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Original Research

Comparison between direct use and PLGA nanocapsules containing drug of traditional Chinese medicine, Tiaojing Zhixue, in treatment of dysfunctional uterine bleeding

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Abstract: Dysfunctional uterine bleeding is menstrual bleeding in abnormal volume, duration, or time, and it is a common problem in women. A wide range of drug therapies, with varying efficacy, is available for women with dysfunctional uterine bleeding. The use of herbal and traditional medicine is one of the ways to treat this disease, which has fewer side effects than chemical drugs. On the other hand, these medicines have less effect on treatment than chemical drugs. Therefore, increasing their effectiveness in the treatment of diseases has always been important. For this purpose, in this study, a comparison was done between direct use and PLGA nanocapsules containing Tiaojing Zhixue, in the treatment of dysfunctional uterine bleeding. First, PLGA nanocapsules containing Tiaojing Zhixue were synthesized by the electrospray technique. Then 80 women with dysfunctional uterine bleeding were treated with this medicine. These people were divided into two groups of 40 people. The first group was treated with 20mg of Tiaojing Zhixue and the other group was treated with PLGA nanocapsules containing Tiaojing Zhixue for eight months. The duration and frequency of bleeding from one month before the start of treatment and during the eight months after the start of treatment (second, fourth, and eighth month) were assessed in two groups. The two groups were homogeneous in terms of mean frequency of bleeding and mean duration of bleeding before starting treatment. The positive response in the PLGA nanocapsules treatment group (75%) was higher than the direct use drug treatment group (42.5%) ($P < 0.01$). The rate of side effects was the same in each group. Due to the effectiveness of PLGA nanocapsules in the treatment of dysfunctional uterine bleeding and the lack of side effects, it can be considered as an alternative medicine for the treatment of this disorder.

Key words: Dysfunctional uterine bleeding; Nanocapsules; Tiaojing Zhixue; Traditional chinese medicine.

Introduction

Irregular menstruation without obvious uterine lesions is called dysfunctional uterine bleeding, which accounts for 50% of cases (1). These women are exposed to various diseases such as anemia, hemorrhage during pregnancy and postpartum hemorrhage and their quality of life decreases. Proper diagnosis and management can reduce unnecessary complications and surgical interventions (2, 3). Treatment for these patients includes nonsteroidal anti-inflammatory drugs, hormonal drugs, intrauterine devices, and surgery. Oral contraceptive pills are one of the main treatments for these disorders, which have many side effects such as nausea, headache, acne, cardiovascular disease, vascular clots and cancer (4-6).

Despite the increasing success of conventional medicine, many diseases, especially chronic ones, have not been treated or are inadequately treated. But traditional medicine gives patients the power to choose their health (7). It is believed that their products are inherently harmless and safer than industrial ones. Of course, in relation to the increase in patients 'demand for traditional medicine, it should be noted that surpassing patients' demand for physicians provides opportunities for products and treatments that may be ineffective or even dangerous (8). Therefore, there is a need for optimal medicine today; this means using all the treatments that may benefit a patient. In this context, if traditional medicine is integrated with conventional medicine can be a benefit for the patient, and can eliminate harmful treatments (7). Chinese medicine is one of the oldest and richest traditional medicines. Tiaojing Zhixue is a Chinese medicine that has been studied to treat dysfunctional uterine bleeding (9). One way to increase the effectiveness of this drug is to use new methods. One of these methods is the encapsulation of drugs (7).

Encapsulation of drugs has attracted a lot of attention because it can increase therapeutic efficiency, biocompatibility, and dissolution rate (10). Different encapsulation methods are used to achieve the desired size of active particles, which largely depends on the type of application. In general, the average particle size is in the range of 100-300nm for venous release, 1-5µm for release into the lungs in powder or gas form, and 0.1-10µm for the oral tract (11, 12).

