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Stability and compatibility of methotrexate and dexamethasone in 0.9% sodium chloride for intrathecal injection

Fan Xu[1,2]* and Su Zeng[1]
[1]Laboratory of Pharmaceutical Analysis and Drug Metabolism, Zhejiang Province Key Laboratory of Anti-Cancer Drug Research, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, Zhejiang, China
[2]Kunming General Hospital of Chengdu Military Region, Kunming, Yunnan, China

Abstract: The stability of a mixture of methotrexate and dexamethasone sodium phosphate in 0.9% sodium chloride for intrathecal (IT) injection was assessed. To simulate the clinically used concentrations of the admixtures, 2mL of 0.9% sodium chloride was added into each of two vials of methotrexate. Both solution mixtures were then transferred into an intrathecal injector. A vial of dexamethasone sodium phosphate (5mg in 1mL) was subsequently added directly to the injector to obtain a mixture of 2mg/mL methotrexate and 1mg/mL dexamethasone. Subsequently, stability as assessed by visual evaluation of color changes, regular pH monitoring, monitoring of changes in particulate contents and drug concentrations in the admixture, and detection of impurities with HPLC- UV-TOF/MS before and after mixing. The results showed that admixtures were clear, no color changes were observed and the pH value remained stable under normal fluorescent room light. The ≥25-μm and ≥10-μm particulate content levels were low and within specification limits. The concentrations of methotrexate and dexamethasone exhibited no significant changes. Impurity peaks before and after mixing were not increased and no new degradation products were detected after mixing. Methotrexate and dexamethasone sodium phosphate did not affect each other's stability.

Keywords: Methotrexate, dexamethasone sodium phosphate, 0.9% sodium chloride injection, compatibility, stability.

INTRODUCTION

Complicated neurological involvement has been reported in cases of systemic lupus erythematosus (SLE), with the incidence rate ranging from 14% (for severe cases) to 83% (including mild forms) (Valesini *et al.*, 1994). Moreover, even with early diagnosis and aggressive treatment, management of neuropsychiatric SLE still represents a significant challenge (Valesini *et al.*, 1994). Currently, intravenous administration of dexamethasone combined with cyclophosphamide pulse therapy represents a standard treatment regimen for most cases of SLE (Dhabhai *et al.*, 2005). However, this treatment strategy is not feasible for patients with severe infections or for those with intolerance to systemic hormone therapy.

Valesini *et al.*, (1994) evaluated a new therapeutic approach involving the compatible usage of methotrexate and dexamethasone (fig. 1) by intrathecal injection (IT) for lupus erythematosus encephalopathy and a small portion of the patients were found to benefit from the treatment (West, 1996). The efficacy of this new therapy was further confirmed by other research groups (Wang J, 2014; Zhao WM, 2016; Zhou B, 2002; Zhou HQ, 2008), and this therapy tends to be widely used. It should be noted that an IT route of administration is associated with a high risk for adverse events, and to the best of our knowledge, there have been no studies that have addressed whether an admixture of methotrexate and dexamethasone is compatible and stable for a given period of time. Thus, it is necessary to evaluate the safety of a solution of these two drugs prior to the widespread accessibility of this clinical treatment for SLE.

In the present study, the physical compatibility and chemical stability of a preparation of methotrexate and dexamethasone in a small volume of 0.9% sodium chloride for IT injection was examined at various time points during its storage at room temperature for 12h.

MATERIALS AND METHODS

Chemicals and drugs

Two standards (methotrexate and dexamethasone sodium) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China, purity ≥98%). Methanol and acetonitrile [high performance liquid chromatography (HPLC) grade, Honeywell, Muskegon, MI, USA], Phosphoric acid (HPLC grade, Sigma, St. Louis, MO, USA) and ultrapure-water were used for all analyses.

Methotrexate and dexamethasone sodium phosphate were commercial products suitable for clinical use and were provided in an injection form. The methotrexate injection (5mg, lot #H20044248) was obtained from Haizheng Pharmaceutical Group Co. Ltd. (Taizhou, Zhejiang, China) and the dexamethasone sodium phosphate injection (5mg in 1mL solution, lot #H41021255) was purchased from Tianjin Pharmaceuticals Group Co., Ltd. (Tianjin, Beijing). The saline 0.9% sodium chloride

**Corresponding author:* e-mail: article_fan@126.com

injection (250mL, lot # L111011209) was obtained from Kelun Pharmaceutical Group Co. Ltd. (Chengdu, Sichuan, China).

