Bioidentical compounded hormones: A pharmacokinetic evaluation in a randomized clinical trial

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1. Introduction

With an aging population globally, the world’s total menopausal population, estimated at 476 million in 1990, is expected to rise to 1.2 billion by 2030 [1]. In the United States, approximately 75%-80% of postmenopausal women report vasomotor symptoms [2]. A large proportion of these women also experience vaginal dryness [3], urinary incontinence [3], decline in sexual interest and satisfaction [4], mood fluctuations [5,6], sleep disturbances [7-9], and changes in memory and cognition, for which hormone therapy is often sought. The collective impact of these symptoms on women’s well-being is enormous, and the need for safe treatments is compelling [10].

Conventional hormone therapy consisting of Food and Drug Administration (FDA)-approved products is the standard of care in the United States when treatment is indicated for menopausal symptom relief. However, randomized trials such as the Heart and Estrogen/Progestin Replacement Study [11] and Women’s Health Initiative [12] raised concerns about the safety of conventional hormones like Premarin™ and Provera™, leading to a marked reduction in their use and creating a demand for safer alternatives.

Bioidentical hormones have gained popularity as possible alternatives to conventional hormones. The term “bioidentical”

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Objective: Bioidentical compounded hormone therapy is popular among patients, but providers do not have pharmacokinetic information or dosing guidelines for these preparations. Our objective was to compare the pharmacokinetics of the commonly used compounded preparations with conventional hormonal preparations that are considered bioequivalent in practice.

Methods: We conducted a randomized, blinded, four-arm 16-day clinical trial of forty postmenopausal women assigned to one of three doses of a compounded estrogen cream (Bi-est (80:20); 2.0, 2.5, or 3.0 mg)+compounded oral progesterone 100 mg, or to a conventional estradiol patch (Vivelle-Dot™ 0.05 mg)+Prometrium™ 100 mg. Serum levels of estrone, estradiol, estril, and progesterone were obtained at multiple time intervals during the first 24-h, and at steady-state.

Results: Results were analyzable for 37/40 women. Study medications were well tolerated. The AUC at 24 h and at steady-state for estrogens remained consistently lower for all doses of Bi-est tested relative to the patch. The difference was statistically significant for Bi-est 2.0 mg (AUC-estradiol = 181 vs. 956; p < 0.001) and 2.5 mg (AUC-estradiol = 286 vs. 917; p = 0.001). Estril levels remained low in all study arms. Serum progesterone levels were comparable in conventional vs. compounded groups.

Conclusions: This pharmacokinetic trial showed that the currently used doses of compounded hormones yield lower levels of estrogen compared to the standard-dose estradiol patch. To find comparable doses, further studies are needed. This successfully conducted randomized controlled study attests to the feasibility of using a similar design in the setting of a larger clinical trial.

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Table 1
Study design.

<table>
<thead>
<tr>
<th>No. of patients/arm</th>
<th>Randomization</th>
<th>Active intervention</th>
<th>Placebo</th>
<th>Intervention phase (16 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Estrogen</td>
<td>Progesterone</td>
<td>Patch</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>Bi-est 2.0 mg</td>
<td>Compounded progesterone 100 mg</td>
<td>Serial measurements E1, E2, E3</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>Bi-est 2.5 mg</td>
<td>Compounded progesterone 100 mg</td>
<td>Patch</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>Bi-est 3.0 mg</td>
<td>Compounded progesterone 100 mg</td>
<td>Patch</td>
</tr>
<tr>
<td>10</td>
<td>Estradiol 0.05 mg patch (Vivelle-Dot™)</td>
<td>Micronized progesterone 100 mg capsule (Prometrium™)</td>
<td>Cream</td>
<td></td>
</tr>
</tbody>
</table>

implies a chemical and molecular structure precisely the same as its endogenous human hormone counterpart. Structural similarity by some is believed to correlate with safety, a perception that has led to a sharp increase in the popularity of bioidentical hormones.

Currently there are multiple FDA-approved conventional hormone products that are plant-derived and bioidentical in chemical structure [13]. However, likely due to extensive media coverage, requests from women for bioidentical hormones are predominantly for the compounded preparations. In the absence of adequate research, no clear guidelines exist for choosing the most appropriate type, dose, or regimen of bioidentical compounded hormones. Lacking this data, it is challenging to study these preparations for safety and efficacy.

