In Vitro Study

An In Vitro Study of the Physicochemical Stability of Ropivacaine and Dexamethasone Formulations Containing Different Preservatives: Establishing a Safety Guideline

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Background: Previous studies evaluating the stability of ropivacaine and dexamethasone mixtures used in epidural steroid injections have reported inconsistent findings regarding the presence, morphology, and size of crystals.

Objectives: We sought to evaluate the physicochemical stability of ropivacaine combined with 3 dexamethasone formulations; each formulation had different preservatives. We also sought to identify a preservative that allows for a physicochemically more stable and thus safer co-administration than those prone to crystal formation.

Study Design: Our in vitro study compared the physicochemical stabilities of ropivacaine and 3 dexamethasone formulations containing different preservatives.

Setting: A university medical center laboratory.

Methods: Mixtures were prepared by combining 0.75% ropivacaine with each of 3 dexamethasone formulations (Formulation 1: glycerin, disodium edetate, sodium hydroxide, and phosphoric acid; Formulations 2 and 3: same components plus benzyl alcohol). All were mixed in a 1:1 ratio in polypropylene syringes and stored at 24°C. The mixtures were evaluated for visual and microscopic changes, pH, and drug concentration for up to 2 hours using high-performance liquid chromatography.

Results: No macroscopic crystal formation was observed in any mixtures. The concentration of both drugs as well as the pH of each mixture remained stable for 2 hours post mixing. Mixtures of ropivacaine and dexamethasone without benzyl alcohol showed no linear crystals upon microscopic examination, whereas the ropivacaine and dexamethasone mixture containing benzyl alcohol formed multiple linear crystals (10 μm–20 μm) immediately after mixing, continuing for 2 hours.

Limitations: The physicochemical stability of drugs observed in vitro does not guarantee unchanged pharmacokinetics and pharmacodynamics in vivo.

Conclusion: Preservatives in dexamethasone formulations may affect the physicochemical stability of ropivacaine and dexamethasone mixtures. Formulations without benzyl alcohol may offer greater compatibility, highlighting the need for preinjection evaluation of drug mixtures and the development of clinical guidelines for safe co-administration.

Key words: Benzyl alcohol, dexamethasone, high-performance liquid chromatography, optical microscope, pH, physicochemical stability, precipitation, ropivacaine

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The global rise in the aging population, combined with posture-related musculoskeletal problems, has contributed to a higher incidence of degenerative spinal disease. Epidural steroid injections have become more frequent for managing these conditions (1,2).

In clinical practice, corticosteroids are frequently combined with local anesthetics to enhance both anti-inflammatory and analgesic effects. These combinations are typically prepared by the provider immediately before administration. Evaluating the physicochemical stability of such combinations is essential to ensure safety and efficacy. Dexamethasone, a nonparticulate corticosteroid, is widely used because of its favorable safety profile and solubility (3-6). Among local anesthetics, ropivacaine is often preferred because of its relatively lower cardiotoxicity and central nervous system toxicity, as well as its longer duration of action compared with other amide-based agents (7,8). Given these advantages, ropivacaine and dexamethasone are frequently co-administered via epidural injection.

However, several studies have reported visible or microscopic crystals forming when ropivacaine is mixed with dexamethasone (9-11). The proposed mechanism involves acid–base interactions: local anesthetics—which are weak bases formulated with acid to improve water solubility—are often mixed with corticosteroids prepared at a more alkaline pH. These pH differences can promote precipitation (9-11). In contrast, other studies have reported no evidence of instability in ropivacaine and dexamethasone mixtures (12-14). A 2024 study by Kim, et al (13) highlighted substantial variability among previous findings, particularly regarding crystal formation and the morphology and size of precipitates. Hwang, et al (9) reported linear crystals ranging from 10 μm to 100 μm; Hoerner, et al (11) and Watkins, et al (10) reported linear crystals exceeding 100 μm; Choi, et al (12) reported spherical particles less than 10 μm; and Kim, et al (13) reported no crystal formation.

These discrepancies suggest that factors beyond pH, such as the type of preservative used in commercial dexamethasone formulations, may affect the physicochemical stability of these mixtures. Kim, et al (13) proposed that preservatives play a critical role in determining the compatibility of ropivacaine and dexamethasone combinations. Crystals formed within drug mixtures can occlude blood vessels and potentially cause spinal cord or cerebral infarctions (15,16). Therefore, identifying the underlying causes and implementing preventive strategies are essential.

