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Qualitative and quantitative measures of various compounded formulations of 17-hydroxyprogesterone caproate

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Abstract

Objectives—17-hydroxyprogesterone caproate (17-OHPC) is available both as an FDA – approved medication and as a product prepared for individual patients by compounding pharmacies. Compounding pharmacies may omit the preservative used in the FDA approved formulation or use an alternate preservative and may dispense 17-OHPC in containers that differ from the FDA-approved product. The objective of this study was to assess the stability, microbiological and pyrogen status of 17-OHPC formulations under various compounding and dispensing conditions.

Methods—17-OHPC was prepared by a local compounding pharmacy. The formulations prepared included one identical to the FDA approved product with benzyl alcohol as a preservative, one with benzalkonium chloride as a preservative and one without a preservative. These various formulations were dispensed into either single dose 1 ml plastic syringes or glass vials or 10 ml glass vials. Concentration of 17-OHPC and microbial and pyrogen status were evaluated at various time intervals over the ensuing 19 weeks.

Results—The concentration of 17-OHPC did not change over the duration of study regardless of the dispensing medium used or the absence or presence of any preservatives. The preparations remained microbe and pyrogen free during the study period regardless of the dispensing medium used or the absence or presence of any preservatives.

Conclusions—Products containing 17-OHPC tested in this study were quite stable over the 19-week period of study in different dispensing containers and in absence or presence of a different preservative. The compounded products remained sterile and pyrogen free during the period of observation.

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DISCLOSURE: None of the authors have a conflict of interest

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Keywords

Compounding; 17 hydroxyprogesterone caproate; preterm birth

Introduction

17-alpha hydroxyprogesterone caproate (17-OHPC) has been used to reduce the rate of recurrent preterm birth since the 2003 report of Meis et al from the Maternal-Fetal Medicine Units (MFMU) Network trial demonstrating a 33% reduction in preterm birth with that therapy.¹ Use of the medication has increased following the endorsement by the American Congress of Obstetricians and Gynecologists² and the March of Dimes³ and with coverage of the cost of medication by many insurers.^{4,5} Although the Food and Drug Administration recently provided conditional approval of a product identical to the one used in the MFMU study, compounding pharmacies are not prohibited from continuing to compound 17-OHPC and many continue to do so based on the recent FDA statement that “FDA does not intend to take enforcement action against pharmacies that compound hydroxyprogesterone caproate based on a valid prescription for an individually identified patient unless the compounded products are unsafe, of substandard quality, or are not being compounded in accordance with appropriate standards for compounding sterile products”.⁶ Some compounding pharmacies have prepared formulations of 17-OHPC without preservatives due to the caution issued by FDA about the administration of benzyl alcohol to pregnant women.⁷ The impact of not using a preservative in the formulation of 17-OHPC is unclear. Additionally, the medication is most often dispensed in multi dose glass containers to minimize absorption of the 17-OHPC that is believed to occur with use of plastic containers. Single dose plastic containers provide a benefit to the user in that measurement errors by the patient are eliminated and issues related to sterility are minimized when compared to multi dose vials. Whether dispensing 17-OHPC in single dose plastic containers leads to lower amount of the drug in solution due to absorption problems or the stability and sterility of such products has not been evaluated. Our objective was to evaluate the impact of time, preservative and dispensing modality on the stability, sterility and pyrogen status of compounded 17-OHPC formulations.

Materials and Methods

This study is IRB exempt as it involves no patients or biological materials.

A. Pharmaceuticals and drug preparation

17-alpha-hydroxyprogesterone caproate (17-OHPC), benzyl alcohol (BA), benzalkonium chloride (BC), and benzyl benzoate were purchased from Professional Compounding Centers of America (Houston, TX). Castor oil was purchased from Paddock Labs (Minneapolis, MN). Stock solutions and working standards used for analysis of the chemical stability were dissolved in methanol and stored at -20 °C. Analytical grade buffer reagents and solvents were purchased from Fisher Scientific (Pittsburgh, PA).