To the best of our knowledge, no pharmacokinetic studies have compared conventional with compounded bioidentical hormones. Compounding pharmacists generally consider Bi-est (referring to estradiol plus estradiol) in a dose of 2.5 mg (for example, in a ratio of 80% estradiol plus 20% estradiol, which consists of 2 mg estradiol and 0.5 mg estradiol) as approximately equivalent to a mid-range dose of an estradiol-containing patch such as Vivelle-Dot™ 0.05 mg. Thus, using Bi-est with 80% estradiol and 20% estradiol, and comparing the calculated estradiol equivalence of the compounded preparations with the daily referenced estradiol dose of the patch, we assigned the three strengths of creams to create a reasonable range for comparison.

In order to lay the groundwork for future trials, we conducted a pharmacokinetic study as a randomized, blinded clinical trial. Our intent was to compare estrogen and progesterone levels obtained following the administration of bioidentical compounded hormone therapy preparations both with themselves and with conventional hormone therapy.

2. Methods

2.1. Subjects

The study was approved by the Mayo Clinic Institutional Review Board. Since estradiol is not an approved product in the United States, an IND (investigational new drug) application was filed with the US-FDA. This was a Phase I, blinded, randomized, four-arm study. Subjects were recruited from Rochester, MN, and surrounding areas through advertisements and news media releases. Eligibility was based on the following criteria: women 40–60 years old, naturally postmenopausal (absence of periods for ≥ 1 year or amenorrhea for ≥ 6 months and FSH ≥ 40 IU/L) or surgically postmenopausal (menopause induced by removal of ovaries), able to understand and sign an informed consent, and willing to stay overnight in a Clinical Research Unit (CRU). Eligible women had normal results on screening tests (AST, creatinine, and TSH within 20% of the upper limit of normal) and a negative mammogram within the last 11 months. Presence or absence of menopausal symptoms was not used as an eligibility criterion. Subjects were excluded using the following criteria: estrogen levels >35 pg/mL; >10 years from last menstrual period; a history of cancer of the breast, uterus or ovary, coronary artery disease, stroke, dementia, migraine, deep venous thromboembolism, active liver or gallbladder disease, uncontrolled hypertension, diabetes, or lupus; currently smoking; alcohol or substance abuse; family history of premenopausal breast or ovarian cancer; or postmenopausal breast cancer in ≥ 2 relatives. Recent use of hormone therapy was allowed with an adequate washout period (at least 1 week for vaginal hormones, 4 weeks for transdermal hormones, and 8 weeks for oral hormones). Additional exclusions included peanut allergy, current use of isoflavone-containing products, and drugs or herbs that might affect metabolizing enzymes.

2.2. Study treatment

Eligible women were randomized to one of four treatment arms in a double-blinded fashion. Three study arms included compounded estradiol–estradiol cream (Bi-est) in varying dosage, along with a placebo skin patch, and compounded oral micronized progesterone capsules. The fourth arm included an estradiol-containing patch (Vivelle-Dot™), placebo cream, and commercially available oral micronized progesterone capsules (Prometrium™) (Table 1).

Bi-est (80:20) 2.5 mg was chosen as it is considered equivalent to 0.05 mg estradiol patches in compounding practice. Bi-est was compounded in Vanicream and dispensed in premarked individual syringes by an experienced compounding pharmacist (RAW), who was blinded to the participants’ study group assignment. Women in study arm one received Bi-est 2.0 mg (1.6 mg estradiol and 0.4 mg estradiol), in arm two Bi-est 2.5 mg (2 mg estradiol and 0.5 mg estradiol), and arm three Bi-est 3.0 mg (2.4 mg estradiol and 0.6 mg estradiol). Women in study arm four received Vivelle-Dot 0.05 mg patches.

On day one of the study, participants were admitted to the hospital in the CRU, where they were instructed to apply their first dose under supervision by trained nurses. The creams were applied to a specified area of the forearm by gentle rubbing for 1 min. The Vivelle-Dot™ patch was applied to the skin of the lower abdomen following the manufacturer’s directions. The capsules were administered orally under supervision. Throughout the next 24 h, serial blood samples were obtained at specified intervals to measure the serum levels of estrogen fractions and progesterone (Table 2). After 24 h of the CRU stay, participants were discharged and continued taking study medications as instructed. They returned on the 15th day of the study and were admitted for 12 h during which time serial blood samples were obtained to measure the steady-state
levels of estrogen and progesterone. Participants returned on day 16 for the last set of blood draws. They returned any remaining supply of medications. Compliance was assessed by counting syringes, patches, and capsules that were returned, as well as by self report.