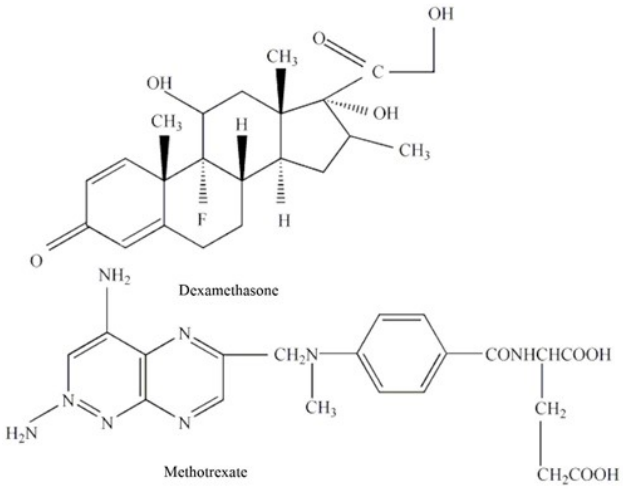

Fig. 1: Chemical structures of dexamethasone and methotrexate. x

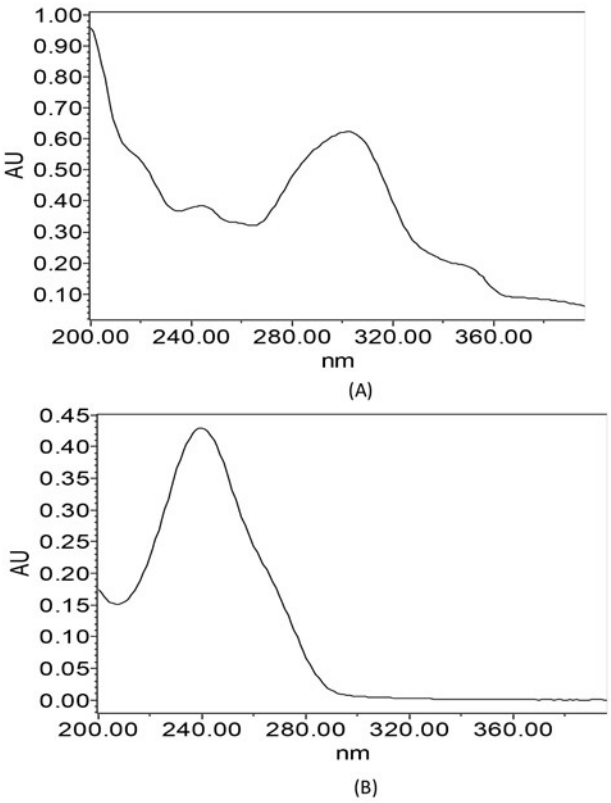

Fig. 2: UV spectra for (A) methotrexate and (B) dexamethasone.

Materials and chromatographic conditions

Quantitative analyses were performed with an 1100 series HPLC machine (Agilent Technologies, Santa Clara, CA, USA) equipped with an online degasser, quaternary pump, autosampler and column compartment, together with a 6220 series time-of-flight mass spectrometry (TOF/MS) machine (Agilent Technologies, Santa Clara, CA, USA) equipped with a standard electrospray ionization source and mass hunter workstation B02.00.

Chromatographic separation was achieved under the following conditions. The separation column was a chromasil-C_{18} column (4.6mm × 150mm, 5µm) maintained at 25ºC. The mobile phase was composed of solutions A (0.05% phosphoric acid in acetonitrile) and B (0.05% phosphoric acid in water) with a solvent flow rate of 1mL/min. The liquid chromatography gradient condition was as follows: 0-15 min in 10-30% solution A; 15-20 min in 30-45% solution A; and hold for 30 min. The injection volume was 10µL with a 10-min re-equilibration interval between successive runs. A diode-array detector was used and elution times were determined at wavelengths of 302 nm and 240 nm. The TOF/MS conditions were set as follows: 40 psi nebulizer, 10L/min drying gas flow rate, 350ºC drying gas temperature, 4000-V capillary voltage and 215-V fragmentor voltage. The MS spectra were acquired in full scan mode over a range of 105-1100 m/z through an extended dynamic range in positive ion mode. The mass axis was calibrated for every scan with the reference solutions for m/z 121.050873 and 922.009798.