We utilized a local pharmacy to prepare six experimental formulations, which are listed in Table 1, and reflect several options afforded to the compounding pharmacies in the preparation of 17-OHPC. Conditions differed by the preservative used (benzyl alcohol 2% or benzalkonium chloride 0.1 % or no preservative) and the dispensing medium (10 ml glass multi-dose vial or 1 ml glass vial or 1 ml plastic syringe). Appropriate amounts of 17-OHPC powder were fully dissolved in benzyl benzoate in presence or absence of the preservatives, benzyl alcohol or aqueous benzalkonium chloride. Castor oil was then added to bring the final concentration of 17-OHPC to 250 mg/ml. Concentrations of benzyl benzoate and castor

oil in the six formulations were 46% and 44–54%, respectively. Each solution was filtered (pore size 0.22 μ m) to remove particulate matter and any bacteria.

All solutions were prepared under a laminar flow hood. We assessed transparency and color of the solutions weekly. Under formulations 1 and 2, three 10 ml glass vials each were prepared. Under formulations 3, 4, 5 and 6, twelve 1 ml plastic syringes and twelve 1 ml glass vials were prepared for each condition to allow measurement of 17-OHPC concentration to be performed in triplicate at baseline and at several time intervals after preparation.

B. Content Analysis

B.1 Timing of drug stability and concentration testing—The concentration of 17-OHPC in each formulation was determined immediately after preparation. Testing was repeated at various times (2, 4 10 and 19 weeks) following preparation (Table 2). To evaluate the stability of the 17-OHPC, we removed an aliquot of the formulation from each syringe or vial and measured the content of 17-OHPC in that aliquot. Samples from all six experimental formulations were tested for drug concentration in triplicate at baseline 2, 4, and 10 weeks after specimen preparation. The 10 ml vials also provided sufficient volume for measurement of concentration in triplicate at 19 weeks.

B.2 Timing of sterility and endotoxin testing—Testing to evaluate microbiological contamination and endotoxin content was performed by Eagle Analytical Services (Houston, Texas) in triplicate at baseline then at 10 and 19 weeks on samples from the 10 ml glass vials. Microbiological and endotoxin testing on the 1 ml syringes were limited to the plastic syringes (conditions 3 and 5) due to volume constraints and costs. Testing on samples from the 1 ml plastic syringes was performed at baseline and 10 weeks after sample preparation. We combined fluid from the 3 identically formulated syringes in order to perform the analyses. Thus, the analyses for microbes and endotoxin from the 10 ml vials were done in triplicate whereas analyses of the 1 ml syringes were performed on pooled samples from the three 1 ml preparations.

The assays were performed at the intervals indicated in Table 2 and the samples were tested for the presence of bacteria, fungi, mold and yeast.

C. Analytical Methods

C.1 Drug concentration—A high performance liquid chromatography (Waters 2695 Separations Module attached to Waters 2998 Photodiode Array Detector) with chromatographic separation of various compounds was performed in a 250 $\times$ 4.6 mm, 5 μ m Symmetry C18 column with an isocratic elution was used. The mobile phase consisted of methanol/water (90:10, v/v delivered at 0.9 ml min⁻¹). The column eluents were monitored at 242 nM. The retention times of 17-OHPC was 7.0 min. The concentration of 17-OHPC in samples was quantified utilizing a standard calibration curve ranging from 1.25 ug/ml to 50 ug/ml. The peak area was plotted against the concentration and a calibration curve was generated using least-squares linear regression analysis. The assay was validated for specificity, precision (coefficients of variation 10%), accuracy (92%), and linearity (1.25 to 50 ug/mL). The limits of detection and the limits of quantitation for 17-OHPC was 5 and 62.5 ng on the column.

C.2 Microbiology—Sterility testing was performed by Eagle Analytical Services (Houston, Texas) according to the USP <71> fourteen (14)-day membrane filtration sterility test procedure. Testing was performed for bacteria, molds, yeast and fungi with tryptic soy

broth medium and thioglycollate medium at 22.5 ± 2.5 °C and 32.5 ± 2.5 °C., respectively (8). Cultures were assessed at days 3, 7 and 14 after plating.

C.3. Pyrogenic Testing—Endotoxin testing was performed by Eagle Analytical Services (Houston, Texas) using the USP(85) turbidimetric testing procedure. Quantities of endotoxin are expressed in endotoxin units per milliliter (EU/mL). The Pyros Kinetix™ Incubating Kinetic Tube Reader (ACC, MA) was utilized, giving detectable endotoxin level down to 0.001 EU/mL.⁹

Results

A. Concentration and stability of 17-OHPC

The concentration of 17-OHPC was stable throughout the 10 weeks study period. This finding was independent of the presence of or type of preservative or the dispensing carrier (Table 3). In the 2 vials with 10 ml solutions, sufficient sample was left to assess concentrations of 17-OHPC at week 19. 17-OHPC at this time point remained completely stable. The coefficient of variation within any single condition ranged between 1-and 4%. There was a difference in measured 17-OHPC at baseline (C.V. = 7%) in the 6 formulations but the compounded products changed little from the baseline over the duration of study.