2.3. Hormone measurements

Estrogen fractions (Estrone E1, E2) were measured by liquid chromatography–tandem mass spectrometry (ThermoFisher Scientific, Franklin, MA and Applied Biosystems–MDS Scix, Foster City, CA). For E1, the intra-assay coefficient of variations (CVs) were 10.9%, 3.4%, 5.1%, and 3.5% at 6.7, 29, 109, and 332 pg/mL respectively, and the inter-assay CVs were 8.1%, 6.9%, 5.2%, 6.3%, and 6.7% at 6.4, 26, 58, 120, and 336 pg/mL respectively. For E2, the intra-assay CVs were 12.4%, 3.1%, 5.0%, and 3.5% at 7.2, 29, 109, and 325 pg/mL respectively, and the inter-assay CVs were 9.2%, 8.6%, 9.0%, 6.6%, and 4.8% at 7.0, 24, 61, 125, and 360 pg/mL respectively. The limit of quantitation for both E1 and E2 was 2.5 pg/mL. Values less than 2.5 pg/mL were reported as <2.5 pg/mL. Estriol (E3) was measured by using the Access® Unconjugated Estriol assay, which was a competitive binding immunoenzymatic assay measured on the Dxi automated immunoassay system (Beckman Instruments, Chaska, MN). The inter-assay CVs were 6.4%, 4.6%, and 4.9% at 1.04, 2.78, and 5.65 ng/mL respectively, and the lowest reportable result was 0.07 ng/mL. Progesterone was measured by a competitive binding immunoenzymatic assay on the Dxi 800 automated immunoassay system (Beckman Instruments, Chaska, MN). The inter-assay CVs were 16.5%, 10.1%, and 7.7% at 0.76, 9.2, and 29.6 ng/mL respectively. The lower limit of the clinically reportable range for progesterone was 0.08 ng/mL.

2.4. Statistical analysis

The concentration of each estrogen fraction at the start of the study period (C0), and at 24 h after treatment application (C24) was measured, as was the difference between the baseline and 24-h concentrations (C24 – C0). We also calculated the average concentration and area under the curve (AUC) for each of the three estrogen fractions over the first 24 h. Due to the marked variability observed in the individual concentration–time profile plots, maximum concentration (Cmax) and time to maximum concentration (Tmax) were not analyzable. The results from each of the three cream groups were compared with the Vivelle-Dot patch group. Progesterone levels were measured at baseline, 24 h, and at the end of study. We calculated the difference between the mean concentration at 24 h and at the end of the study compared to baseline. The combined results from the compounded micronized progesterone groups were compared against the conventional Premarin group.

Subject characteristics were summarized using mean ± standard deviation, median (min, max) for continuous variables, and frequency counts for categorical variables. E1, E2, and E3 parameters described previously were summarized separately for each of the 4 treatment groups. The rank sum test was used to compare each of the Bi-est groups to the Vivelle-Dot patch group. As a secondary analysis, progesterone levels were summarized and compared between the Bi-est arms and Vivelle-Dot group using the rank sum test. In all cases, two-tailed p-values ≤ 0.05 were considered statistically significant. The frequency of adverse events was summarized according to treatment group and compared across groups using the Fisher exact test.

3. Results

3.1. Participants

Fifty-two postmenopausal women were screened: 12 of those were excluded and all 40 women who were randomized into the trial completed the study (Fig. 1). The average age of the study participants was 54 years; their average BMI was 29 kg/m² (Table 3). The compliance rate for use of Bi-est creams and Vivelle-Dot patch was 100%. Only one participant returned a single unused progesterone capsule. Two participants whose initial screening estradiol levels were in the postmenopausal range had elevated baseline estradiol levels on repeat testing before administration of the study medications (82 pg/mL and 151 pg/mL respectively) and were excluded. A third participant with non-physiologic estradiol levels (1500 pg/mL), presumably from contamination with estrogen cream at the venipuncture site, was excluded after the study medications were administered. Data were analyzable for 37/40 women.

3.2. Estrogen pharmacokinetics

Average concentrations and AUC were calculated for estrogen fractions after initial dosing and at steady-state (Tables 4 and 5). The Bi-est cream groups showed a slight trend toward increasing serum levels of estrogen with an increasing dose of estrogen. However, participants had wide fluctuations in their estradiol level with Bi-est, both after the initial administration as well as at steady-state (Figs. 2 and 3). In contrast, the estrogen absorption with Vivelle-Dot patches was more consistent across the ten participants given the patch, and the pattern was similar to what is reported in the literature (Fig. 2).

