Investigation of the Bioaccessibility of Progesterone (Micronized and Non-micronized), Using an *In Vitro* Model of the Human Gastrointestinal System

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Abstract

The aim of this study is to evaluate the bioaccessibility of two progesterone oral formulations (micronized progesterone 100 mg SR capsules and milled progesterone 100 mg SR capsules), using an in vitro model of the human GI tract. Results show that overall bioaccessibility of micronized progesterone (21.8%) was significantly greater than that of milled progesterone (1.9%), $p < 0.001$. The higher total bioaccessibility could potentially be due to the smaller particle size of micronized progesterone (< 6 μm), in comparison to that of milled progesterone (approximately 92 μm). One may hypothesize that oral compounded micronized progesterone has the potential for greater bioavailability because of the significantly higher bioaccessibility of micronized progesterone in comparison to milled progesterone. Knowledge of this study's results may be useful for practitioners when justifying the viability of using micronized progesterone over milled progesterone in the management of postmenopausal symptoms.

Keywords: Bioaccessibility, bioavailability, dissolution, Loxoral, menopause, particle size, pharmaceutical compounding, progesterone

1. Introduction

In the United States, the standard of care for women seeking treatment for postmenopausal symptoms is the use of hormones, such as estradiol and its counterpart progesterone, that are approved by the Food and Drug Administration (FDA) (Sood*et al*., 2013). However, progesterone is a highly lipophilic molecule, and as such has poor aqueous solubility and absorption in the Gastrointestinal (GI) tract. Particle size reduction through micronization is a common method that can be utilized to increase the solubility and GI absorption of drugs. The micronization of progesterone decreases the average diameter of progesterone particles and thereby increases the surface area of the drug, facilitating aqueous dissolution in the small intestine (Bolaji *et al*., 1993). Dissolution is a process that involves the release of drug molecules when the solid phase of a dosage form establishes contact with the liquid phase (e.g. gastric fluid) (Chu *et al*., 2012). When progesterone's dissolution increases, it is likely that the drug's bioaccessibility, the portion of drug that is released into the GI tract and is available for absorption, also increases. This could potentially result in greater drug bioavailability, the amount of drug reaching systemic circulation (Hargrove *et al*., 1989).

Micronized progesterone is commercially available in the United States as Prometrium® and is approved for the prevention of endometrial hyperplasia in nonhysterectomized postmenopausal women receiving conjugated estrogen (AbbVie Inc., 2013). Results from the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial recommend that oral micronized progesterone be the first choice for estradiol-opposing therapy in postmenopausal women (Miller *et al*., 1995; Barrett-Connor *et al.*, 1997).

Though oral micronized progesterone has a more favorable absorption and bioavailability profiles in comparison to those of synthetic and natural progesterone, the pharmacokinetics of micronized progesterone is known to be highly variable among patients (Fitzpatrick & Good, 1999; Liu *et al.*, 2010). Therefore, the use of a formulation of micronized progesterone that can be tailored to individual patients may be a valuable therapeutic alternative to consider. Pharmaceutical compounding involves the preparation of customized medications to meet the specific needs of individual patients (U.S. Pharmacopeial Convention, 2014). Compounded medications may be personalized to include special combinations of active substances in particular strengths, as well as specific raw materials and organoleptic characteristics adjusted to the patient's specific symptoms and comorbidities. Micronized progesterone can be compounded into capsules for oral administration. The purpose of this study is to evaluate the bioaccessibility of two progesterone oral formulations (micronized progesterone 100 mg Sustained-Release (SR) capsules and milled progesterone 100 mg SR capsules), using an *in vitro* model of the human GI tract. Both micronized and milled progesterone were compounded with Loxoral, a proprietary excipient blend containing isomalt that can be combined with a variety of active pharmaceutical ingredients and incorporated into capsules for oral administration. This can be used in the preparations of oral immediate-release or SR capsules, with the latter being in combination with Methocel® E4M (PCCA, 2014), an excipient consisting of cellulose polymers that allows for slow release of medications (Zur, 2013).

2. Materials and Methods

2.1 Materials

Micronized progesterone (lot numbers: C148624, C158636, and C159277), Methocel® E4M Premium CR (lot number: C155691), and Loxoral (lot number:1309189) were obtained from PCCA (Houston, TX, USA). Milled progesterone (lot number: 89043/B) was obtained from MEDISCA, Inc. (Plattsburgh, NY, USA).

