Pharm Res (2015) 32:2180–2191
DOI 10.1007/s11095-015-1686-2

EXPERT REVIEW

Long-Acting Injectable Hormonal Dosage Forms for Contraception

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Received: 4 November 2014 / Accepted: 20 March 2015 / Published online: 22 April 2015

ABSTRACT Although great efforts have been made to develop long-acting injectable hormonal contraceptives for more than four decades, few long-acting injectable contraceptives have reached the pharmaceutical market or even entered clinical trials. On the other hand, in clinical practice there is an urgent need for injectable long-acting reversible contraceptives which can provide contraceptive protection for more than 3 months after one single injection. Availability of such products will offer great flexibility to women and resolve certain continuation issues currently occurring in clinics. Herein, we reviewed the strategies exploited in the past to develop injectable hormonal contraceptive dosages including drug microcrystal suspensions, drug-loaded microsphere suspensions and *in situ* forming depot systems for long-term contraception and discussed the potential solutions for remaining issues met in the previous development.

KEY WORDS contraception · *in situ* forming depot systems · microcrystals · microspheres · steroidal progestogens

INTRODUCTION

Four generations of steroidal progestogens have been developed as active pharmaceutical ingredients (APIs) for hormonal contraceptives. The first-generation progestogens include progesterone which is the major naturally occurring human progestogen, norethisterone (NET), ethynodiol diacetate, norethynodrel, lynestrenol (LYN), medroxyprogesterone acetate (MPA), and megesterol acetate (MGA) (Fig. 1) (1). To reduce the cost and the side effects such as bone loss (2,4) associated with the first generation progestogens, second-generation progestogens to include levonorgestrel (LNG) and norgestrel (mixture of two stereoisomers dextroorgestrel and levonorgestrel) (Fig. 2) were introduced into the market in the 1970s (5,6). Currently, LNG is widely used progestogen in long acting contraceptives (5). In the 1980s, the third-generation progestogens including desogestrel, gestodene, norgestimate, and etonorgestrel (Fig. 3) were introduced to the market as oral pills for contraception in patients with diabetes or lipid disorders due to their minimal impact on blood glucose levels and the lipid profile (5–7). To minimize the side effects related to androgenic, glucocorticoid and estrogenic receptor interactions, the fourth generation progestogens including drospirenone, and dienogest (Fig. 4) were introduced into the market about 10 years ago (8,9). Drospirenone is currently used in oral contraceptives in combination with an estrogen such as ethinylestradiol. In addition to the contraceptive effect, drospirenone is also reported to have potentials to reduce body weight, blood pressure and low-density lipoprotein levels, and enhance high-density lipoprotein levels (10). Table I summarizes the solubility of the various contraceptive APIs in water.

The above contraceptive APIs are usually formulated into oral pill, injectable, implant and/or vaginal ring/device dosage forms for contraception (11). Among the four types of dosage forms, the last three can provide sustained API release and offer contraceptive protection to women for months or even years (12,13). For example, Sino-implant (II)® rod, Norplant® capsule and Jadelle® rod implants can provide birth control for 4–7 years (7). However, these implants require surgical removal once they are no longer effective, which has low patient compliance. In addition, such a long duration of contraceptive protection is undesirable for some women.

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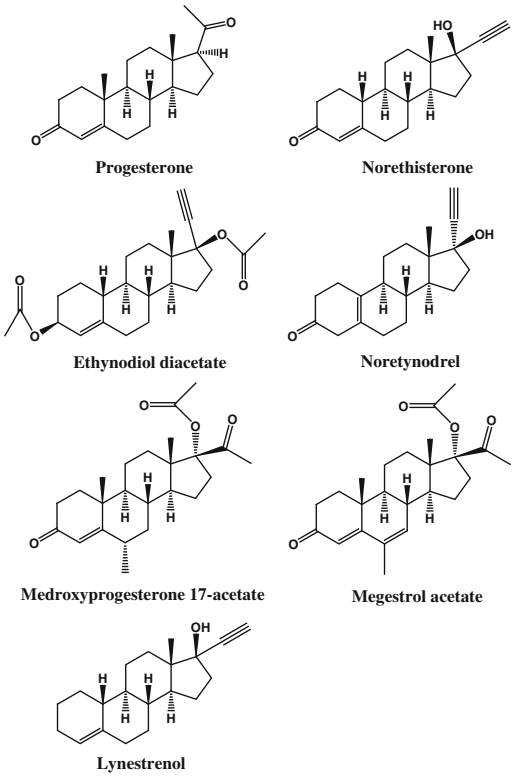

Fig. 1 Chemical structures of first generation progestogens used for contraceptives.

On the other hand, injectables can provide 1 or 3 month contraception and this short term contraception can increase flexibility to women. The currently available injectables are Cyclofem and Lunelle for 1 month contraception, and Depo-Provera®/Depo-subQ Provera 104® for 3 month contraception. These products are injected *via* the intramuscular or subcutaneous routes. Injectables of this type, however, require frequent (at least four times per year) clinic visits which may cause discontinuation

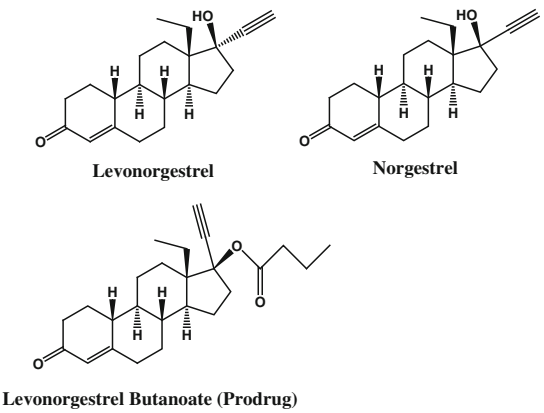

Fig. 2 Chemical structures of second generation progestogens used for contraceptives.