3.2.1. Estradiol

In the Bi-est groups, for the majority of subjects a peak estradiol level was absent after a single-dose administration (Fig. 2). The 24-h E2-AUC was smaller in all Bi-est groups compared to the estradiol patch group, with the difference being statistically significant for Bi-est 2.0 and 2.5 mg. The steady-state E2-AUC (Days 15, 16) was also smaller for all Bi-est groups compared to the patch, with the difference being statistically significant for the Bi-est 2.0 and 2.5 mg groups (Table 4).

3.2.2. Estrone

Estrone (E1) levels in the Bi-est groups remained lower than for the patch, both after initial administration of the medication, as well as at steady-state (Fig. 4). The 24-h E1-AUC and
steady-state E1-AUC were smaller across all Bi-est groups compared to the patch, and the difference was statistically significant for the Bi-est 2.0 and 2.5 mg arms (Table 5).

3.2.3. Estriol
Most participants had E3 levels below the lower limit of detection of the assay, both at baseline and at steady-state. All E3 assays were run in triplicate to evaluate the reliability of these results. Acknowledging the limitation of a wide coefficient of correlation due to very low serum values, average E3 values were comparable at baseline. The 24-h median AUC for the Bi-est 2.0 mg arm and the steady-state median AUC for the Bi-est 2.0 and 2.5 mg arms were significantly lower than the patch ($p < 0.05$) (Fig. 5).

3.3. Progesterone pharmacokinetics
Progesterone levels were analyzed in the 37/40 women whose estrogen levels were confirmed to be menopausal. The levels at

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**Table 3**
Baseline characteristics of study participants.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Bi-est 2.0 mg (n = 10)</th>
<th>Bi-est 2.5 mg (n = 7)</th>
<th>Bi-est 3.0 mg (n = 10)</th>
<th>Vivelle-Dot 0.05 mg (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>53.7 ± 4.3</td>
<td>55.1 ± 3.6</td>
<td>52.9 ± (3.8)</td>
<td>53.8 ± 2.7</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>54 (44, 59)</td>
<td>55 (50, 59)</td>
<td>53 (48, 59)</td>
<td>54 (48, 57)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.9 ± 5.1</td>
<td>28.8 ± 9.3</td>
<td>29.3 ± 6.5</td>
<td>28.1 ± 5.8</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>30 (23, 38)</td>
<td>27 (18, 42)</td>
<td>28 (21, 44)</td>
<td>28 (20, 38)</td>
</tr>
<tr>
<td>Prior conventional hormone therapy use</td>
<td>No</td>
<td>6</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Prior compounded hormone therapy use</td>
<td>No</td>
<td>10</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
</tbody>
</table>
baseline were comparable in all 4 groups. The levels increased comparably at 24 h and at steady-state with administration of both the compounded and conventional bioidentical progesterone preparations; no intergroup differences were noted (Table 6).

3.4. Effect of BMI on estrogen

There was wide variability in the BMI of the study participants. Since the enzymes in body fat are involved in estrogen metabolism, we plotted E3 levels against BMI. We used the Vivelle-Dot patch group, as this group had no exogenous E3 administered and thus any rise in E3 was assumed to be from interconversion. The coefficient of correlation between BMI and E3 was noted to be −0.33 at steady-state, and the p value was not statistically significant (Fig. 6).

3.5. Safety

The study medications were well tolerated. No patient discontinued the study due to medication side effects. The majority (57%) of reported symptoms were deemed to be unrelated to study
medications (e.g., upper respiratory infection, gastroenteritis). Of the remaining symptoms, most were mild to moderate, and most (71%) resolved by day 23 at the time of final follow-up phone visit. The reported symptoms included abdominal bloating and pain (n = 6, 15%), upper respiratory infection (n = 5, 12.5%), fluid retention (n = 3, 7.5%), gastroenteritis (n = 3, 7.5%), skin irritation (n = 3, 7.5%), progestosterone withdrawal bleed (n = 2, 5%), and insomnia, dizziness, fatigue and vaginal discharge in one patient each (n = 1, 2.5% each).

### 4. Discussion

Our study was designed as a comparative prospective pharmacokinetic trial, with the goal to compare the pharmacokinetics of the conventional and compounded hormonal preparations that are considered bioequivalent in clinical practice. To the best of our knowledge, this is the first study of its kind in the literature.

Contrary to our expectations, we found that the dose of Bi-est 2.5 mg, commonly prescribed in lieu of the estradiol 0.05 mg patch,
yielded much lower estrogen levels compared to the patch. Even a higher dose of Bi-est 3.0 mg yielded lower estrogen levels than the 0.05 mg Vivelle-Dot patch, although the difference didn’t reach statistical significance.