2.2 Particle Size Testing

Particle size distribution testing of micronized progesterone was performed by Intertek Allentown (Allentown, PA, USA) using a Horiba LA-910 laser diffraction instrument. Three lots (C148624, C158636, and C159277) of micronized progesterone were analyzed and the mean and median particle size distributions were determined using laser diffraction volume-based calculations. One sample of milled progesterone (lot number: 89043/B) was analyzed in duplicate for particle size distribution by Micromeritics Pharmaceutical Services (Norcross, GA, USA) using a Saturn DigiSizer II instrument.

2.3 Preparation of Progesterone SR Capsules

Micronized and milled progesterone SR capsules used for dissolution and bioaccessibility testing were prepared by mixing appropriate amounts of micronized (lot number: C148624) or milled (lot number: 89043/B) progesterone powders, Methocel® E4M (lot number: C155691), and Loxoral (lot number: 1309189) together, with trituration, in a mortar and pestle. The powdered mixtures were then encapsulated in size 1 capsules using the hand filling method. In this method, the caps and bases of empty capsules were first separated from one another. By hand, the base of each capsule is then pressed into the powder layer with thickness that is one-third the length of the capsule. The pressing is repeated to gather more powder until resistance is felt, indicating that the capsule is full. Sealing of the capsule is then done by pressing the cap onto the base of the capsule (Allen, 1999).Details relating to the compositions of each micronized or milled progesterone capsule are shown in Tables 1 and 2.

2.4 Assessment of Micronized Progesterone Dissolution Using the US Pharmacopoeia (USP)-2 System

The examination of micronized progesterone release from Loxoral/Methocel® E4M capsules was performed in duplicate (n=2) using a simulated gastric fluid/gastrointestinal fluid (SGF/SGIF) model employing a USP Apparatus 2 with the rotating paddle method, as described previously, with minor modifications (Hamoudi *et al.*, 2011). Each micronized progesterone capsule was introduced into SGF without pepsin and after 60 minutes, simulated intestinal fluid without enzymes was added. Samples were withdrawn at 30, 60, 90, 240, 480, 720, and 1,440 minutes and were filtered upon withdrawal through a 0.45 μm Tuffryn filter. Progesterone was assayed directly using high-performance liquid chromatography at a wavelength of 242 ηm, and the cumulative amount of progesterone released was calculated.

2.5 Assessment of Micronized and Milled Progesterone Bioaccessibility Using the TIM-1 System

The bioaccessibility of micronized and milled progesterone was examined using the dynamic *in vitro* TIM-1 GI model, which simulates the human GI tract from the stomach to the small intestine.

The TIM-1 model comprises of a stomach compartment and 3 small intestinal compartments containing fluids (gastric acid, gastric enzymes, bile, pancreatic juice, and bicarbonate) that mimic the physiological conditions encountered during transit through the GI tract. Analysis of the bioaccessibility of micronized and milled progesterone oral capsules using the TIM-1 system was performed in duplicate (n=2) at 37°C as described previously, with minor modifications (Dickinson *et al.*, 2012). The test conditions simulated the fasted state in healthy human adults, including the pH profile of the stomach, stomach emptying, concentration of secreted bile, pancreatic enzymes, and pH of the small intestine compartments. In order to measure the fraction of progesterone available for absorption, fluid samples were collected from the stomach, duodenum, jejunum, and ileum compartments at 60, 120, 180, 240, and 300 minutes using a semi permeable Spectrum® membrane filtration unit with a cutoff of 0.05 µm. The ileum effluent, which simulates fluid containing drug that would enter the colon and be unavailable for absorption, was collected each hour for a total of 5 samples per run and was diluted 1:1 with ethanol to dissolve any undissolved progesterone. Upon completion of the experiment, remaining progesterone that was not absorbed was measured by sampling of the residues in the stomach, duodenum, jejunum, and ileum compartments, which were collected separately. The compartments were washed with ethanol, and the wash fluids were collected and pooled with the corresponding residues. Progesterone concentrations in each sample were measured using liquid chromatography-mass spectrometry and the total amount of progesterone recovered from each compartment was calculated with correction for recovery. The overall bioaccessibility of the two formulations of oral progesterone tested was calculated by combining the bioaccessibilities of the jejunum and ileum compartments over time.