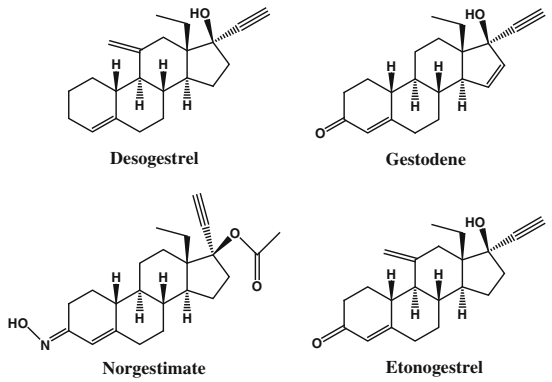

Fig. 3 Chemical structures of third generation progestogens used for contraceptives.

and prolonged return to fertility due to users' difficulty in complying with the multiple-injection schedule. Meanwhile, the side effects of current available contraceptive injectables are well noticed in the clinical sides which include bone loss, menstrual irregularities, nausea, weight gain, mood changes, headaches, and breast tenderness (2,4,7,14). Therefore, there is a desire for long-acting injectable contraceptives which could provide contraception for longer than 3 months after one shot and have less side effects so that better adherence and continuation rates can be achieved (15,16). Meanwhile, adding this new option into the method mix will also increase contraceptive prevalence, and decrease the patient load burden on clinical facilities and community-based programs. In recognition of these potential advantages, great efforts have been made in the past decades to develop injectable longer-acting contraceptive dosage forms.

Drug microcrystal suspensions, drug-loaded microsphere suspensions, and *in situ* forming depot systems are among the most common injectable dosage forms to achieve sustained release of APIs (2,17–19). Each dosage form has its own advantages and disadvantages which are summarized in Fig. 5. Drug microcrystals have advantages of low cost due to less process steps and materials involved, and using water as an

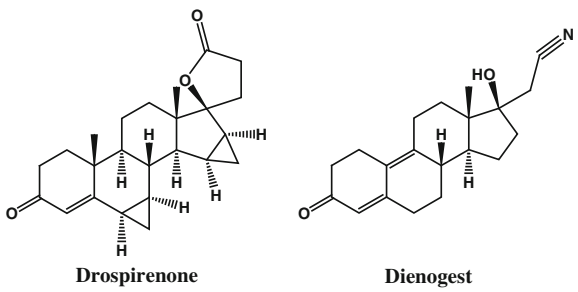

Fig. 4 Chemical structures of fourth generation progestogens used for contraceptives.

Table I Contraceptive Steroids and their Solubility in Water

Active pharmaceutical ingredient	Solubility in water (mg/L)
Medroxyprogesterone acetate	22.2[a]
Progesterone	8.81[a]
Levonorgestrel	2.05[a]
Norethisterone	7.04[a]
Norethisterone enanthate	n/a, practically insoluble
Norgestimate	5.31[a]
Levonorgestrel cyclobutylcarboxylate	n/a, practically insoluble
Levonorgestrel butanoate (LNG-B)	0.84[a]
Ethynodiol diacetate	3.97[b]
Desogestrel	3.01[b]
Dihydroxyprogesterone acetophenide	n/a
Norethindrone enanthate	n/a
Gestodene	5.81[b]
Lynestrenol	n/a
Drospirenone	2.25[b]; 13.0 (10)

[a] Experimental data reported at www.drugbank.ca

[b] Predicted data reported at www.drugbank.ca

administration vehicle, but needs reconstitution and are difficult to achieve sustained release of APIs for more than 3 months. Microspheres have the advantages of using water as a vehicle, and capability of achieving more than 3 month release of APIs due to the retarding effect of polymeric matrix (4,20–29). However, they need reconstitution and some toxic organic solvents used in the fabrication of microspheres might be trapped inside and the manufacturing is generally costly for well-defined microspheres. The possibility of microsphere migration from the site of injection might also be a concern (18). *In situ* forming depots have advantages of simplicity of manufacture, reconstitution un-necessary, and versatility. However, they have disadvantages of using organic solvents as vehicles, forming depots *in vivo* with non-precise shapes and sizes, and requiring drugs with broad therapeutic windows (19,30). All the above three dosage forms have been used to develop long-acting injectable contraceptives. Contraceptives in microcrystal forms such as Depo-Provera® and Depo-subQ Provera 104® have been marketed as commercial products for 3-month contraceptive protection. Although long-acting injectable contraceptives are not commercially available in the forms of microsphere suspensions and *in situ* forming depot systems, long-acting leuprolide injections are currently sold in the forms of both microspheres (LUPRON DEPOT®) and *in situ* forming depot system (ELIGARD®) to treat advanced prostate cancer in men. In the following discussions, we will systematically