There are many methods for producing small particles and many of them are used in drug formulations,

the most commonly used methods are solvent evaporation from the emulsion, fluidized bed coating, sedimentation, spray drying, etc. Although these particle production methods have been used successfully, each of them has its drawbacks and may not be suitable for specific compounds and applications (13). Many of these methods have disadvantages such as low encapsulation efficiency or slow separation of particles from the aqueous phase (14). Although these reduction methods are very efficient for molecules with crystalline structures, they are inefficient due to the complexity of these techniques for biological unstable molecules such as porous and hollow particles, non-spherical particles, nanoparticle composites, and surface materials (encapsulated) (15).

A good solution for the production of nanoparticles or micron particles from biologically active molecules is electrohydrodynamic microprocessing, a process in which a liquid jet is broken down into droplets by electric forces (16). Biocompatible and biodegradable polymers are increasingly finding their way into medical applications such as the long-term release of peptides or protein therapies and vaccines using micro-spheres made of Poly-lactic-co-glycolic acids (PLGA)(17). Poly-lactic-co-glycolic acid (PLGA) is widely used as a carrier in drug delivery systems due to its biocompatibility to glycolic acid and lactic acid throughout the body. PLGA and electrospray have been used for drug delivery purposes with successful particle production (18, 19).

Therefore, the aim of this study was to the comparison between direct use and nanocapsules of PLGA containing Tiaojing Zhixue, to find out the effectiveness of integrating traditional Chinese medicine with the new method of pharmacy in the treatment of dysfunctional uterine bleeding.

Materials and Methods

Patients

This study was performed as a randomized clinical trial. The effect of the independent variable, ie, direct use and nanocapsules of PLGA containing Tiaojing Zhixue, was investigated on the dependent variable, ie dysfunctional uterine bleeding. The statistical population was women with dysfunctional uterine bleeding. Inclusion criteria were: patient satisfaction to participate in the study, more than 6 months of dysfunctional uterine bleeding, no use of hormonal drugs and antidepressants one month before the study, no use of calcium channel blockers two weeks before, No previous allergic reaction to traditional medicine, specially Tiaojing Zhixue, no history of kidney disease, diabetes, hypothyroidism and depression, no serious physical or mental health problems, being able to answer questions, and not taking any medication at the time of the study.

In order to determine the sample size, 40 patients in each group were studied which total participants were 80 patients. Subjects were randomly divided into two groups of direct use treatment and nanocapsules of PLGA containing drug treatment. After two months, the patients were referred and the necessary information about the severity and average duration of daily bleeding was collected by the researcher in a questionnaire. The duration of treatment in both groups was 8 months. Patients also did not know about the kind of drug. The daily dose of Tiaojing Zhixue was 20mg. Patients were re-examined two, four, and eight months later and the duration and severity of bleeding were assessed.

Preparation of PLGA nanocapsules containing Tiaojing Zhixue

In order to prepare a polymer solution for electrospray, a synthetic copolymer of polylactic-glycolic acid (PLGA) with a ratio of lactic acid to glycolic acid of 50/50 with an average molecular weight of 24-38kDa. The solvents were ethanol with a purity of more than 99.9%, acetone with a purity of 99.9% and acetic acid with a purity of 99.9% were purchased from Merck, Germany.

Spraying systems are based on voltage source, syringe pump and collector. Solutions containing polymer and drug were loaded into a 1ml syringe and sprayed into the produced capsules. Produced capsules from the liquid jet were collected on aluminum foil.

The size and morphology of the nanocapsules were examined by scanning electron microscope (SEM). For each sample, the size of the capsules was measured at a voltage of 15kv from SEM images. The sample was coated with a thin layer of gold for 90 seconds before imaging.

To measure the drug release rate, a certain weight of the drug-containing nanocapsules in 10ml of phosphate buffer solution (pH=7.4) and 37°C was subjected to continuous stirring for 24 hours. The phosphate buffer solution was kept constant at 37°C. The precipitated capsules were removed and resuspended in 10ml of fresh phosphate buffer solution. At a certain time interval (1h) a certain volume of capsules was centrifuged at 4000rpm for 20 min. The precipitated particles were removed and their release was measured at 280nm by an ultraviolet spectrophotometer. Because the Tiaojing Zhixue contains diosmin, the UV-vis adsorption was considered at 280 nm (20).