The primary objective of our in vitro study was to test the hypothesis—that the type of preservative used in commercial dexamethasone formulations may affect the physicochemical stability of these mixtures—by comparing the physicochemical stability of ropivacaine mixed with 3 commercially available dexamethasone formulations containing different preservatives. The secondary objective was to evaluate the relative safety of these mixtures based on preservative composition and to identify a dexamethasone formulation that may provide safer co-administration.

Methods

Drug Preparation

Ropivacaine hydrochloride (0.75%, Ropivacaine Kabi, 7.5 mg/mL, Fresenius Kabi); the first dexamethasone sodium phosphate formulation (5 mg/mL, Yuhan Corporation); the second dexamethasone sodium phosphate formulation (5 mg/mL, Jeil Pharmaceutical Co., Ltd.); and the third dexamethasone sodium phosphate formulation (5 mg/mL, Humedics Co., Ltd.) were all commercially obtained (Table 1).

Compound Mixtures Preparation

Mixtures were prepared by combining undiluted 0.75% ropivacaine with undiluted dexamethasone solutions from each of the 3 manufacturers in a 1:1 ratio (Table 2).

The preservative composition varied among the dexamethasone formulations. The first dexamethasone formulation contained glycerin, disodium edetate, sodium hydroxide, and phosphoric acid. The second and third dexamethasone formulations contained the same components, along with benzyl alcohol. All drug mixtures were transferred into polypropylene syringes. To simulate clinical practice, syringes were stored at room temperature (24°C) without light protection. The final concentrations in each mixture were 3.75 mg/mL for ropivacaine and 2.50 mg/mL for the dexamethasone formulations.

To ensure analytical accuracy, 5 replicate mixtures were prepared for each of the 3 drug combinations, yielding a total of 15 mixtures. All mixtures were prepared under aseptic conditions.

Evaluating the Analgesic Mixtures' Stability

Physical Evaluation

Visual Evaluation of Appearance, Solution Clarity, and Color

At each time point—immediately post mixing and at one and 2 hours post mixing—2 mL of the sample were withdrawn from each drug mixture. To evaluate the physical properties, the samples were

transferred to colorless silicate glass test tubes and examined against both white and black backgrounds for changes in color, turbidity, visible precipitates, or crystal formation.

Microscopic examination was conducted using an optical microscope to detect the presence of fine crystals. Samples were drawn directly from the stored polypropylene syringes and placed on glass slides for immediate observation.

Physicochemical stability was defined as the maintenance of the original appearance of the solution (clear, colorless, and free of particulate matter) throughout the 2-hour observation period (17).

Chemical Assessment

pH Measurement

The pH of each drug mixture was measured at 3 time points: immediately post mixing and at one and 2 hours post mixing. At each time point—immediately post mixing and at one and 2 hours post mixing—2 mL of the sample were withdrawn from each of the 5 replicate drug mixtures.

Measurements were obtained using a digital pH meter (Orion Star A212; Thermo Scientific). For each group, 5 independent replicate mixtures were prepared, and the pH of each replicate was measured once at each time point. The mean (SD) values were calculated, and these values were used to identify any changes in the chemical characteristics of the mixtures over time.

Drug Concentrations Evaluation

High-performance liquid chromatography (HPLC) was used to assess drug concentration stability and to detect potential decomposition peaks. Before mixture analysis, HPLC was conducted on the individual drug components to identify their respective peaks.

Samples (10 µL) were collected from each mixture immediately post mixing and again at one and 2 hours. Reverse-phase HPLC was performed using an Agilent 1200 Series system (Agilent Technologies) equipped with a G1311A quaternary pump, G1322A vacuum degasser, G1329A autosampler, and G1315C ultraviolet–visible light detector. Separation was achieved with a Luna 5-µm C18 column (inner diameter, 250 × 10 mm). The mobile phase consisted of a gradient elution with 0.05% trifluoroacetic acid in water and 0.05% trifluoroacetic acid in acetonitrile at a flow rate of 2 mL/min. Gradient conditions were as follows: from 0 to 30 minutes, acetonitrile concentration increased from 30% to 70%; from 30 minutes to 50 minutes, the mobile phase consisted of 10% water and 90% acetonitrile. Ultraviolet-visible light detector wavelengths were set to 270 nm, 290 nm, and 310 nm to detect each drug. The column was maintained at room temperature, and injection volume was 10 µL. For each mixture, the concentration of each drug immediately post mixing was defined as 100%, and the change in drug concentration over time was calculated. Data from 5 replicates for each combination were used to calculate the mean

Table 1. *Concentration, chemical formula, molecular weight, and pH of each drug used.*