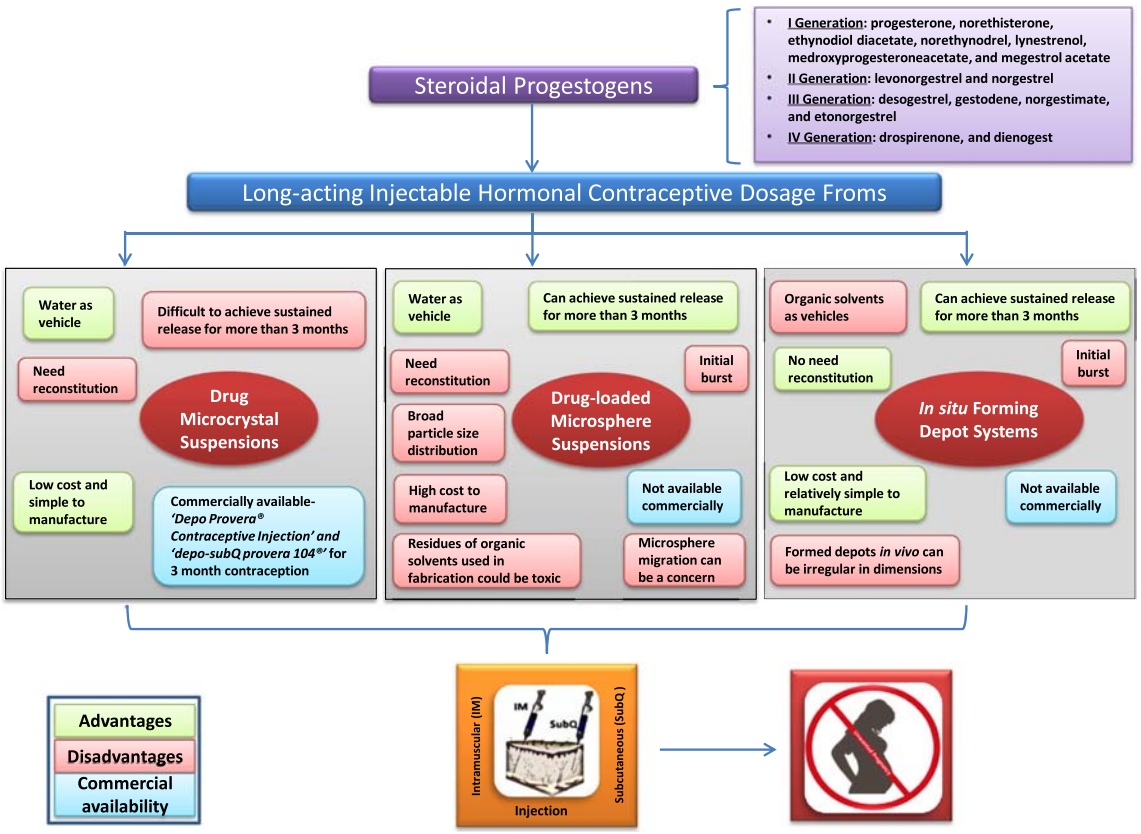

Fig. 5 Flow chart of the advantages, disadvantages, and commercial availability of long-acting injectable hormonal contraceptive dosage forms including drug microcrystal suspensions, drug-loaded microsphere suspensions and *in situ* forming depot systems.

review the progress and challenges in the development of the three injectable dosage forms for long-acting contraception.

DRUG MICROCRYSTAL SUSPENSIONS

In a drug microcrystal suspension dosage form, the components are only API microcrystals and injection vehicle water so that this dosage form is easy and low cost to be manufactured. The microcrystal suspension dosage form can achieve longer therapeutic effects than the API alone (non-crystal form) due to the slow dissolution of the API from the drug crystals into the body fluid. When prodrugs are used to form the drug microcrystals, the combination of dissolution of the prodrugs from the crystals and subsequent hydrolysis/cleavage of the prodrugs into active entities controls the release of the API into the body fluid. Great efforts have been made to develop injectable suspensions of contraceptive steroidal progestogen microcrystals containing either the active drug or prodrug obtained *via* esterification of the hydroxyl groups of the active drug. Depending on the contraceptive API types and microcrystal sizes, up to 3 month contraception can be achieved (31,32). For examples, medroxyprogesterone acetate, the most well-known steroid drug, has been formulated into injectable microcrystals for 3-month contraceptive protection. It has been marketed as Depo-Provera® (Table II) and Depo-subQ Provera 104® (Table III) administrated by intramuscular and subcutaneous injections, respectively. Norethisterone enanthate, an ester of norethisterone (NET), has been developed as injectable microcrystals for 2 month contraception (32,35). Levonorgestrel has been formulated into prodrugs levonorgestrel cyclobutylcarboxylate and levonorgestrel butanoate (LNG-B) which have lower water solubility than LNG (Table I) and have been studied in rats, rhesus and cynomolgus monkeys, and women (2). Both the prodrugs could suppress ovulation for 5–6 months in women at a single dose of 50 mg in the form of microcrystal suspensions. LNG-B demonstrated better performance in terms of overall toxicity, pharmacokinetic profiles and pharmacodynamic effects in phase I clinical trials (2,36). Currently, several companies are developing LNG-B crystal suspensions for long-acting contraceptives (37,38). For example, CONRAD is trying to formulate LNG-B for preclinical and clinical studies aiming at contraception for 4 months after a single injection (38).

Table II Formulation of Depo-Provera® for Intramuscular Injection (1 mL) (33)

Ingredient	Amount (mg)
Medroxyprogesterone acetate	150
Polyethylene glycol 3350	28.9
Polysorbate 80	2.41
Sodium chloride	8.68
Methylparaben	1.37
Propylparben	0.15
Water for injection, sodium hydroxide, hydrochloric acid	q.s.

DRUG-LOADED MICROSPHERE SUSPENSIONS

Microspheres made of natural or synthetic polymers such as polyesters have the advantage of using water as a vehicle to form suspensions for long term (over months) sustained release of different drugs (39–44). Through extensive investigation over past several decades, it has been acknowledged that many factors of microspheres such as their chemical and physical structure (composition, chirality and crosslinking density), drug loading and distribution, physical property (size, shape, size distribution, and molecular weight), and fabrication and sterilization processes significantly affects drug release profiles including release rate, duration of action and initial burst, and microsphere overall performance *in vitro*, in animals, and in humans (20,23,27,45–50). In the following, we will discuss how these factors have been investigated for the development of microspheres as injectables for long-term contraception.