To determine the thermal properties of the samples, polymer powder and drug and their solutions mixture were measured by differential scanning calorimeter (DSC). 5mg of the samples were treated with dry nitrogen in an aluminum pan. Samples were heated from ambient temperature to 250°C with a scan rate of 10°C/min. Thermogram analysis confirms that the amount of samples remains constant and that no effective amount of solvent remains in the samples.

Statistical analysis

The collected data were analyzed using SPSS (version 17) software, and Chi-square, t-test and correlation analysis at the significance level of $P \leq 0.05$.

Results

PLGA nanocapsules containing Tiaojing Zhixue

The difference voltage between the spray nozzle and the ring plays a key role in determining the spray pattern (21). The applied voltage and the distance from the nozzle to the plate have essentially the same effect because the electric field strength is measured by these parameters. Voltage controls the size and morphology of the capsules. In this study, a voltage of 15 kV with

a constant current of 0.2µl/min was used to synthesize PLGA nanocapsules. The morphology of the PLGA nanocapsules containing Tiaojing Zhixue by scanning electron microscope (SEM) showed that the nanoparticles were synthesized correctly and their size was approximately between 100-200 nm (Figure 1). Therefore, the selected voltage was the right voltage to produce nanoparticles in the size of between 100 and 200 nanometers.

Differential scanning calorimetry was used to investigate the thermal and natural properties and intramolecular interaction of the PLGA-encapsulated drug (Figure 2). The physical condition of the drug and PLGA can affect the stability and release of the drug from the capsules. The drug can be present in crystal or amorphous form in the crystal or amorphous PLGA matrix. Reactions between the drug and PLGA can cause a change in thermal peak. In PLGA/Drug sample = 0.45/0.25, heat absorber peak at 47°C indicates Tg for PLGA and the other peak is related to the drug which has been transferred to a lower temperature, which indicates the presence of the drug in the structure and the interaction between them. The boiling point of 47 °C in mixtures represents the Tg of the polymer.

Measurement of UV-vis absorption showed that the drug was present in the nanocapsules and was not destroyed by spraying. Release of drug from a biodegradable polymer is done by three mechanisms: 1- Disposal of surface drugs, 2- Drug release from the polymer matrix, 3- Gradual degradation of the polymer matrix. The sudden release of the drug in the early minutes could be attributed to the presence of free drug molecules on the surface, without binding. The release process slowed down over time, and according to the graphs, after 24 hours at 37°C, 90% of the total drug was released into the phosphate buffer (Figure 3). Therefore, the entire drug remaining in the polymer matrix was slowly released until the total PLGA was destroyed. A large reduction in drug release between 40-50% indicated a high degree of drug binding to nanocapsules.

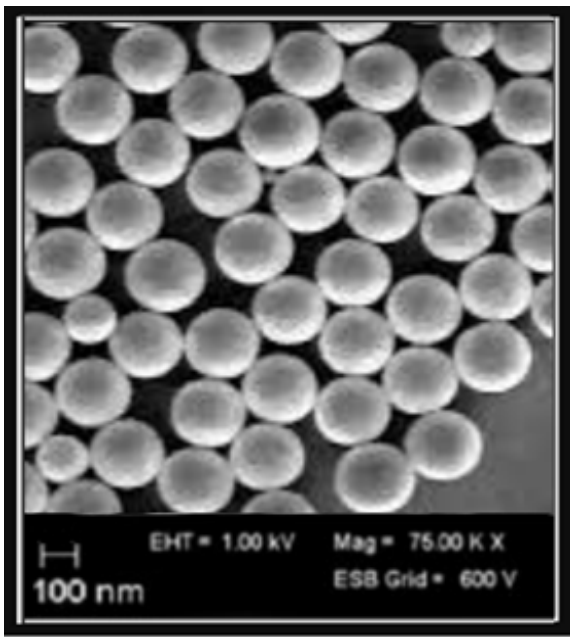

Figure 1. Scanning electron microscope (SEM) of PLGA nanocapsules containing Tiaojing Zhixue