B. Sterility and Pyrogenic Testing

Cultures were negative at baseline and at 10 weeks after compounding in all samples including those without preservative, those with benzyl alcohol and those with benzalkonium chloride. Endotoxin assays performed in triplicate on formulations 1 and 2 at 10 weeks were below the pyrogenic level thought to be indicative of contamination (350 EU/ml for 70 kg person) (Table 4). Endotoxin assays in formulations 3 and 5 which were performed on pooled samples from the three 1ml plastic syringes remained pyrogen-free at 10 weeks. Endotoxin assays in formulations 1 and 2, which were performed in triplicate, were within the normal range at 10 weeks

Discussion

The 17-OHPC formulation currently approved by the Food and Drug Administration (FDA) for preterm birth prevention is identical to the product (delalutin) approved in 1956 by the agency for the maintenance of pregnancy. It is composed of 250 mg of 17OHPC in castor oil (1 mL) with 46% benzyl benzoate and 2% benzyl alcohol. The benzyl alcohol is added as a preservative while the benzyl benzoate enhances the dissolution of 17-OHPC in the castor oil.¹⁰

Compounding pharmacies may make an identical product for preterm birth prevention or may choose to alter the product by omitting the preservative or making some other modification that does not alter the quantity of the active ingredient. We have demonstrated that 17-OHPC concentration is remarkably stable over 10 weeks whether it is formulated with benzyl alcohol or benzalkonium chloride as preservatives or in the absence of any preservatives. Stability of 17-OHPC was seen independent of the container material or size that it was stored in. Even 19 weeks after preparation, the concentration of drug changed little in the two formulations tested. Pyrogen free status is maintained over long periods in single dose plastic syringes even without preservative. The medication is generally dispensed in 5 ml multi-dose vials so with weekly injections, the contents in a vial are normally consumed within 5 weeks.

The observation that the concentration of 17-OHPC changes minimally over the period of study provides reassurance that a properly compounded medication does not deteriorate or precipitate over time in either a plastic or glass container. Added reassurance regarding compounded drug safety is provided by the demonstration that if the compounded medication is properly prepared sterility is maintained after preparation and that the compounded formulation remains pyrogen-free for prolonged periods of time. The finding that sterility is maintained and that pyrogens are not detected regardless of the presence or absence of preservative provides alternatives in the compounding of 17-OHPC. Likewise, the findings of our study may enhance patient convenience given the similarity in findings whether a single dose syringe is used or a multidose vial is used. Since the medication is generally given from 16 to 36 weeks, as many as 20 injections are administered. If 1 ml plastic syringes are used, the patient can receive up to ten 1 ml syringes and be confident that the drug will not deteriorate or precipitate in that time frame. Delivering the product in a 1 ml syringe will also minimize patient dosing errors and reduce the risk of contamination associated with repeated use of multi-dose vials. It is known that 17-OHPC is very lipophilic and will adhere to plastic avidly. In principle, this could lead to a considerable percentage of drug being lost on a container. Although this concept has led to the use of glass containers our findings do not support this concern. The concentrations of 17-OHPC used clinically are very high and loss of 17-OHPC is minimal if any as demonstrated by the stable concentrations reported here.

We have also noted a substantial variation in the plasma concentration of 17-OHPC in women with twin, triplet or singleton gestation.^{11,12} We can be confident that the variation seen in these subjects is not likely due to degradation of the drug, precipitation or adsorption onto the container in which it was dispensed.

The finding that 17-OHPC is stable and retains sterility without pyrogenic activity independent of preservative use has many potential benefits. In a recent publication, the Food and Drug Administration cautioned about administration of benzyl alcohol to pregnant women and infants.⁷ This caution was motivated by reports of adverse outcomes in newborns receiving benzyl alcohol in various 'flush' solutions.^{13,14} It is not clear if intramuscular administration of medications with benzyl alcohol as a preservative is encompassed in this FDA warning, nor is it clear if intramuscular administration of benzyl alcohol as a preservative poses any risk to the fetus. Some compounding pharmacies omit benzyl alcohol as a preservative in their compounded 17-OHPC product. Our results suggest that this omission does not pose any additional risk of infection to the mother receiving such a product.