Drug	Premixing Concentration (mg/mL)	Chemical formula	Molecular weight	pH mean (SD)
Ropivacaine (ropivacaine hydrochloride)	7.5	C28H36N2O8	274 (310)	4.86 (0.09)
First dexamethasone formulation (dexamethasone disodium phosphate)	5	C18H22ClNO4	392 (516)	7.36 (0.03)
Second dexamethasone formulation (dexamethasone disodium phosphate)	5	C18H22ClNO4	392 (516)	7.72 (0.18)
Third dexamethasone formulation (dexamethasone disodium phosphate)	5	C18H22ClNO4	392 (516)	7.57 (0.04)

Table 2. *Drug combinations evaluated in this study and differences in preservatives among dexamethasone formulations.*

	0.75% Ropivacaine (7.5 mg/mL)	Dexamethasone (5.0 mg/mL)	Dexamethasone Preservatives	Total amount (mL; mixing ratio)
Mixture 1	4 mL	First dexamethasone formulation 4 mL	(Glycerin, natriumedetat, natriumhydroxid, phosphoric acid)	8 mL (1:1)
Mixture 2	4 mL	Second dexamethasone formulation 4 mL	(Glycerin, natriumedetat, natriumhydroxid, phosphoric acid, benzyl alcohol)	8 mL (1:1)
Mixture 3	4 mL	Third dexamethasone formulation 4 mL	(Glycerin, natriumedetat, natriumhydroxid, phosphoric acid, benzyl alcohol)	8 mL (1:1)

(SD). Concentration stability was defined as the maintenance of 90%–110% of the initial drug concentration consistent with the United States Pharmacopeial Convention Monograph (18).

Analytical Validation

Method validation was performed in accordance with the International Conference on Harmonization (ICH) guidelines for analytical procedures (19). Parameters evaluated included linearity, accuracy, precision, and repeatability.

Calibration

The linear relationship between peak area and drug concentration was determined using linear regression analysis across a predefined concentration range. Calibration curves were constructed using 4 drug concentrations, each analyzed in quadruplicate. Method accuracy was assessed based on the calibration curves.

Accuracy

Accuracy and precision were evaluated using the relative standard deviation (RSD) or coefficient of variation of accuracy (CVa), calculated as the difference between theoretical and experimental concentrations. Measurements were repeated 4 times for each drug concentration. The CVa of each drug was calculated separately for each combination.

Repeatability

HPLC analysis, pH measurement, and visual and microscopic inspections of crystal formation were conducted by the same investigator under identical conditions to ensure consistency. Five replicate analyses were performed for each mixture using homogeneous samples. Repeatability was evaluated by calculating the mean (SD) of replicate results, expressed as the RSD or coefficient of variation of repeatability (CVr). CVr values for each drug were calculated separately for each combination.

Results

Physical Stability

Appearance, Clarity, and Color

All 3 ropivacaine and dexamethasone mixtures remained visually clear, colorless, and free of visible particles or precipitates for up to 2 hours post mixing.

However, optical microscopy of samples taken directly from polypropylene syringes and mounted on glass slides revealed distinct differences among the mixtures. In the combination of ropivacaine and the first dexamethasone mixture, no newly formed crystals were observed, except for spherical bubbles smaller than 10 μm. In contrast, ropivacaine with the second and third dexamethasone mixtures containing benzyl alcohol as a preservative showed numerous dense, linear crystals measuring 10 μm–20 μm, which appeared immediately post mixing and continued at one and 2 hours post mixing (Fig. 1).

Chemical Stability

pH Stability

The pH values of all 3 mixtures remained stable throughout the study period; no notable changes were observed over time. At each measurement point, pH values differed by less than 2.1% from the values measured immediately post mixing (Table 3).

Concentration analysis

The concentration of each drug in each mixture was determined by integrating the area under the chromatographic peaks. The retention times of ropivacaine, the first dexamethasone formulation, the second dexamethasone formulation, and the third dexamethasone formulation were approximately 9.2, 11.9, 12.0, and 11.9 minutes, respectively (Fig. 2). In addition, chromatograms of the mixtures containing ropivacaine with the second and third dexamethasone formulations consistently revealed a minor additional peak at 12.7 minutes (Fig. 2). However, these peaks were identical in area to those observed during the calibration of the second and third dexamethasone standards; no novel or unexpected peaks suggestive of degraded products were detected.

For each mixture, the concentration of each drug at time zero (immediately post mixing) was defined as 100%. Relative concentration ratios at subsequent time points were calculated from 5 replicate measurements, and the mean (SD) values were used for analysis. Time-dependent changes in mean concentration ratios of each drug were plotted to illustrate stability over time (Fig. 3).

