased the size of crystals formed in the mixture (22,35). The information provided by the manufacturers differed among the studies and, in some cases, was not provided. Therefore, we conclude that each study produced different results because of the differences in pH

between ropivacaine and dexamethasone produced by different manufacturers. In support of this argument, Milner et al (36) reported that ropivacaine precipitated at a pH > 6.0. However, Hwang, et al (21) and Choi et al (14) reported that ropivacaine had no apparent crystals at pH 6.1 – 6.2. Hwang et al (21) reported the formation of ropivacaine crystals at pH 6.8. Therefore, the pH at which ropivacaine precipitates may vary, depending on the manufacturer.

In addition, differences in the pH of the mixtures may have led to different results. In contrast to previous studies, Melton et al (26) found that when a mixture of 0.5% ropivacaine (17.95 mL) and 2 mL dexamethasone (4 mg/mL) was subjected to nuclear magnetic resonance analysis, the mixture was stable for up to 48 hours after mixing. This might have been because ropivacaine and dexamethasone were mixed in a 9:1 ratio (26); therefore, the mixture was acidified, and ropivacaine did not precipitate. Additionally, this study showed that higher ropivacaine concentrations resulted in lower rates of decrease in concentration over time. This may be because the mixture tended to become more acidic as the concentration of ropivacaine increased, which may reduce the alkalinization of ropivacaine.

However, in our study, crystal formation in the mixture could not be explained by the pH difference alone. Crystal formation and a decrease in the concentration of ropivacaine occurred in a solution (average pH 6.90) in which ropivacaine and betamethasone stock solutions were mixed at a 3:1 ratio. If crystals were formed because ropivacaine was alkalized, crystal formation and a decrease in ropivacaine concentration would have occurred in the 1:1 mixture of ropivacaine and dexamethasone stock solutions (average pH 6.94). However, crystal formation and concentration reduction were not observed in this mixture. Furthermore, these results differ from those of other studies in that ropivacaine crystals were formed by ropivacaine alkalinization in a mixture at pH 6.8 – 6.9 (14,21).

Considering these results, we hypothesized that, in addition to pH, other factors influence crystal formation in mixtures of ropivacaine and steroid preparations. Differences in the steroid preservatives for each product could be a contributing factor.

The dexamethasone preparation used in our study contained water for injection and glycerin, disodium edetate, sodium hydroxide, and phosphoric acid as preservatives (Supplementary File 1). The betamethasone preparation used in this study contained phenol and sodium hydrogen sulfite as preservatives, water for injection, sodium hydroxide, and disodium edetate (Supplementary File 2). No preservative peaks were observed during the HPLC analysis of dexamethasone. However, in the HPLC analysis of betamethasone, a peak attributable to various preservatives was observed (Fig. 1).

At similar pH levels, the mixture of ropivacaine and dexamethasone was physicochemically stable; therefore, the physicochemical instability of the mixture of ropivacaine and betamethasone may be attributed to the presence or absence of these preservatives, in addition to pH-dependent crystallization. These factors may explain why the results of our ropivacaine and dexamethasone stability study differ from those of other studies. Hwang et al (21) reported that crystals of 10 μm – 100 μm were formed in a mixture of ropivacaine and dexamethasone when Daewon dexamethasone (Dexamethasone Sodium Phosphate Injection®, 5 mg/mL; Daewon Pharmaceutical Co., Ltd.) was used. Unlike the formulation used in our study, this formulation of dexamethasone contained benzyl alcohol as a preservative (Supplementary File 3). In summary, differences in preservatives may have led to heterogeneity in study results. A study comparing the crystal formation and concentration reduction using different commercial preservatives should be performed to confirm this hypothesis.

The results of our study suggest that several aspects of each drug should be considered in clinical practice. The combination of ropivacaine and betamethasone should be avoided as much as possible because of its physicochemical instability. Combinations of ropivacaine and dexamethasone can be used carefully because our study showed that ropivacaine and dexamethasone mixtures do not form crystals and are physicochemically stable. When ropivacaine and dexamethasone are necessary, the smallest possible amount of dexamethasone should be used in the mixture to minimize any increase in pH. Based on our study, we also identified the need for pharmaceutical companies to produce standardized preparations and to be forthcoming regarding the constituents of their formulations.

Limitations

Our study has some limitations. First, the drugs used in the ropivacaine and dexamethasone mixtures were physicochemically stable for up to 2 hours after mixing. However, in vitro, stability does not guarantee that pharmacokinetics or pharmacodynamics are unaltered in vivo; clinical trials are necessary to confirm this. Second, despite having the same ingredients, local anesthetic and nonparticulate steroids may have different

formulation characteristics owing to their preservatives. Therefore, these results cannot be generalized to other commercially available formulations. To overcome this limitation, we will conduct a follow-up study using commercially available dexamethasone formulations. Third, this study was conducted at a room temperature of 24°C. Therefore, further research is required on the physicochemical stability at different temperatures.

Conclusion

The mixture of ropivacaine and dexamethasone used in our hospital was physicochemically stable for up to 2 hours after mixing. Crystals of at least 10 μm were observed in the mixture of ropivacaine and betamethasone; the concentration of ropivacaine decreased following mixing, confirming that it was physically and chemically unstable. However, these results cannot be generalized to other commercially available formulations. Therefore, additional studies comparing different commercially available preparations are required.

Availability of Data and Materials

The datasets generated and analyzed in this study are available from the Open Science Framework repository (https://osf.io/hmd5q/ or DOI 10.17605/OSF.IO/HMD5Q).

Authors' Contributions

All authors had full access to all data and are responsible for the integrity of the data and accuracy of the data analysis. HK, SSC, and CHL designed the experiments. All the authors were involved in the experiments. All authors searched and reviewed the literature and wrote the first draft of the manuscript. HK, SSC, and CHL revised the intellectual content of the manuscript and approved the final version.

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