Another potential benefit of our findings relate to side effects from 17-OHPC treatment. Local skin reactions are the most common side effect reported with the use of 17-OHPC.¹ The impact of benzyl alcohol on these reactions is unclear but our findings suggest that the incidence and cause of local skin reactions can now be assessed by comparing the rate of local skin reaction in women receiving 17-OHPC with or without preservative or with a different preservative such as benzalkonium chloride.

In 1956, 17-OHPC first gained FDA approval for pregnancy support in women with threatened miscarriage.¹⁰ Due to its potent and long lasting progestational effect, the medication was also used to treat uterine cancer.¹⁵⁻¹⁷ The drug was voluntarily withdrawn in 1989 by Bristol Myers Squibb due to market circumstances. Interest of the medication was renewed with publication of the MFMU study, which demonstrated a 33% reduction in recurrent preterm birth in women receiving 250mg weekly from 16- 36 weeks.¹ As the 17-OHPC formulation used in the MFMU trial was only available via compounding pharmacies, these pharmacies became the primary suppliers of the drug. A New Drug

Application (NDA) was submitted by KV Pharmaceuticals in 2010 using the data from the MFMU trial. The FDA provided conditional approval of the medication based on that single trial. Generally two independent studies are required to gain FDA approval unless “there is compelling evidence”. The FDA’s conditional approval requires a second collaborating study, which is currently underway. Generally, once a product gains FDA approval, compounding pharmacies are restrained from compounding an identical or substantially similar product for general sale. Due to the high cost of the FDA approved product (initially \$1500/dose) and the ensuing public and congressional outcry, the FDA issued a statement allowing compounding pharmacies to continue compounding 17-OHPC without repercussions from the FDA as long as certain conditions were met.⁶ As a result of this FDA statement, compounding pharmacies may, with certain restrictions, continue to provide 17-OHPC for preterm birth prevention.⁶ The results of our study provide considerable comfort to the physician and patient and latitude to compounding pharmacies providing 17-OHPC. This study was not designed to address the pros and cons of using a compounded product versus an FDA approved product but rather was intended to address the impact of preservative and container on a compounded product prepared in a standard fashion by a compounding pharmacy that follows regulatory guidelines in preparation of the compounded product. The study addresses scientific and not regulatory issues. Individual compounding pharmacies continue to bear the burden of assuring that appropriate materials and methods are reliably employed in compounding any medication

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References

1. Meis PJ, Klebanoff M, Thom E, et al. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. *N Engl J Med.* 2003; 348:2379–2385. [PubMed: 12802023]
2. American College of Obstetricians and Gynecologists. ACOG Committee Opinion. Use of progesterone to reduce preterm birth. *Obstet Gynecol.* 2003; 102(5 Pt 1):1115–1116. [PubMed: 14672496]
3. http://www.marchofdimes.com/pregnancy/preterm_labor_progesterone.html
4. http://www.nchealthystart.org/cgi-bin/advprint/print.cgi?doc=http://www.nchealthystart.org/aboutus/incite/incite_feb_07.html
5. http://www.aetna.com/cpb/medical/data/500_599/0510.html
6. <http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm249025>
7. US Food and Drug Administration. [Accessed October 05, 2012] Safety: heparin sodium injection. Jun. 2010 Available at: <http://www.fda.gov/Safety/MedWatch/SafetyInformation/ucm219000.htm>
8. <http://www.eagleanalytical.com/sitecontent/589/rapid-scan-rdi-bacteria-mold-and-fungal-test/category/462/about-testing.aspx>
9. <http://www.eagleanalytical.com/sitecontent/588/endotoxins/category/462/about-testing.aspx>
10. www.fda.gov/ohrms/dockets/ac/06/slides/2006-4227S1-01-01-Adeza.pdf
11. Caritis SN, Simhan HN, Zhao Y, et al. Relationship between 17-hydroxyprogesterone caproate concentrations and gestational age at delivery in twin gestation. *Am J Obstet Gynecol.* 2012;207. [Epub ahead of print.]. [PubMed: 23220510]