2.6 Statistical Analysis

All results are displayed as mean ± Standard Deviation (SD). A two-way analysis of variance with Bonferroni post hoc correction was performed to compare the bioaccessibility of micronized and milled progesterone over time in the TIM-1 system. A Student's t-test was performed to compare residues of micronized and milled progesterone in each compartment within the TIM-1 system following each run. All statistical analyses were performed using GraphPad Prism version 5.0b for Mac OS X (GraphPad Software, San Diego, CA, USA).

3 Results

3.1 Particle Size Analysis

In order to determine the particle size distributions of micronized and milled progesterone, a sample of each powder was analyzed using laser and light scattering analysis. Results from particle size analysis can be found in Table 3. For micronized progesterone, mean and median particle sizes were 4.97 to 5.75 µm and 4.36 to 5.09 µm, respectively, with a SD of 2.86 to 3.43 µm. For milled progesterone, mean and median particle sizes were 92.31and 89.87 µm, respectively, with a SDof 0.45 µm.

3.2 Dissolution

The dissolution profile of compounded oral micronized progesterone was determined using a SGF/SGIF model. The proportion of micronized progesterone that was dissolved from Loxoral/Methocel® E4M capsules increased rapidly up to 480 minutes and achieved a maximum dissolution of 9.83% at 1,440 minutes (Figure 1).

3.3 Bioaccessibility Comparison

The bioaccessibility of micronized and milled progesterone was analyzed using the TIM-1 system. Results show that the total bioaccessibility of micronized progesterone (21.8%) was significantly higher than that of milled progesterone (1.9%), $p < 0.001$. The higher total bioaccessibility of micronized progesterone compared with milled progesterone was significant from 120 minutes throughout the remainder of the study (Figure 2). This increase stemmed from the increased bioaccessibility within the jejunum from 120 minutes to 240 minutes (Figure 3) and within the ileum from 240 to 300 minutes (Figure 4). Both micronized and milled progesterone formulations demonstrated similar levels of progesterone that were not absorbed after transit through the TIM-1 system (30 mg and 37 mg progesterone, respectively). However, the total amount of progesterone residue within the TIM-1 system after transit was slightly higher for milled progesterone (86.7%) than for micronized progesterone (72.1%). Additionally, progesterone residue was significantly higher for micronized progesterone in the duodenum compartment and trended higher for milled progesterone in the ileum compartment, although this latter difference was not statistically significant (Figure 5).

Finally, the total amount of progesterone in the ileum effluent (i.e., the fluid content emptied from the small intestine into the large intestine that is not available for absorption) was significantly lower for micronized progesterone than milled progesterone (Figure 6).

4. Discussion

The bioavailability of progesterone is known to be highly dependent upon particle size, vehicle, and route of administration (Hargrove *et al*., 1989; Fitzpatrick & Good, 1999). Micronization of progesterone is known to increase the accessible surface area of the active pharmaceutical ingredient, potentially increasing drug dissolution and allowing for greater absorption within the GI tract (Bolaji *et al*., 1993; Chu *et al*., 2012). This may lead to greater serum progesterone concentrations and enhanced efficacy. Results from particle size analysis show that oral compounded micronized progesterone has an inherently smaller particle size (< 6 μm) than oral compounded milled progesterone (approximately 92 μm). The smaller particle size seen with micronized progesterone is advantageous because when particle size is reduced, this increases surface area and potentially enhances drug dissolution (Chu*et al*., 2012).

When comparing bioaccessibility of micronized progesterone to that of milled progesterone, overall bioaccessibility of micronized progesterone was significantly higher than that of milled progesterone, $p < 0.001$. The higher total bioaccessibility could be due to the smaller particle size of micronized progesterone, which may have allowed for increased drug dissolution within the GI tract. Since drug bioaccessibility is the portion of the drug that is available for absorption in the small intestine, and drug bioavailability is the amount of drug that actually reaches the systemic circulation (Hargrove *et al.,* 1989), it is likely that an increase in bioaccessibility may lead to an increase in drug bioavailability. Based on the *in vitro* results of this study, one may hypothesize that oral compounded micronized progesterone has the potential for greater bioavailability because of the significantly higher bioaccessibility of micronized progesterone in comparison to milled progesterone. The increase in bioavailability may potentially lead to increased efficacy of the progesterone formulation in the management of postmenopausal symptoms. However, such prediction is only theoretical and future *in vivo* studies are needed to establish such *in vitro in vivo* correlation.