Microsphere Chemical and Physical Structure and Drug Loading

Starting in the late 70s, injectable microsphere contraceptive systems have been developed for long-term contraception,

Table III Formulation of Depo-subQ Provera 104® (0.65 mL) for Subcutaneous Injection (34)

Ingredient	Amount (mg)
Medroxyprogesterone acetate	104
Polyethylene glycol	18.688
Polysorbate 80	1.950
Povidone	3.250
Sodium chloride	5.200
Monobasic sodium phosphate. H2O	0.451
Dibasic sodium phosphate. 12H2O	0.382
Methionine	0.975
Methylparaben	1.040
Propylparaben	0.098
Water for injection	q.s.

pioneered by Beck and co-workers using poly(lactide) (PLA, MW 90,000 Da), and/or poly(lactide-co-glycolide) (PLGA, 96:4, 92:8, 87:13 and 74:26; MW about 40,000 Da) to encapsulate NET *via* oil-in-water (o/w) emulsion/solvent evaporation process (48). NET microcrystals were successfully encapsulated into PLA and PLGA microspheres by suspending the NET microcrystals in polymer acetone/chloroform co-solvent solutions followed by adding this suspension into 5 wt% polyvinyl alcohol (PVA) solution to construct the o/w emulsion, and then solvent evaporation. Afterwards, PLGA became the most popular polymer for fabricating microspheres to encapsulate contraceptive APIs including LNG, gestodene and ethinyl estradiol using o/w or w/o/w emulsion/solvent evaporation technique (20–23,25,27,50,51). Poly(ε-caprolactone) (PCL) is a second popular polymer for fabricating microspheres to encapsulate contraceptive APIs including LNG, ethinylestradiol, norgestrel, progesterone and β-estradiol using o/w or w/o/w emulsion/solvent evaporation technique (49,52–56). Besides PLA, PLGA and PCL, diblock copolymers of lactide and ε-caprolactone (49,53), triblock copolymers of ε-caprolactone, lactide and glycolide (54), and diblock copolymer of ethylene glycol and D,L-lactide (57) have also been used to encapsulate contraceptive APIs such as progesterone, estradiol, norgestrel, and LNG *via* o/w emulsion/solvent evaporation. Furthermore, casein (25,58,59), chitosan (60), serum albumin (61), gelatin (62) and poly(3-hydroxy butyrate) (63) were other materials used to fabricate progesterone-, LNG- or estradiol-contained microspheres. The microspheres were fabricated by w/o and o/w emulsion followed by crosslinking using glutaraldehyde, emulsion polymerization using glutaraldehyde as a crosslinker, phase separation followed by crosslinking using formaldehyde, and o/w emulsion followed by solvent evaporation technique, respectively. Table IV summarizes the polymers, APIs, sizes, *in vivo* release durations, animal models, and fabrication processes of the microspheres developed for contraception.

The composition of the microspheres controls the biodegradation rate of the microspheres, and in turn affects the contraceptive API release profiles from the microspheres. For example, as the hydrolytic degradation rate of the polymers PGA, PLA and PCL increased in the order of PGA > PLA ≫ PCL, the microspheres made of the triblock polymers consisting these three polymer blocks released LNG at a rate increasing with increasing the amount of the three polymer blocks in the order of PGA > PLA ≫ PCL (64). PCL possessed a good permeability for steroidal drugs but undergoes slow biodegradation, resulting in the release of LNG from the PCL matrix to be controlled by diffusion (64–66). On the other hand, PLA and PGA had lower permeability to steroidal drugs but undergo homogenous erosion, resulting in the release of LNG from the PCL matrix to be controlled mainly by erosion (64,67). It was also observed that PLGA microspheres degraded faster and NET was released from the microspheres faster when the amount of hydrophilic glycolide component was increased (27). The chirality of polymers, crosslinking density and drug loading of microspheres also play important roles in controlling contraceptive API release profiles. It was reported that microspheres composed of D,L-lactide caused more uniform release rate and less initial burst for progesterone and β-estradiol than those composed of L-lactide (49). Microspheres composed of casein and chitosan released progesterone slower with increasing the amount of crosslinker glutaraldehyde which resulted in higher crosslinking density and increased barrier for drug diffusion, and decreasing drug loading (58,60). Microspheres with higher amount of unencapsulated drug present on the surface would show higher initial burst (20,50,52).

Microsphere Fabrication and Sterilization Processes

In the initial attempts to develop contraceptive API-loaded microspheres, the conventional emulsion technique with simple stirring or high shear stirring was used to fabricate the microspheres (20–22,27,48,49,52,56,58,60). In these emulsion systems, the droplet formation reached a steady-state size equilibrium or size distribution due to continuous droplet collision, coalescence, and re-division. Therefore, the size and size distribution could not be well controlled. The resulting microspheres generally had a wide size distribution and further sieving was required to get narrow size distributed microspheres (27,68,69). The quality of microspheres depended on emulsion types, solvents, temperature, and surfactants/stabilizers. It was reported that when LNG was encapsulated into PLGA microspheres using o/w emulsion/solvent evaporation technique, poor quality microspheres with a large amount of LNG crystals on the surface were obtained (20). A later report from Zeng and co-workers demonstrated that poor quality PLGA microspheres were obtained when LNG crystals (<44 μm) were suspended in PLGA polymer dichloromethane solutions and the solvent evaporation was processed at room temperature (~25°C), while high quality microspheres were fabricated when LNG microcrystals were suspended in PLGA acetone/chloroform co-solvent (3:1 *v/v*) solution and low temperature (0–5°C) was used for solvent evaporation (21). When Vamasadhara and co-workers used w/o/w emulsion/solvent evaporation technique to fabricate LNG and ethynylestradiol encapsulated PLGA and PCL microspheres, they successfully obtained the microspheres with 10–25 μm diameter and smooth spherical surfaces (22,52,56). They also noted that when PVA was used as a surfactant/stabilizer in the continuous phase, it would provide a thin layer covering the