12. Caritis SN, Sharma S, Venkataramanan R, et al. Pharmacology and placental transport of 17-hydroxyprogesterone caproate in singleton gestation. *Am J Obstet Gynecol*. 2012;207. [Epub ahead of print.]. [PubMed: 23220510]
13. Gershanik J, Boecler B, Ensley H, McCloskey S, George W. The gasping syndrome and benzyl alcohol poisoning. *N Engl J Med*. 1982; 307:1384–1388. [PubMed: 7133084]
14. CDC MMWR Weekly Neonatal deaths associated with use of benzyl alcohol—United States. Jun 11. 1982 31:290–291. Available at: <http://www.cdc.gov/mmwr/preview/mmwrhtml/00001109.htm>.
15. Junkmann K. Estrogens with prolonged action. *Exp Pathol Pharmacol*. 1953; 220(5):358–64.
16. Medroxyprogesterone acetate (Depo-Provera) vs. hydroxyprogesterone caproate (Delalutin) in women with metastatic endometrial adenocarcinoma Piver MS, Barlow JJ, Lurain JR, Blumenson. *Cancer*. 1980; 45(2):268. [PubMed: 6985833]
17. Reifenstein EC. The treatment of advanced endometrial cancer with hydroxyprogesterone caproate. *Gynecol Oncol*. 1974; 2:377. [PubMed: 4616884]

Table 1

Formulation According to Container and Preservative

Formulation	Container	Preservative
1	10 ml multi-dose glass vial	Benzyl Alcohol
2	10 ml multi-dose glass vial	Benzalkonium Chloride
3	1 ml plastic syringe	None
4	1ml glass vial	None
5	1 ml plastic syringe	Benzalkonium Chloride
6	1 ml glass vial	Benzalkonium Chloride

All six formulations contained 250 mg/ml 17-hydroxyprogesterone caproate dissolved in castor oil. Each container was filled to the designated capacity

Table 2

Time of Quality Assurance Assays

Test	Weeks After Drug Preparation				
	Baseline Testing	2	4	10	19
17-OHPC Concentration	X	X	X	X	X [*]
Microbiology	X ^{**}			X ^{**}	
Endotoxin	X ^{**}			X ^{**}	

* Testing only performed for conditions 1 and 2 (10 ml multi-dose glass vials)

** Testing for conditions 1 and 2 performed in triplicate. Testing for conditions 3 (1 ml plastic syringe without preservative) and 5 (1 ml plastic syringe with benzalkonium chloride) were performed on pooled specimens

Table 3

17-OHPC Concentration Over Time with Various Formulations

Formulation	Baseline	17-OHPC concentration (mg/mL) *					
		Week 2	Week 4	Week 10	Week 19	Mean (SD)	
Benzyl alcohol in 10-mL vial	251±6	273±3	271±9	270±9	260±8	265±9	
benzalkonium chloride in 10-mL vial	262±2	258±5	258±4	263±7	257±5	260±5	
No preservative in 1-mL plastic syringe	225±3	226±3	230±2	225±6	NA	226±2	
No preservative in 1-mL glass syringe	229±7	226±8	232±9	228±13	NA	229±3	
benzalkonium chloride in 1-mL plastic syringe	267±8	268±5	272±10	275±9	NA	270±4	
Benzalkonium chloride in 1-mL glass syringe	251±13	272±5	257±14	269±6	NA	262±10	

* Values are mean ± SD (n=3 for each formulation at each time point)

Table 4

Endotoxin assessment of different formulations of 17-OHPC.

Formulation	Baseline [*]	Week 10 [*]	Week 19 [*]
Benzyl alcohol in 10-mL glass vial	< 1 /< 1 /< 1	< 1 /< 1 /< 1	3.5 /< 1 /< 1
Benzalkonium chloride in 10-mL glass vial	< 1 /< 1 /< 1	< 1 /< 1 /< 1	34 /< 1 /< 1
No preservative in 1-mL plastic syringe ^{**}	< 1 ^{**}	< 1 ^{**}	NA
No preservative in 1-mL glass vial	NA	NA	NA
Benzalkonium chloride in 1-mL plastic syringe [*]	< 1 ^{**}	< 1 ^{**}	NA
Benzalkonium chloride in 1-mL glass vial	NA	NA	NA

^{*}
= EU/ml^{**}
based on samples pooled from 3 syringes