5. Conclusions

Although progesterone has shown to be useful in women for the management of postmenopausal symptoms (Miller *et al*., 1995; Barrett-Connor *et al.*, 1997), the low solubility and poor drug absorption within the GI tract limit the ability of this drug to exhibit its full therapeutic effects following oral administration (Bolaji*et al.,* 1993). This study demonstrates the potentials for increased solubility and absorption of progesterone, *in vitro,* through particle size reduction, as total percentage of micronized progesterone available for absorption within the GI tract was significantly greater than that of milled progesterone. Knowledge of this study's results could potentially be useful for practitioners when justifying the viability of using micronized progesterone over milled progesterone in patients suffering from postmenopausal symptoms. Additionally, oral compounded micronized progesterone may serve as a valuable treatment alternative to consider in patients who are unable to tolerate or obtain adequate symptomatic relief from commercially available micronized progesterone formulations. Compounded micronized progesterone could potentially offer a more personalized approach to hormone replacement therapy.

6. Acknowledgements

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Table 1: Composition of a Single Micronized Progesterone 100 mg SR Capsule

Ingredient	**Quantity (mg)**
Micronized progesterone, USP	100
Methocel® E4M Premium CR (Hypromellose USP)	115.20
Loxoral	54.88

Table 2: Composition of a Single Milled Progesterone 100 mg SR Capsule

Ingredient	**Quantity (mg)**
Milled progesterone, USP	100
Methocel® E4M Premium CR (Hypromellose USP)	115.20
Loxoral	116.82

Table 3: Comparison of Particle Size between Micronized and Milled Progesterone

Sample	**Mean Particle Size (µm)**	**Median Particle Size (µm)**	**Standard Deviation (µm)**
Micronized progesterone	4.97 to 5.75	4.36 to 5.09	2.86 to 3.43
Milled progesterone	92.31	89.87	0.45

Figure 1: Dissolution profile of micronized oral progesterone in a simulated GI fluid model. Compounded oral micronized progesterone was incubated for 60 minutes in SGF, followed by incubation for up to 1440 minutes in SGIF. Samples were withdrawn over a 24-hour period and assayed for the amount of progesterone released. Results are displayed as mean ± SD. SGF = simulated gastric fluid; SGIF = simulated gastrointestinal fluid.

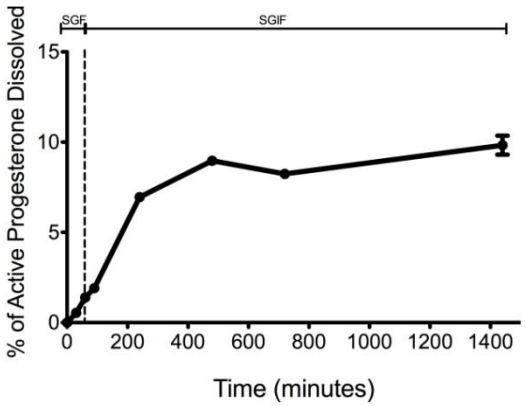

Figure 2: Total bioaccessibility of oral micronized progesterone and oral milled progesterone over time in the TIM-1 simulated GI tract model system. Progesterone capsules (100 mg) were subjected to the stomach (60 minutes), duodenum (10 minutes), jejunum (80 minutes) and ileum (150 minutes) compartments. Samples were withdrawn at 60, 120, 180, 240, and 300 minutes and assayed for progesterone concentration. The amount of progesterone recovered from each compartment was corrected for recovery and is displayed as mean ± SD. *** p < 0.001; ** p < 0.01; * p < 0.05.