Table IV Contraceptive API-Loaded Microspheres

Polymer (MW, Da)	API encapsulated	Size (μm)	*In vivo* release duration (animal model)	Fabrication technique	Reference
PLA (90,000)	NET	10–240	180 days (baboons)	o/w emulsion/solvent evaporation	(48)
PLGA (96:4, 92:8, 87:13 and 74:26) (~40,000)	NET	90–125, 90–106, 45–90, 63–90[a]	150–210 days (rats & baboons)	o/w emulsion/solvent evaporation	(27)
PLGA (87:13) (~40,000)	LNG	40–95[a]	180 months (baboons)	o/w emulsion/ solvent evaporation	(20)
PLGA	NET	<250	N/A	o/w emulsion/solvent evaporation	(50,51)
PLGA(90:10) (177,000)	LNG	10–110	126 days (rats)	Modified o/w emulsion/ solvent evaporation	(21)
PLGA (70,000)	LNG and ethinylestradiol	10–25	105 days (rats)	w/o/w double-emulsion/ solvent evaporation	(22)
PLGA (50:50) (12,000)	Gestodene and ethinyl estradiol	30–100	35 days (rats)	o/w emulsion/ solvent evaporation	(23)
PLGA (53:47) (14,000)	LNG	46.26 (mean)	28 days (rabbits)	o/w emulsion/ solvent evaporation	(25)
PCL (40,000)	LNG and ethinylestradiol	8–25	15 weeks (rats)	w/o/w double-emulsion/ solvent evaporation	(52,55,56)
PCL-b-PLA (90:10, 75:25, 60:40) (~40,000)	norgestrel	40–300/400/500	N/A	o/w emulsion/solvent evaporation	(53)
Poly-D,L-lactide-co-ε-caprolactone	Progesterone/ estradiol	5/10–100/150	N/A	o/w emulsion/ solvent evaporation	(49)
ABA, ABC, ACB[b]	LNG	70–120	N/A	o/w emulsion/solvent evaporation	(54)
Poly (ethylene glycol-b-poly (D,L-lactide)	LNG and estradiol	30 (mean)	6 months (mice)	Solvent evaporation	(57)
Chitson (315,000)	Progesterone	45–300	5 months (rabbits)	w/o emulsion then crosslinked by glutaraldehyde	(60)
Casein	Progesterone	75–180	5 months (rabbits)	w/o emulsion then crosslinked by glutaraldehyde	(58)
Casein	LNG	103.85 (mean)	28 days (rabbits)	o/w emulsion then crosslinked by glutaraldehyde	(59)
Serum albumin	progesterone	100–200	20 days (rabbits)	Emulsion polymerization using glutaraldehyde as a crosslinker	(61)
Gelatin	LNG and estradiol	10–40	50 days (mice)	Phase separation then crosslinked by formaldehyde	(62)
Poly(3-hydroxy butyrate) (230,000)	LNG	28.7–85.8	1–3 months (mice)	o/w emulsion/solvent evaporation	(63)

[a] Specific size range achieved by sieving

[b] A: poly(ε-caprolactone); B: poly-DL-lactide; C: polyglycolide

[c] The authors of the paper call the process "precipitation". But based on the description, the process should be o/w emulsion/solvent evaporation

microspheres which helped to reduce the coalescence during the fabrication process (52).

When fabricated microspheres were not uniform, initial burst release was observed in the *in vitro*, *in vivo* and clinical evaluations of the microspheres for contraception (21,22,27,54,56,68,70). Even though this initial burst release might be not very harmful to the users, it might cause API concentration beyond the therapeutic window and reduce the amount of API available for contraception and the duration of release (71–73). Among the methods studied for minimizing the initial burst release, coating or core/shell structure is one of the most straightforward strategies (74,75). This additional layer of shell could be created by a dipping, mixing or emulsion process (42). It was reported that simply modifying the prototype NET-loaded PLGA microspheres by adding a polymer/chloroform solution into the suspension of the prototype microspheres in alcohol aqueous solution significantly reduced the initial burst and extended the release duration of NET for 40–50 days longer than the prototype microspheres with a similar size range and NET loading (76). The reduced initial burst and extended release duration of NET from the modified microspheres was because the polymer coating layer added an additional barrier for the drug to diffuse/penetrate through.

In recent years, more sophisticated technologies such as extrusion through needles, membranes (77,78) and microfabricated microchannel devices (*e.g.* microfluidic devices) (79,80), and dripping using electronic force, ultrasonic excitation and vibration (46,81–83) were used to produce uniformly sized droplets in the emulsion systems. Monodispersed polyester microspheres have been fabricated for drug release using dripping/spraying with ultrasonic excitation, and

extrusion using microfluidic devices (45–47,82). It would be reasonable to assume that well-defined polyester microspheres with core/shell structures and narrow particle size distribution could be fabricated to encapsulate contraceptive APIs for reduced initial burst release and extended contraception period, although no attempts have been made in this direction.