The pH value of the admixtures was measured with a precision pH meter (Model pHS-3C, Leici Instrument Co., Shanghai, China). A laser injection micro particle analyzer (Model ZWF-J6, Tianhe Medical Instrument Co., Tianjin, China) was used in accordance with the specifications of the Pharmacopoeia of People's Republic of China for small-volume injections. The compatibility criteria included: ≤6000 particles ≥10µm in size per container and ≤600 particles ≥25µm per container.

Admixture preparation and storage

To simulate the concentrations commonly used in clinical practice, 2mL of 0.9% sodium chloride was added into each of two vials of methotrexate (5 mg/vial). Both solution mixtures (a total of 4mL containing 10 mg methotrexate) were then transferred into an intrathecal injector. A vial of dexamethasone sodium phosphate (5 mg in 1mL) was subsequently added directly to the injector to obtain a mixture of 2mg/mL methotrexate and 1mg/mL dexamethasone. After adequate mixing, the admixtures were stored at 20-25°C for 12h without protection from light.

Compatibility testing

Immediately after sample preparation and at specific time intervals of storage (e.g., 0, 1, 2, 4, 6, 8 and 12h), physical compatibility was assessed by visual observation of clarity, precipitation, and color change. Changes in pH and the appearance of particulate matter were also recorded. Chemical stability was simultaneously assessed by quantifying the amount of methotrexate and

Table 1: Observations over 12 h of the pH, appearance and particulate matter of a methotrexate and dexamethasone admixture in 0.9% sodium chloride that was prepared for injection

Time (h)	pH	Appearance	Particulate matter (per container)	
			≥ 10 µm	≥ 25 µm
0	7.18	Colorless	4632.5	75.0
1	7.18	Colorless	4700.0	60.0
2	7.19	Colorless	3100.0	32.5
4	7.19	Colorless	2592.5	17.5
6	7.19	Colorless	2025.0	0
8	7.17	Colorless	367.5	0
12	7.15	Colorless	50.0	0

Table 2: Percentages of methotrexate and dexamethasone detected in at various time points following admixture preparation.

Time (h) after mixing	Methotrexate (%)	Dexamethasone (%)
0	100.00	100.00
1	99.41	99.55
2	98.83	98.99
4	99.41	99.60
6	98.37	98.74
8	99.16	99.65
12	99.07	99.65

The amounts of each drugs at time-zero were considered to be 100%.

dexamethasone sodium phosphate present in the admixtures. For all admixtures, the concentrations detected within 12h were compared with the initial drug concentration. Impurities and degradation products were determined by HPLC-UV-TOF/MS, comparing the pre- and post mixing chromatograms and mass spectra. Chemical compatibility was defined as follows: (1) 95-105% retention of the initial concentrations; (2) free of impurity [i.e., no chromatographic peaks that are not attributable to the control solutions (on the basis of relative retention times) and with an area <0.10% of that for the total response]; (3) no new degradation products being formed after mixing.

RESULTS

Chromatography analysis

The sample analysis results are summarized in a three-dimensional plot of the diode array detector (DAD) signal versus the maximal absorption wave bands for methotrexate and dexamethasone sodium phosphate, which were at 301±1 nm and 240±1 nm, respectively. Representative UV chromatograms of the main drugs are shown in fig. 2.

Under the chromatographic conditions described, methotrexate and dexamethasone were separated completely from each other. The retention times were 8.5 min for methotrexate and 20.2 min for dexamethasone (fig. 3). The main impurity peaks were separated from those of intact drugs and identified by MS. Furthermore, while both drugs were found to undergo substantial degradation following their exposure to either 1 N nitric acid or 1 N sodium hydroxide at 60°C for 5h, none of the degradation products were found to interfere with the elution peaks of the intact drugs. These results confirm that the HPLC method was stable.