As shown in Fig. 3, the concentration of each drug in the ropivacaine and dexamethasone mixtures remained within 90%–110% of their initial values for up to 2 hours post mixing. No additional peaks in-

dicative of degraded products were detected in any mixtures.

Linear regression analysis of concentration–time data indicated that both ropivacaine and dexamethasone retained more than 95% of their initial concentrations throughout the 2-hour observation period in all mixtures.

Analytic Validation

Calibration

Calibration was conducted using linear regression analysis for each drug concentration. The mean regression equations for ropivacaine, the first dexamethasone formulation, the second dexamethasone formulation, and the third dexamethasone formulation were $y = 476.9(x) + 0.6$ (mean R^2: 0.9999); $3095.4(x) - 42.8$ (mean R^2: 0.9998); $3029.6(x) - 23.1$ (mean R^2: 0.9999); and $3109.6(x) - 70.1$ (mean R^2: 0.9999), respectively. The calibration curves used to establish these equations are shown in Fig. 4.

Linearity and calibration

All drugs demonstrated a strong linear relationship between peak area and concentration, with excellent correlation coefficients (R^2 values). These equations were subsequently used to determine concentrations in the mixture.

Accuracy

The accuracy of the HPLC analytical method was validated by analyzing 4 known concentrations of each drug, measured in quadruplicate, in accordance with the ICH guidelines (19). The mean experimental values closely matched theoretical concentrations with RSD values below 1%, indicating high precision. The coefficients of variation for accuracy (CVa = RSD × 100) for ropivacaine, the first dexamethasone formulation, the second dexamethasone formulation, and the third dexamethasone formulation were 0.7–1.1% (accuracy ≥ 98.9%); 1.5% (accuracy ≥ 98.5%); 1.3% (accuracy ≥ 98.7%); and 1.2% (accuracy ≥ 98.8%), respectively.

In all combinations, CVa values for all 4 analyzed items (ropivacaine and the 3 specific dexamethasone formulations) remained below 1.6%, demonstrating acceptable analytical accuracy.

Repeatability

Repeatability was assessed by analyzing 5 repli-

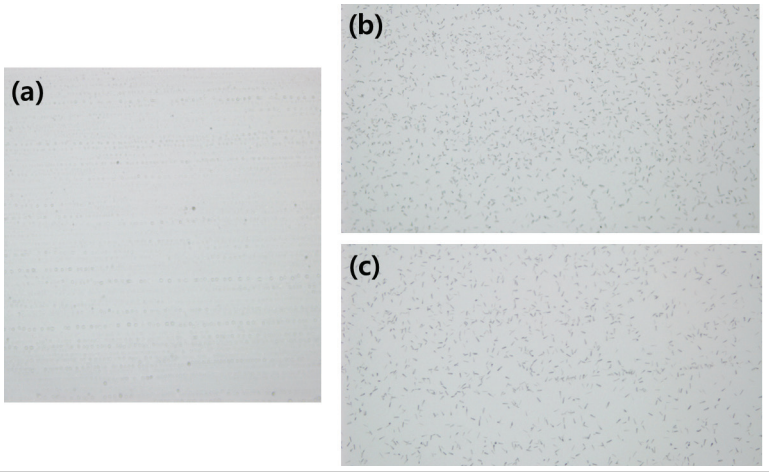

Fig. 1. *Microscopic images of ropivacaine and dexamethasone mixtures. (a) Optical microscopy image of the mixture of ropivacaine and the first dexamethasone formulation immediately post mixing (x 100); no newly formed crystals were observed, except for spherical bubbles smaller than 10 μm. (b) Optical microscopic image of ropivacaine and the second dexamethasone formulation immediately post mixing (x 100). (c) Optical microscopic image of ropivacaine and the third dexamethasone formulation immediately post mixing (x 100); numerous densely arranged linear crystals measuring 10 μm–20 μm were observed.*

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

	Mixture composition	Immediately	Time post mixing One hour	2 hours
Mixture 1	Ropivacaine + first dexamethasone formulation	6.82 (0.02)	6.79 (0.04)	6.81 (0.04)
Mixture 2	Ropivacaine + second dexamethasone formulation	6.80 (0.07)	6.66 (0.03)	6.69 (0.03)
Mixture 3	Ropivacaine + third dexamethasone formulation	6.87 (0.03)	6.87 (0.03)	6.74 (0.08)

Measured pH. Data are expressed as the mean ± SD.