The process of sterilization opted can also affect the release profile of the contraceptives. When Beck *et al.* evaluated the effect of sterilization by gamma radiation (2.8–3.5 mrad) on the *in vitro* release profile of LNG-loaded microspheres, they observed that the microspheres released LNG faster after sterilization (20). A major problem connected with the long acting injectable contraceptives is the abnormality of the menstrual bleeding cycle, which can be associated with non-synchronous release of contraceptives from the injectables with the normal cyclic profile of endogenous progesterone. Beck *et al.* conceptualized that by coating microspheres with different polymers and then blending them together into a single injection, repeat release of contraceptives would be achieved to better control menstrual draining than the common steady-state release (76).

Microsphere Physical Property

Microsphere size is an effective means for modulating contraceptive API release kinetics (20,47,48) and as well as microsphere injectability (20). Primarily, the size of microspheres determines the surface area to volume ratio, and thus directs the extent of surface available for releasing the contraceptive APIs through diffusion. Smaller microspheres have higher surface area to volume ratio and less diffusion path length for the APIs, and subsequently result in quicker API release rate and shorter release duration. This effect was demonstrated in a study where the release rate of NET and progesterone increased with decreased size of PLGA (27) and chitosan (60) microspheres, respectively. The size of microspheres inversely affects the efficiency of the injectability: the bigger the size is, the more difficult the microspheres can be injected through clinically used needles. The optimal size range for yield and efficiency of injection is between 20 and 90 μm (20). The mean size of microspheres can be controlled by the concentration of polymers used for fabricating the microspheres. The higher the polymer concentration is, the higher the viscosity of the organic phase in emulsion droplets is, and thus the bigger the mean size is and the more contraceptive APIs are encapsulated into the microspheres (52,55). Molecular weight (MW) of the polymers used for fabricating microspheres is another important factor affecting contraceptive API release. The higher is the MW, the slower the polymers degrade and the longer the APIs are released (64). For example, microspheres composed of PLGA with lower MW (12,000 Da) led to 35-day release of ethinyl estradiol, while PLGA with higher MW (70,000 Da) resulted in 105-day release of ethinyl estradiol (22,23).

In Vitro Evaluation

Although *in vitro* drug release kinetics does not always have a clear correlation with the *in vivo* release in animals and humans (84), it is still used as a fast approach to screen formulations. Two different release/dissolution media have been extensively used in the *in vitro* release studies of contraceptive-loaded microspheres: aqueous ethanol solution (20,21,48,53,76), water with and without PBS (pH 7.4) (22,49,54,56,58,60). The use of ethanol accelerated the release of contraceptive API from the microspheres. These two release media have been used to study the effects of microsphere chemical and physical structure, physical property, drug loading and distribution, and fabrication and sterilization processes on the *in vitro* release of contraceptive API from microspheres that have been discussed in the previous sections, and will not be repeated again here. Ethanol alone is probably not enough to catch the initial burst and higher pH and some surfactants may be needed for accelerated release study to differentiate polymers in term of initial burst.

In Vivo Evaluation

Female baboons, rats, rabbits and mice have been used as animal models to evaluate serum API levels and contraceptive effect of microspheres after single intramuscular injection. Beck and co-workers evaluated their NET-loaded microspheres on female baboons. Their PLA microspheres (10–240 μm) could sustain the release of NET in female baboons for about 180 days and the ovulation was inhibited through the 6-month treatment period for all three dose levels tested: 75, 50, 25 mg of NET equivalent (48). *In vivo* evaluation of their NET-loaded PLGA microspheres on baboons showed that the NET release duration was dependent on the particle size and the LA to GA ratios of PLGA polymers, and the release of NET presented a two phase pattern (27). Beck and co-workers also designed LNG–loaded PLGA(87:13) microspheres and found that the microspheres released LNG in baboons for up to 6 months with 3–6 month ovarian function suppression, depending on the dose level (20). A biphasic release pattern was seen in LNG release from the microspheres in a similar way as the NET-loaded microsphere systems. In rat studies, LNG- or LNG and ethinylestradiol-loaded PLGA and PCL microspheres were found to maintain constant LNG blood level at 0.2–2 ng/mL for 15 weeks to 5 months (21,22,56). In rabbit studies, it was found that progesterone was maintained a blood level of 1–2 ng/mL for about 5 months (60), 5 months (58) and 20 days (61) by microspheres made of chitosan, casein and serum albumin, respectively (58,60); and LNG was maintained a blood level of 0.1–0.5 ng/mL for 28 days by microspheres made of casein (25). In mouse study, LNG- or LNG and estradiol-loaded poly (ethylene glycol-b-poly (D,L-lactide) (57), gelatin (62) or

poly(3-hydroxy butyrate) (63) microspheres showed 6 month, 50 day, and 1–3 month contraceptive effect, respectively. The above pharmacokinetic and contraceptive effect results of the contraceptives released from the microspheres in different animal models are summarized in Table IV.