We found that patterns of estrogen absorption with Bi-est creams were highly variable and showed no consistent peak of absorption. Vivelle-Dot, on the other hand, showed a pattern of absorption similar to what is reported in the literature [14]. These differences may be explained by the interindividual variations in skin physiology, blood flow, and hormone metabolism. More importantly, use of different vehicles of administration may also explain these differences, as seen from studies of estrogen in hydroalcoholic gels [14,15]. Our results cannot be compared directly to another cream-based preparation owing to non-availability of any such FDA-approved hormone cream.

FDA-approved E3 assays test the high E3 levels of pregnancy. Their reliability in detecting the extremely low postmenopausal E3 levels is unproven. Acknowledging this limitation, our results showed a small rise in E3 in all treatment groups, regardless of whether E3 was administered exogenously or not. We observed a surprising trend toward increased E3 levels in the E2 patch group compared to the E2+E3 cream groups, which may be partly explained by endogenous conversion from higher E2 in the patch group to higher E3 levels. Overall, we observed only a small increase in E3 and hypothesize the possibility of a rapid conversion of E3 to some of its metabolites, or interference in its absorption due to Vanicream. Thus, future studies testing E3 metabolites and utilizing other vehicles of absorption are needed. In our study, progesterone levels were comparable at several time points after initial dosing as well as at steady-state in all groups, thereby suggesting that oral absorption of natural micronized progesterone is comparable across compounded and conventional preparations.

From a clinical perspective, the finding of small increments in estradiol levels with low-dose Bi-est formulations raise the question of how much symptomatic benefit attributed to these doses is derived from a placebo effect. Our findings also raise the question as to whether the observed estrogen levels with the compounded formulations in current doses are sufficient to potentiate any bone benefits in menopausal women, which needs to be studied in future clinical trials.

There are certain limitations to our study. First, our choice of particular formulations and dosing was empiric because there were no evidence-based guidelines available to direct us. Second, our results can be generalizable to Vanicream-based compounded formulations only. We chose Vanicream as the compounding medium as this is commonly used in the compounding pharmacy at our institution. Third, although we measured E3 levels in triplicate using a state-of-the-art automated immune assay, the test has significant variability and poor reliability at very low postmenopausal levels. Lastly, since we tested multiple medication doses, we chose the multiple arm randomized controlled design without cross-over due to time and cost issues.

Our study has several strengths. First, this is the first study of its kind to compare the pharmacokinetics of compounded and conventional bioidentical hormones. Second, the study design was randomized, controlled, and blinded, which optimizes the reliability of results. All study medications were prepared and dispensed by the same experienced compounding pharmacist (RAW),
thereby minimizing variation in compounding. The study utilized a strict protocol for medication application, thus reducing the potential for variability in results attributable to inconsistent administration of the product. Finally, the successful design and conduct of this study supports the feasibility of conducting research comparing compounded hormone therapies with well-studied, gold-standard hormone therapy products. This is important as the lay public, as well as medical providers, are increasingly seeking reliable information regarding these widely used products.

In conclusion, this pharmacokinetic trial provides information regarding bioidentical compounded preparations and bioidentical conventional hormonal preparations that are considered bioequivalent in practice. More studies are needed to evaluate clinical as well as pharmacokinetic differences between compounded formulations, using various vehicles or administration and dosages. The knowledge gained from this study provides the groundwork for future trials to determine the safety and efficacy of bioidentical compounded hormones. This successfully conducted randomized, controlled, blinded multi-group study attests to the feasibility of using a similar design in the setting of a larger clinical trial.

Contributors

Richa Sood, Lynne Shuster, Darrell Schroeder conceptualized and designed the study. The manuscript was drafted by Richa Sood, Lynne Shuster, Deborah Rhodes, Dietlind Wahner-Roedler, Rebecca Bahn. Laboratory services and analysis were carried out by Ravinder Singh. Pharmaceutical services were provided by Roger Warndahl. Analysis and interpretation of data were done by Richa Sood, Darrell Schroeder, Ravinder Singh, Lynne Shuster, Rebecca Bahn, Deborah Rhodes. Statistical analysis was carried out by Darrell Schroeder. All authors have read and approved the final version of the manuscript.

Competing interest

The authors declare no conflict of interest.

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Role of funding source

Solvay was not involved with the study design, data collection and analysis, or writing of the paper.

Ethical approval

The study was approved by the Mayo Clinic institutional Review Board.

References