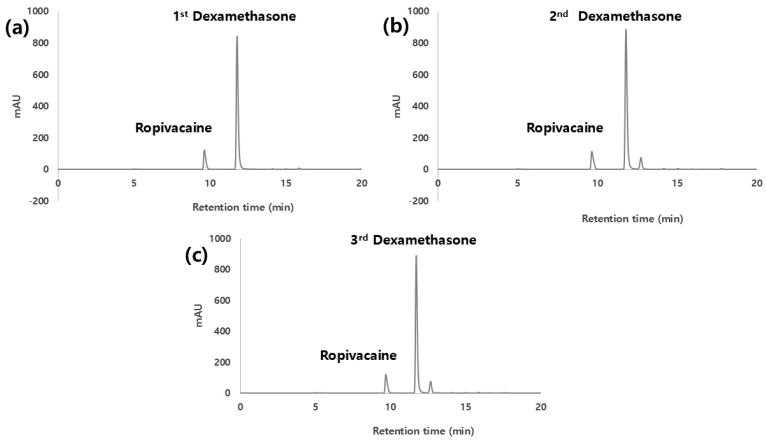

Fig. 2. *High-performance liquid chromatography peaks of each mixture immediately post preparation. (a) Ropivacaine and the first dexamethasone formulation. (b) Ropivacaine and the second dexamethasone formulation. (c) Ropivacaine and the third dexamethasone formulation.*

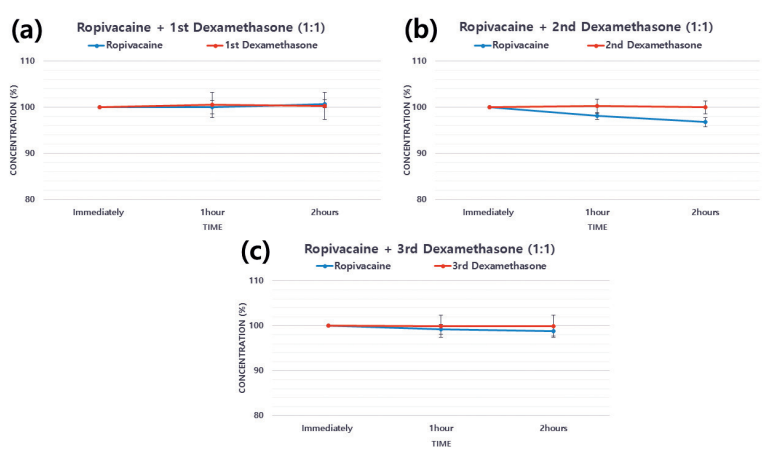

Fig. 3. *Rate of change in the concentration of each drug over time in ropivacaine and dexamethasone mixtures. (a) Ropivacaine and the first dexamethasone formulation. (b) Ropivacaine and the second dexamethasone formulation. (c) Ropivacaine and the third dexamethasone formulation.*

cates of each mixture. The coefficients of CVr (CVr = RSD × 100) for ropivacaine, the first dexamethasone formulation, the second dexamethasone formulation, and the third dexamethasone formulation were 1.2–2.2% (repeatability ≥ 97.8%); 2.8% (repeatability ≥ 97.2%); 1.9% (repeatability ≥ 98.1%); and 2.9% repeatability (≥ 97.1%), respectively.

In all mixtures, CVr values of all drugs were below 3.0%, indicating excellent repeatability of the analytical method.

Discussion

Our study evaluated and compared the physicochemical stability of ropivacaine and dexamethasone mixtures prepared using 3 commercially available dexamethasone formulations that had different preservatives.

All mixtures remained clear, with no visible crystal formation on macroscopic inspection. Drug concentrations remained stable for up to 2 hours post mixing. However, optical microscopy revealed distinct differences. In the mixture containing the first dexamethasone formulation—which did not have benzyl alcohol as a preservative—no linear crystals larger than 10 µm were observed throughout the 2-hour observation period. In contrast, mixtures prepared using the second and third dexamethasone formulations—both of which contained benzyl alcohol—had numerous linear crystals measuring 10 µm–20 µm that appeared immediately post mixing and continued for 2 hours.

Physicochemical instability in drug mixtures is most commonly attributed to pH differences between the combined agents (20-23). Previous studies reporting instability in ropivacaine and dexamethasone mixtures have primarily attributed crystal formation to pH differences, particularly due to the alkalinization of ropivacaine when mixed with dexamethasone (9-12).

However, previous findings on the physicochemical stability of these mixtures have been inconsistent, particularly regarding the observed presence, morphology, and size of the crystals. Based on these discrepancies, we hypothesized that crystal formation in ropivacaine and dexamethasone mixtures may be influenced not only by pH differences but also other factors, specifically, the type of preservative in the dexamethasone formulations.