Clinical Trials

Phases I, II and II clinical trials have been conducted for the NET-loaded PLA and PLGA microspheres (4,28,29,68,69). The phase I clinical trials (69) of these NET-loaded PLA microspheres (size range: 60–240 μm, or 90–212 μm) in 63 women at three centers showed that the release of NET lasted for 6 months after one intramuscular injection, while the serum levels of NET varied in proportion to the dose injected and the suppression of ovarian function was also highly dose dependent. Suppression of ovulation for 6 months could be achieved for doses ranging from 1.33 to 3.45 mg of NET/kg for NET-loaded PLA microspheres with size 60–240 μm (27). In order to avoid the build-up of PLA at the injection sites due to its slow degradation, Beck and coworkers designed the second generation NET microspheres using PLGA (68). NET-loaded PLGA microspheres (63–90 μm or 90–106 μm) fabricated from PLGA with 86–88% LA and 14–12% GA were clinically tested in women. Microspheres with sizes of 63–90 μm could suppress ovarian function for 3–4 months at dose of 75 mg or 100 mg of NET, while the 90–106 μm microspheres suppressed ovarian cyclicity for an average of 22 weeks with the shortest one of 14 weeks and longest one of 26 weeks at similar dose levels (85,86).

IN SITU FORMING DEPOT SYSTEMS

In situ forming depot systems appeared in the field of controlled drug release as an alternative to microspheres in order to overcome the disadvantages noticed for microspheres such as the need of reconstitution, the possibility of microsphere migration after injection, and high fabrication cost, low encapsulation efficiency for some APIs (87–90). These *in situ* forming depot systems generally are injectable polymer or lipid solutions/suspensions having low viscosity before injection, and solidify into semi-solid or solid depots after administration into the body (91). The mechanisms of solidification include phase separation induced by pH (92–94), solvent exchange (95,96) or temperature (97), solubility change, and physical or chemical crosslinking (98). The phase separation systems involving solvent exchange mechanism have attracted considerable attention in the development of long-acting injectables as commercially available materials can be used for the development (30,99,100). Dunn *et al.* pioneered this concept in 1990 by employing biodegradable polyesters such as PLGA and PLA in water miscible and solvents such as N-methyl-2-pyrrolidone (NMP) and dimethyl sulfoxide (95,99,101). Upon subcutaneous injection, the solvents in the formulation dissipated into the surrounding tissue and meanwhile aqueous biological fluids penetrated into the formulation. In turn, phase separation and precipitation of the biodegradable polyesters occurred, and a depot was formed at the injection site. API in the formulation was entrapped within the depot and then released *via* diffusion and bio-erosion mechanisms. The concept *of in situ* depot formation was soon adopted to develop long-acting injectable contraceptives (102,103). The University of Tennessee Health Science Center (UTHSC) has been actively developing injectable contraceptives for the past 20 years (102–104). Their studies started with Precirol ATO 5 as a depot matrix, Labrafil 1944 CS as a solvent/plasticizer and LNG as an API for contraceptive (104). The injectability of such systems was strongly dependent on the Precirol concentration and the size of the needle used. Lower maximum force for injection was observed for lower Precirol concentrations and larger gauge needles. The same trend was observed for the *in situ* forming depot systems developed from PLGA/PLA (102). *In vitro* release kinetics demonstrated that the release rate of LNG from the resulting depots was affected by the Precirol concentrations used in the formulation and the LNG crystal sizes (104). Moreover, zero order release of LNG was achieved up to 30 days *in vitro* for formulations containing 10 wt% Precirol, and 2 wt% LNG with drug crystal size of 6 μm (104). *In vivo* investigation demonstrated that the durations of suppression on rat estrous cycles after single subcutaneous injection were significantly influenced by the LNG contents in the 10 wt% Precirol formulation with 20, 27 and 41 consecutive days for rats receiving 1 mL of the formulations containing 0.25, 0.50 and 2.00 wt% LNG content, respectively (104). These short contraception durations might be attributed to the fast degradation of the formed depots which disappeared in the injection sites around 35 to 45 days post-injection (104).

In order to achieve longer contraception protection after a single injection, the UTHSC evaluated polyesters such as PLGA (various ratios of LA to GA) and PLA with different intrinsic viscosity (molecular weight) as the depot matrix (102,103). They studied the effects of varying drug loading, polymer concentration, polymer inherent viscosity and copolymer composition on *in vitro* release of LNG. Increasing the LNG loading from 1 to 8% *w/w* increased the cumulative amount of LNG released *in vitro* on day 30 from 1.195 to 3.4045 mg per gram of formulation made from PLGA50:50. The cumulative amount of LNG released on day 30 decreased from 2.06 to 0.628 mg per gram of formulation when PLGA50:50 concentration increased from 20 to 80% *w/w*. Increasing the PLGA50:50 inherent viscosity from 0.15 to 1.07 dL/g decreased the cumulative amount of drug released on day 30 from 14.17 to 0.935 mg per gram of formulation. *In vitro* drug release rate was found to decrease with increasing

proportion of lactide in lactide-glycolide copolymers. Drug release from the system was due to a combination of diffusion and polymer degradation. *In situ* forming depot systems prepared from PLGA50:50/NMP (33:67 *w/w*) with different LNG contents were further investigated *in vivo* on Sprague–Dawley albino female rats for contraceptive effect after Sub-Q injection. The duration of *in vivo* activity of the LNG released from the *in situ* formed depots was evaluated by monitoring the estrous cycles of the female rats. The sizes of the *in situ* formed depots decreased with time and the depots disappeared completely from the injection site between day 45 and 70 after injection. Formulations containing 2 and 4% *w/w* LNG suppressed the rat normal estrous cycles for 65.5 and 103.5±8.5 days at a dose level of 1 ml/kg, respectively (102,103). The formulation containing 2 wt% of LNG in 32.67 wt% PLGA (inherent viscosity of 0.59) and 65.34 w% NMP was further evaluated on Japanese quails with Depo-Provera® as a control (105). The dose level was 40 mg API equivalent per kg and the injection site was the left pectoral musculature. Quails treated with the LNG-containing *in situ* forming depot system stopped laying eggs for 67±4.1 days, while resume ovarian recrudescence (evidenced by egg laying) on day 59 after treatment (one bird; range, 59–70 days). For the birds treated with Depo-Provera®, two of seven birds stopped egg-laying for 19 and 49 days, respectively, while for the other five birds, the average duration of not egg-laying was 5.8±2.3 days (range, 2–8 days). The contraceptive duration observed on these quail was consistent with that observed on rats for the same formulation (102,105). The formulation containing 4 wt% LNG was further evaluated at the dose level of 40 mg/kg body weight (equivalent to 1 mL/kg body weight) on Feline queens *via* subcutaneous injection (106). Blood and fecal samples were collected weekly for the first 5 weeks to build the base lines. Initial treatment injections were given at the beginning of week 6, and all queens received a second injection with the same dosage at the beginning of week 16 (68 days after initial treatment). The results demonstrated that the follicular activity and estrogen secretion in the domestic cats could be suppressed for at least 36 weeks after the two injections of the formulation (106).