The calibration curves for methotrexate and dexamethasone sodium phosphate (e.g., plots of peak area versus concentration) included concentrations that ranged from 20-300µg/mL. As shown in fig. 2, both calibration curves were linear with a regression coefficient of 0.9999. The calibration equations were Ym=33300 X-160000 (n=3) and Yd=16000 X-78000 (n=3), respectively, where y is the peak-area of the drugs and x is the corresponding concentration of each drug. The intra-day and inter-day precisions (% CV) and accuracies were determined at low, medium, and high concentrations of methotrexate (50, 100 and 200µg/mL) and dexamethasone sodium phosphate (50, 100 and 200µg/mL) by replicate analyses, respectively. Intra-day and inter-day precisions were found to be within 0.22%, 0.33% and 0.09% for methotrexate and methylprednisolone sodium succinate.

Stability studies

No changes in color, clarity, or the presence of visible precipitates were observed in any of the prepared admixtures over the 12h period examined at 20-25°C. The pH values of the admixtures prepared in 0.9% sodium chloride for injection also remained constant and ranged from 7.15 to 7.18 throughout the study period (table 1).

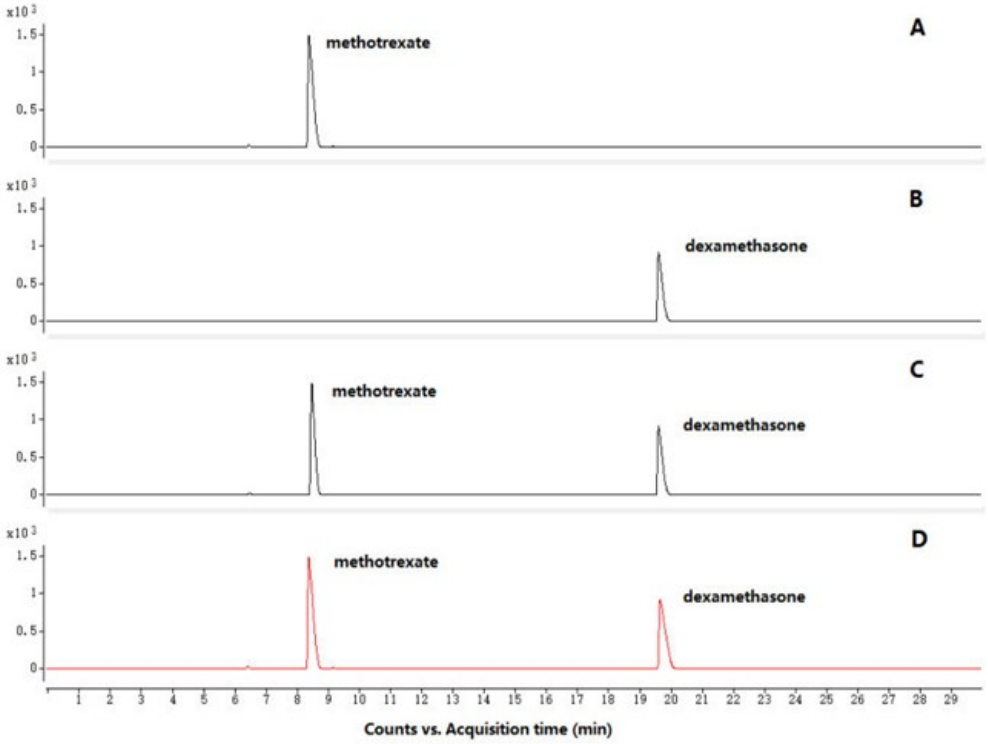

Fig. 3: UV chromatograms for methotrexate injection (A), dexamethasone sodium phosphate injection (B), a freshly prepared methotrexate and dexamethasone sodium phosphate mixture in 0.9% sodium chloride injection (0 h) (C), and a methotrexate and dexamethasone sodium phosphate mixture in 0.9% sodium chloride injection after mixing (12 h) (D).