Supporting this hypothesis, Kim, et al (13) and Choi, et al (12) reported stable ropivacaine and dexamethasone mixtures and used the same first dexamethasone formulation (Yuhan Dexamethasone) as evaluated in

our study. This formulation, which contains glycerin and disodium edetate but is free of benzyl alcohol, has been consistently associated with a lack of crystal formation.

In contrast, Hwang, et al (9) reported linear crystal formation ranging from 10 µm to 100 µm following mixing;, they used a dexamethasone phosphate injection (5 mg/mL; Daewon Pharmaceutical). This formulation contained the same components as the Yuhan formulation, but it also included benzyl alcohol and sodium bisulfite as preservatives.

These differences suggest that preservatives—particularly benzyl alcohol or sodium bisulfite—may have contributed to crystal formation when mixed with ropivacaine.

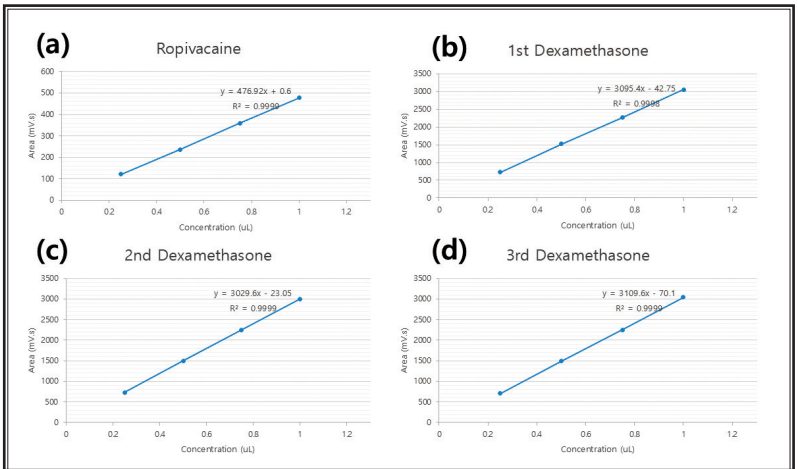

Fig. 4. *Calibration curves for each drug concentration. (a) Ropivacaine. (b) The first dexamethasone formulation. (c) The second dexamethasone formulation. (d) The third dexamethasone formulation.*

Benzyl alcohol is widely used both as a preservative and a cosolvent in injectable formulations. One possible interaction between benzyl alcohol and ropivacaine is a shift in pH. Because benzyl alcohol is weakly acidic, it may alter the pH of ropivacaine, which remains most stable in the pH range of 4–6. Depending on the concentration of benzyl alcohol, pH variations may reduce the solubility of ropivacaine, thereby promoting visible turbidity or microprecipitation in benzyl alcohol-containing formulations.

To test this hypothesis, we compared mixtures containing the first dexamethasone formulation (without benzyl alcohol) with the second and third formulations (both including benzyl alcohol but not sodium bisulfite).

No linear crystals larger than 10 µm were observed in the ropivacaine and first dexamethasone formulation mixture. In contrast, both the ropivacaine and second dexamethasone formulation mixture and the ropivacaine and third dexamethasone formulation mixture had numerous linear crystals measuring 10 µm –20 µm, which continued throughout the 2-hour observation period. These findings suggest that crystal formation is likely due to attributable interactions with benzyl alcohol, which was present only in the second and third formulations.

Further supporting the role of benzyl alcohol in crystal formation, the analysis of drug concentrations in all 3 mixtures showed that both ropivacaine and dexamethasone remained chemically stable for up to 2 hours, regardless of the presence or absence of crystals. This suggests that crystal formation was not due to degradation or instability of the active ingredients, but was instead the result of physicochemical interactions involving excipients, most notably benzyl alcohol.

We considered the possibility that sodium bisulfite, another preservative in the Daewon dexamethasone formulation used by Hwang, et al (9), may have contributed to crystal formation when combined with ropivacaine. Supporting this hypothesis, a 2024 study by Kim, et al (13) reported the formation of linear crystals measuring 50 µm –100 µm in mixtures of ropivacaine and betamethasone. The betamethasone formulation in that study, Huons betamethasone, contains sodium bisulfite as a preservative.

These findings led to the hypothesis that preservatives such as sodium bisulfite may interact with ropivacaine, promoting the formation of linear crystals. To verify this hypothesis, the Daewon dexamethasone formulation was initially considered for inclusion in our study (9). However, because production of this formulation was discontinued in 2022, we were unable to obtain this formulation. This formulation was the only dexamethasone preparation previously available in the Republic of Korea that contained sodium bisulfite as a preservative.