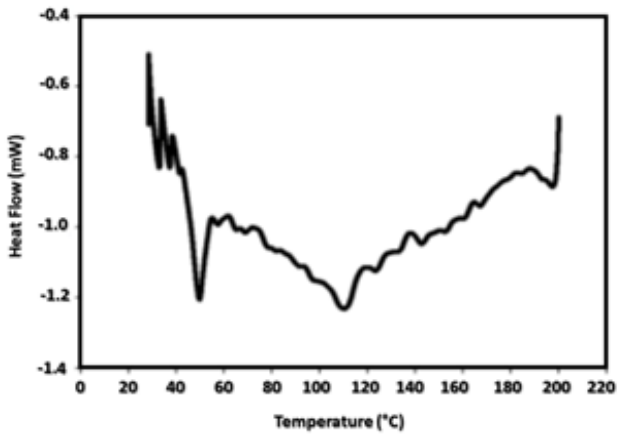

Figure 2. Thermogram of differential scanning calorimetry (DSC) for nanocapsules of PLGA/drug, 0.45/0.25.

The effect of Tiaojing Zhixue medicine

The demographic characteristics of the patients are presented in Table 1. In the present study, in terms of the severity of uterine bleeding before starting treatment, fifteen patients (37.5%) in the direct use of drug group and 12 patients (30%) in the group receiving PLG nanocapsules containing the drug had very severe uterine bleeding. The rest of the group had severe uterine bleeding. Therefore, the two groups were homogeneous in terms of uterine bleeding severity (P=0.318). The two groups were homogeneous in terms of the duration of uterine bleeding on the day before treatment (P=0.448).

The difference between the two groups in terms of severity of uterine bleeding was significant on the day after the second month of treatment (P=0.018), the fourth month after treatment (P=0.045), and the eighth month after treatment (P=0.01) (Figure 4). The changes in the mean duration of uterine bleeding after two, four, and eight months of treatment in the two groups are shown in Table 2.

In the nanocapsule treatment group, after two months of treatment, 23 cases of bleeding had decreased, but 18 cases had not changed. After four months, 29 cases of bleeding had decreased, but 11 cases had not changed. After eight months, the bleeding had decreased in 30 cases, but 10 cases had not changed. This result indicated a 75% positive clinical response.

In the treatment group with direct use of the drug, after two months of treatment, 10 cases of uterine bleeding had decreased, but 30 cases had not changed. After four months of treatment, the severity of bleeding had decreased to 12 cases, and after eight months, the seve-

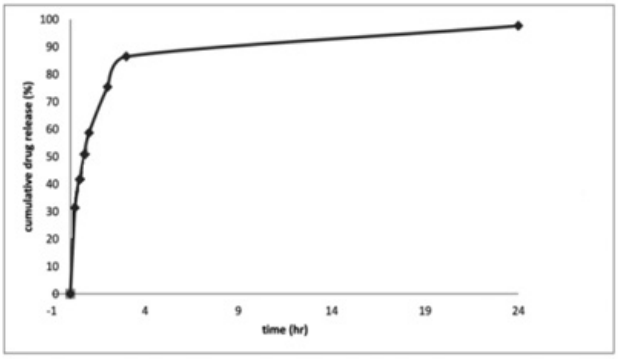

Figure 3. Drug release curve for nanocapsules of PLGA.

Table1. The demographic characteristics of the patients in two groups.