Based on the above *in vivo* evaluations, cottontop tamarins, a small new world nonhuman primate, were used to further evaluate the contraceptive effect of the PLGA-based formulations (107). The formulation was slightly modified to contain 24 wt% PLGA50:50 as the depot matrix and 72 wt% NMP/triethyl citrate (TEC) (90:10, v:v) as the solvent, and denoted as F1. F1 formulation was subcutaneously injected into cottontop tamarins at an LNG dose level of 45.53±2.52 mg/kg. The normal ovarian cycles of the cottontop tamarin was suppressed for 102.0±20.9 days indicated by the fecal PdG and E_1C concentrations. In order to extend the suppression duration, the type and intrinsic viscosity of the polyester used in the formulation were adjusted to increase the viscosity of the formulation and slow the degradation of the *in situ* formed depots. Two new formulations were obtained: formulation F2 containing 4 wt% LNG, 8 wt% PLGA50:50, 8 wt% PLGA85:15, 8 wt% PLA and 72 wt% NMP/TEC(90:10); and formulation F3 contained 4 wt% LNG, 4.8 wt% PLGA50:50, 19.2 wt% PLA and 72 wt% NMP/TEC(9:1). After subcutaneously injected at LNG dose levels of 46.60±3.30 (for F2) and 48.08±3.39 (for F3) mg/kg, the F2 and F3 formulations suppressed the normal ovarian cycles of cottontop tamarins for 120.4±46.0 days and 134.8±32.8 days, respectively. With further modification of the formulation F3 including increasing LNG content from 4 to 6 wt%, reducing the weight percentage of the mixture solvent NMP/TEC (9:1) from 72 to 70 wt%, a formulation F4 was obtained. After subcutaneously injected at the LNG dose levels of 71.89±8.90 mg/kg, the F4 formulation extended the suppression of the normal ovarian cycles of cottontop tamarins to 198.8±70.3 days. However, both the F3 and F4 formulations did show some initial burst. The initial burst release could reduce the API available for the contraceptive, in other words the release duration could be longer if the initial burst release could be eliminated. In addition, the polymeric solution formulations had problems with relatively broad duration of the contraception. It has been documented that the initial burst release and/or duration of the API release could be adjusted by varying the polymer/solvent ratio, using a blend of polymers with different end groups, molecular weight, and degradation properties, and adjusting the hydrophobicity/hydrophilicity of the solvents (100,108). It has been reported that zero-order release of LNG was achieved *in vitro* from *in situ* forming depot systems containing PLA (MW 11,000) and a mixture of benzyl benzoate and benzyl alcohol for 3 months with almost no initial burst and less than 40% of loaded LNG released out after 3 months (109). Moreover, in order to minimize the initial burst release, the API could also be encapsulated into microparticles and then dispersed/suspended in the *in situ* forming depot systems (108,110). It has been demonstrated that by encapsulating naltrexone base in PLA microparticles prepared *via* melting/fusion/grounding process, the *in situ* forming PLGA/NMP depot systems dispersed with these naltrexone/PLA microparticles showed significantly reduced initial burst compared with the PLGA/NMP systems loaded with naltrexone base (108).

SUMMARY AND PERSPECTIVES

The previous efforts to develop long-acting injectable contraceptives have generated a lot of positive data supporting the feasibility of long-acting injectable contraceptives for more than 6 months' contraceptive protection after one intramuscular or subcutaneous injection. However, due to one or another reason, currently there are not many efforts to continue such investigations with a targeting goal of commercializing

the products. With new technologies for fabricating well-defined microspheres/ microparticles, the understanding of the roles of excipients in *in situ* forming depot systems, and funding available from foundations like the Bill & Melinda Gates Foundation, there is a great potential for a longer-acting injectable contraceptive to reach the market in the near future (111). The Family Health International (FHI) 360 has received funding from the Bill & Melinda Gates Foundation, and is currently supporting four research groups including the authors' group at the University of Tennessee Health Science Center to develop long-acting injectable contraceptives in the forms of drug-loaded microspheres, *in situ* forming depots and drug conjugated silica microparticles, aiming at providing 6 months' contraception with only one single injection.

ACKNOWLEDGMENTS AND DISCLOSURES

We thank our former graduates Dr. Zhi-Hui Gao, Dr. Shilpa Thosar and Dr. Yichun Sun, our former research associate Dr. Wen Qu, and our collaborators William R. Crowley, James F. Reger, Catharine J. Wheaton, Donald Neiffer, Bill Lasley, and Gary Anderson for their efforts in our studies on *in situ* forming depots for contraception at UTHSC. We also thank Dr. Wen Qu for his discussion and comments on the manuscript.

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