Sodium bisulfite is typically present in aqueous solutions with an acidic pH range of 3.5–5.5 and is widely used as a reducing agent to prevent oxidation, particularly for stabilizing compounds such as epinephrine and local anesthetics. Theoretically, sodium bisulfite may induce physicochemical instability when mixed directly with ropivacaine. First, although sodium bisulfite

is acidic, it can act as a buffering agent in the highly acidic environment of ropivacaine formulations (pH ≤ 4), potentially increasing the pH of the mixture. This pH shift promotes the conversion of ropivacaine hydrochloride (ionized form) into its nonionized free-base form, which is significantly less water-soluble and more prone to precipitation or crystal formation. Second, depending on the concentration ratios and acid–base equilibrium of the mixture, a further pH decrease cannot be ruled out. This may alter the ionization state of ropivacaine, promoting the formation of a poorly soluble, free base form.

In both scenarios, conversion to the free-base form may result in precipitation or microcrystal formation, ultimately compromising the physicochemical stability of the mixture. Therefore, steroid formulations containing sodium bisulfite as a preservative may pose an instability risk when combined with ropivacaine.

According to the United States Pharmacopeia (USP) and the US Food and Drug Administration (FDA) drug labeling information, many commercially available injectable dexamethasone formulations contain sodium bisulfite and benzyl alcohol as preservatives. Although these preservatives were included to ensure product stability and prolong shelf life, our findings suggest that they may contribute to physicochemical instability and increase the risk of precipitation when mixed with ropivacaine. Therefore, during the formulation development, particularly for products intended for co-administration with ropivacaine, preservative-related stability factors should be carefully evaluated to ensure compatibility and safety.

Limitations

The limitations of our study should be considered when interpreting the findings. First, the physicochemical stability of the drug mixtures was evaluated only under in vitro conditions. Therefore, these findings do not guarantee that the pharmacokinetic and pharmacodynamic properties of the drugs remain unchanged in vivo. Additional clinical studies are required to confirm the safety and efficacy of these mixtures for clinical use. Second, undiluted ropivacaine (0.75%) was used instead of the diluted form (0.2%) commonly used in clinical practice. This approach was selected to examine the potential for crystal formation under conditions that promote precipitation. The use of a higher concentration allowed for more sensitive detection of physicochemical changes. Third, although microscopic crystals were observed in some mixtures, structural analyses such as nuclear magnetic resonance or x-ray diffraction were not performed. Incorporating such techniques in future research may help identify the preservative-related causes of crystallization and guide formulation optimization. Finally, all experiments were conducted at a controlled room temperature (24°C). Because temperature is a critical factor affecting solubility and crystal formation, the stability observed in our study may not be fully representative of other clinical preparations or storage environments. Further investigations under variable temperature conditions are warranted to provide a more comprehensive assessment of stability.

Conclusion

Our study demonstrates that preservatives in dexamethasone formulations may influence the physicochemical stability of mixtures containing ropivacaine. Because most commercially available dexamethasone injections contain benzyl alcohol and sodium bisulfite, our findings suggest that preservative-free formulations or those with minimal excipients may provide greater stability when combined with ropivacaine.

To ensure safety and efficacy in clinical use, future regulatory frameworks should incorporate physicochemical compatibility testing into the regulatory approval criteria for formulations intended for co-administration. Furthermore, hospitals and pharmaceutical manufacturers should establish standardized stability guidelines to ensure the safe and effective use of these mixtures in clinical practice.

Authors' Contributions

All authors had full access to the data and take responsibility for the integrity and accuracy of the analysis. HK, SYL, and CHL designed the study. All authors participated in conducting the experiments, reviewing the literature, and drafting the initial manuscript. HK, SYL, and CHL revised the manuscript for important intellectual content and approved the final version for submission.