Variable	PLGA nanocapsules	Direct use	Significant level
	Mean and SD	Mean and SD	
Mean age (year)	30.03±1.5	31.33±1.2	*P*=0.128
Duration of Bleeding (year)	2.5±0.6	2.6±0.4	*P*=0.211
Weight (Kg)	63±3	65±2	*P*=0.067

Table2. Changes in the duration of uterine bleeding after two, four, and eight months in the two groups (days).

Variable	PLGA nanocapsules	Direct use	Significant level
	Mean and SD	Mean and SD	
Bleeding duration after two months of treatment (day)	2.81±0.91	2.65±0.13	*P*=0.500
Bleeding duration after four months of treatment (day)	1.95±1.12	2.51±0.46	*P*=0.024
Bleeding duration after eight months of treatment (day)	1.28±0.35	2.09±0.6	*P*=0.013

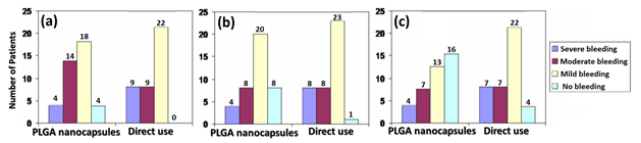

Figure 4. Comparison of two treatment groups in terms of severity of uterine bleeding; (a) After two months, (b) After four months, (c) After eight months.

rity of bleeding had decreased in 17 cases but not in 23 cases. The results of this group also showed a 42.5% of positive clinical response.

In terms of side effects, 52.5% of the complication was observed in the nanocapsule treatment group and 58.5% in the direct use of drug treatment group, which was not statistically significant (P = 0.105). The most common complication was a headache in the nanocapsule group and nausea in the direct use of the drug group.

Discussion

Dysfunctional uterine bleeding is one of the most common problems before and after menopause in women, which causes several problems for patients that must be carefully evaluated and treated (22). There are different ways to treat this disease, and the use of traditional medicines is one of these methods that have led to its treatment for a long time. But the problem with traditional medicines is that they are less effective than chemical drugs. The presence of other compounds along with the active ingredient reduces the effectiveness of these medicines. On the other hand, when they enter the gastrointestinal tract, a large amount of them are destroyed by stomach acid (23). Therefore, finding a way that can increase the effectiveness of traditional medicines has always been interesting to researchers (7).

In recent years, much attention has been paid to nanoparticles as drug carriers, because they can serve as a drug delivery system due to controlling and slowing the release of drugs, smaller particle size than cells, biocompatibility, and increasing the therapeutic efficacy of drugs (24). Therefore, nanotechnology has been very effective in the evolution of drug delivery, and materials such as nanocarriers are known to be very effective in diagnosing and treating a variety of diseases with higher benefits than conventional methods (25).

One of the most common non-toxic nanocarriers used for drug delivery is nanocapsules (26). The formulation of nanocapsules is very important for improving the biocompatibility of drugs. Electrospray is used as a new method for the production of nanocapsules containing drugs (27). There have already been numerous reports in many studies on the use of nanoparticles for various applications (28-37). The present study showed that PLGA nanocapsules containing Tiaojing Zhixue, which is made by electrospray technique, increase the efficacy and effectiveness of Tiaojing Zhixue up to 32.5% in patients with dysfunctional uterine bleeding. For all human dysfunctions, it is necessary to conduct comprehensive and complete genetic studies to determine the genetic nature of these dysfunctions (38-40).

In this study, PLGA nanocapsules containing Tiaojing Zhixue with controllable size and morphology were successfully produced by the electrospray technique. These PLGA nanocapsules were then used to treat dysfunctional uterine bleeding. For this purpose, 80 patients with dysfunctional uterine bleeding were selected and divided into two groups of 40 patients. One group received Tiaojing Zhixue directly. The other group received this medicine through PLGA nanocapsules. The treatment lasted for 8 months. The results showed that PLGA nanocapsules increased the efficiency of Tiaojing Zhixue to 32.5%. Therefore, the combination of traditional medicine and modern pharmaceutical methods could help the treatment of dysfunctional uterine bleeding.

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