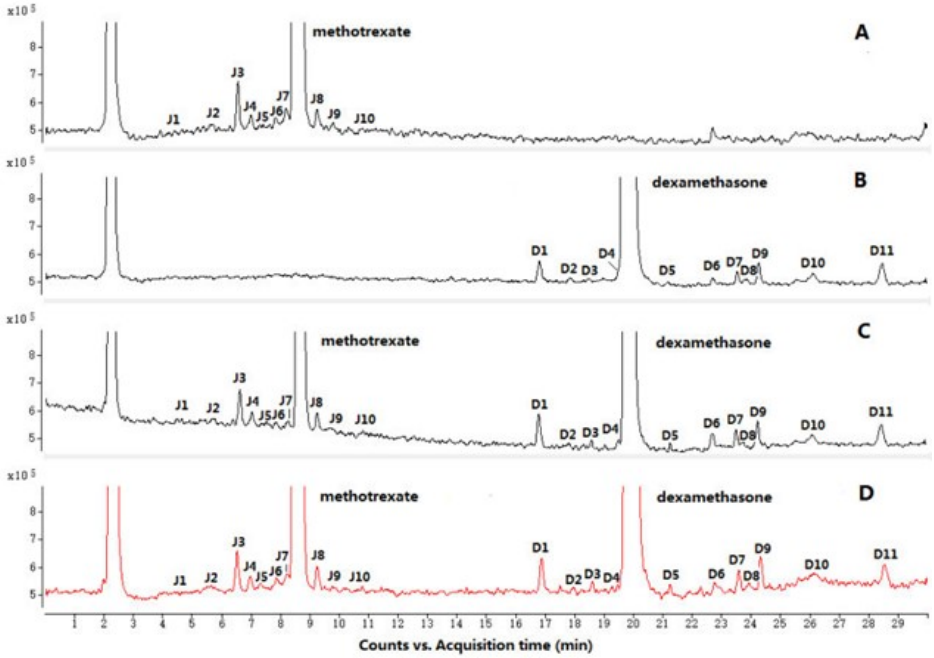

Fig. 4(1): TOF/MS chromatograms for methotrexate injection (A), dexamethasone sodium phosphate injection (B), a freshly prepared methotrexate and dexamethasone sodium phosphate mixture in 0.9% sodium chloride injection (0h) (C) and a methotrexate and dexamethasone sodium phosphate mixture in 0.9% sodium chloride injection after mixing (12h) (D). Peaks D1-D8 are the original mass spectrometry impurity peaks obtained for dexamethasone sodium phosphate injection. Peaks J1-J10 are the original mass spectrometry impurity peaks obtained for methotrexate injection.

The numbers of particles ≥ 25μm and ≤25μm yet ≥10μm, per milliliter were few and were within the specification limit (table 1). In the chemical compatibility test, the concentrations of methotrexate and dexamethasone sodium phosphate remained around 99.07% and 99.65%, respectively, in 0.9% sodium chloride after being stored for 12 h (table 2). A total of 18 impurities were detected, a number equal to the sum of impurities found in solutions of each drug alone, indicating that no new degradation products emerged following mixing (fig. 4).

DISCUSSION

Physical compatibility testing yielded test solution pH values that were near neutral and stable. The density (per ml) of particles ≥25μm and between 10μm and 25μm were low, within specifications and these densities decreased with time. These results indicate that methotrexate is a sterile powder for injection that is difficult to dissolve completely in a short period of time.

In chemical compatibility testing, we employed HPLC-UV for quantitative studies of methotrexate and dexamethasone; this method has better detection stability than TOF/MS and a robust UV response to the two target drugs. The maximal absorption wave bands for methotrexate and dexamethasone were used for dictation. Meanwhile, in consideration of its sensitivity and ability for molecular weight determination, HPLC-TOF/MS was used to identify impurities and degradation products whether or not they absorb UV energy. To improve detection efficiency and reduce variability, we merged the above methods, creating a novel HPLC-UV-TOF/MS chemical compatibility testing method. Using this method, we found that the concentrations of methotrexate and dexamethasone sodium phosphate were stable. Comparison of pre- versus postmixing chromatograms and mass spectra demonstrated that new impurity peaks emerged indicating that no new degradation products formed following mixing (fig. 4). Altogether, our compatibility and stability analysis results showed that methotrexate and dexamethasone sodium phosphate were compatible in 0.9% sodium chloride solution and thus likely suitable for IT injection.

CONCLUSION

The results of the present study demonstrated that an admixture of 10mg methotrexate and 5mg dexamethasone sodium phosphate in 5mL 0.9% sodium chloride that was prepared for injection and stored at room temperature (20-25°C) under natural light conditions underwent no significant changes in pH, apparent particulate matter, or contents. Thus, this mixture appears to be stable and compatible for at least 12 h at room temperature and these data support the clinical use of methotrexate and dexamethasone mixtures for IT injection.

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