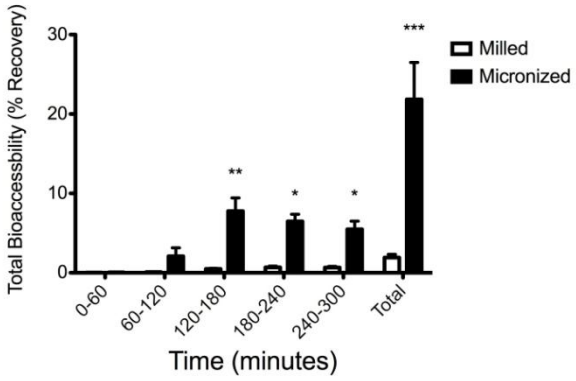

Figure 3: Bioaccessibility of oral micronized progesterone and oral milled progesterone in the jejunum compartment over time in the TIM-1 simulated GI tract model system. Progesterone capsules (100 mg) were subjected to the stomach (60 minutes), duodenum (10 minutes), jejunum (80 minutes) and ileum (150 minutes) compartments. Samples were withdrawn at 60, 120, 180, 240, and 300 minutes and assayed for progesterone concentration. The amount of progesterone recovered from each compartment was corrected for recovery and is displayed as mean ± SD. *** p < 0.001; ** p < 0.01.

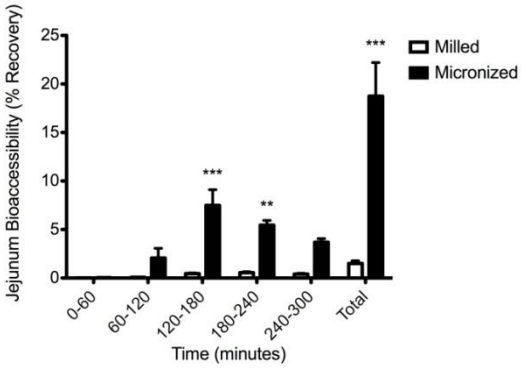

Figure 4: Bioaccessibility of oral micronized progesterone and oral milled progesterone in the ileum compartment over time in the TIM-1 simulated GI tract model system. Progesterone capsules (100 mg) were subjected to the stomach (60 minutes), duodenum (10 minutes), jejunum (80 minutes) and ileum (150 minutes) compartments. Samples were withdrawn at 60, 120, 180, 240, and 300 minutes and assayed for progesterone concentration. The amount of progesterone recovered from each compartment was corrected for recovery and is displayed as mean ± SD. *** p < 0.001; * p < 0.05.

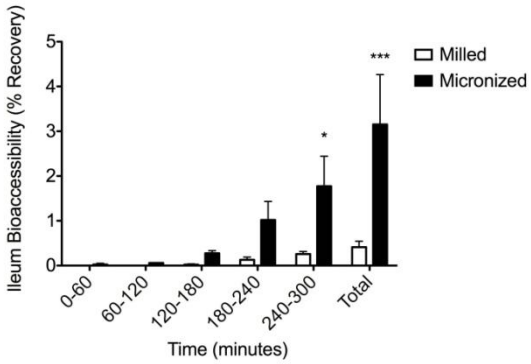

Figure 5: Micronized progesterone residue and milled progesterone residue present in specific compartments of the TIM-1 simulated GI tract system after completed TIM-1 runs. Residues from each compartment were collected separately upon completion of each TIM-1 run. The amount of progesterone remaining undissolved was measured and was corrected for recovery and is displayed as mean ± SD. * p < 0.05.

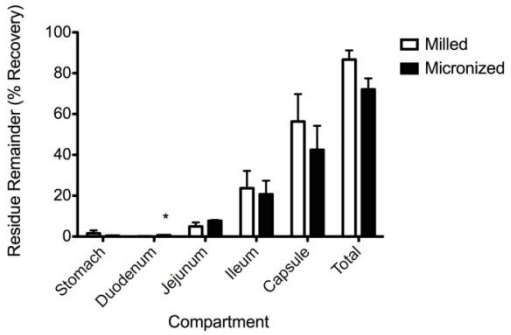

Figure 6: The non-bioaccessible portion of micronized and milled progesterone present within the ileum effluent over time in the TIM-1 simulated GI tract model system. Progesterone capsules (100 mg) were subjected to the stomach (60 minutes), duodenum (10 minutes), jejunum (80 minutes) and ileum (150 minutes) compartments. Ileum efflux samples were withdrawn at 60, 120, 180, 240, and 300 minutes and assayed for progesterone concentration. The amount of progesterone recovered from each compartment was corrected for recovery and is displayed as mean ± SD. * p < 0.05.