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References

1. Manchikanti L, Kaye AD, Soin A et al. Comprehensive evidence-based guidelines for facet joint interventions in the management of chronic spinal pain: American Society of Interventional Pain Physicians (ASIPP) guidelines for facet joint interventions 2020 guidelines. *Pain Physician* 2020; 23:S1-S127.
2. Sencan S, Edipoglu IS, Celenlioglu AE, Yolcu G, Gunduz OH. Comparison of treatment outcomes in lumbar central stenosis patients treated with epidural steroid injections: Interlaminar versus bilateral transforaminal approach. *Korean J Pain* 2020; 33:226-233.
3. Lee GY, Lee JW, Lee E, et al. Evaluation of the efficacy and safety of epidural steroid injection using a nonparticulate steroid, dexamethasone or betamethasone: a double-blind, randomized, crossover clinical trial. *Korean J Pain* 2022; 35:336-344.
4. Heesen M, Klimek M, Imberger G, Hoeks SE, Rossaint R, Straube S. Co-administration of dexamethasone with peripheral nerve block: Intravenous vs perineural application: Systematic review, meta-analysis, meta-regression and trial-sequential analysis. *Br J Anaesth* 2018; 120:212-227.
5. Donohue NK, Tarima SS, Durand MJ, Wu H. Comparing pain relief and functional improvement between methylprednisolone and dexamethasone lumbosacral transforaminal epidural steroid injections: A self-controlled study. *Korean J Pain* 2020; 33:192-198.
6. Derby R, Lee SH, Date ES, Lee JH, Lee CH. Size and aggregation of corticosteroids used for epidural injections. *Pain Med* 2008; 9:227-234.
7. Piper SL, Kim HT. Comparison of ropivacaine and bupivacaine toxicity in human articular chondrocytes. *J Bone Joint Surg Am* 2008; 90:986-991.
8. Verlinde M, Hollmann MW, Stevens MF, Hermanns H, Werdehausen R, Lirk P. Local anesthetic-induced neurotoxicity. *Int J Mol Sci* 2016; 17:339.
9. Hwang H, Park J, Lee WK, et al. Crystallization of local anesthetics when mixed with corticosteroid solutions. *Ann Rehabil Med* 2016; 40:21-27.
10. Watkins TW, Dupre S, Coucher JR. Ropivacaine and dexamethasone: A potentially dangerous combination for therapeutic pain injections. *J Med Imaging Radiat Oncol* 2015; 59:571-577.
11. Hoerner E, Stundner O, Putz G, Steinfeldt T, Mathis S, Gasteiger L. Crystallization of ropivacaine and bupivacaine when mixed with different adjuvants: A semiquantitative light microscopy analysis. *Reg Anesth Pain Med* 2022: rapm-2022-103610.
12. Choi EJ, Kim DH, Han WK, et al. Non-particulate steroids (betamethasone sodium phosphate, dexamethasone sodium phosphate, and dexamethasone palmitate) combined with local anesthetics (ropivacaine, levobupivacaine, bupivacaine, and lidocaine): A potentially unsafe mixture. *J Pain Res* 2021; 14:1495-1504.
13. Kim H, Choi SS, Kang TH, Byun SY, Lee CH. Physicochemical stability and compatibility of mixtures of ropivacaine with dexamethasone or betamethasone for epidural steroid injections: An in vitro study. *Pain Physician* 2024; 27:E89-E98.
14. Melton MS, Sposito J, Ribeirio A, Nielsen K, Tucker M, Kein S. Determination of compatibility and stability of a ropivacaine–dexamethasone admixture using nuclear magnetic resonance analysis, abstract. In: Anaesthesiology, Chicago, IL, 2011: Program and abstracts of American Society of Anesthesiologists Annual Meeting, October 15-19, 2011.
15. Kennedy DJ, Dreyfuss P, Aprill CN, Bogduk N. Paraplegia following image-guided transforaminal lumbar spine epidural steroid injection: Two case reports. *Pain Med* 2009; 10:1389-1394.
16. Lyders EM, Morris PP. A case of spinal cord infarction following lumbar transforaminal epidural steroid injection: MR imaging and angiographic findings. *AJNR Am J Neuroradiol* 2009; 30:1691-1693.
17. Trissel LA, Martinez JF. Physical compatibility of melphalan with selected drugs during simulated Y-site administration. *Am J Hosp Pharm* 1993; 50:2359-2363.
18. The United States Pharmacopeia, 30th rev., and the National Formulary, 25th ed. United States Pharmacopeial Convention, Rockville, MD, 2007: p. 2123.
19. Validation of analytical procedures: Methodology ICH (Q2B), Nov 1996 (CPMP/ICH/281/95). Available at: www.emea.eu.int
20. Devarajan PV, Gore SP, Chavan SV. HPTLC determination of ketorolac tromethamine. *J Pharm Biomed Anal* 2000; 22:679-683.
21. Kim YS, Lee CH, Kim AR, et al. Microbiological and physicochemical stability of fentanyl, oxycodone, hydromorphone, ketorolac, ramosetron, and ondansetron for intravenous patient-controlled analgesia: An in vitro study. *Pain Physician* 2021; 24:E829-E837.
22. Fulling PD, Peterfreund RA. Alkalinization and precipitation characteristics of 0.2% ropivacaine. *Reg Anesth Pain Med* 2000; 25:518-521.
23. Lee CH. Physicochemical stability of mixtures of non-steroidal anti-inflammatory drugs such as ketorolac and diclofenac and antiemetics such as ondansetron and ramosetron: An in vitro study. *Korean J Pain* 2025; 38:103-115.