EXPERT OPINION



# Leuprolide acetate: pharmaceutical use and delivery potentials

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***Introduction:*** Thanks to recent advances in biotechnology, the use of peptides and proteins as drugs has become a concrete clinical reality, and consequently an interesting challenge has emerged for non-parenteral drug delivery. Leuprolide is a synthetic nonapeptide agonist to the luteinizing hormone-releasing hormone (LH-RH) receptor with principal clinical applications for prostate cancer. Although a large number of formulations available, they mainly consist in depot subcutaneous injections or implantable devices. Both of these routes of administration present multiple limitations considering the large clinical applications of this active substance.
***Area covered:*** The objective of this review is to critically discuss the formulations currently available on the market for leuprolide optimization and to consider how drug delivery plays an important role in improving the bioavailability of this compound.
***Expert opinion:*** Due to its physicochemical properties and its economical market, leuprolide is an interesting candidate for drug delivery to improve the efficacy of existing treatments, dose adjustments, and patient compliance and safety.

**Keywords:** drug delivery, leuprolide acetate, LH-RH agonist, protein delivery, routes of administration

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## 1. Introduction

The scientific advancements of the last decades showed the therapeutical potentials of peptides and proteins as innovative drug substances. These molecules are nowadays already largely used and often they are considered as standard clinical practice. In spite of their large therapeutic applications, these drugs present complex limitations in their practical usage, mainly due to their poor stability and absorption [1]. For this reason although different routes were considered as alternative to the parenteral administration [2-4], it still remains the more effective method nowadays. The challenge represented by protein and peptide delivery becomes even more important now that, thanks to the advancement of molecular biology, new peptidic active substances are constantly discovered; the luteinizing hormone-releasing hormone (LH-RH) agonists represent an example of these progress.

The discovery of the structure of gonadotropin releasing hormone (GnRH) or LH-RH led to the development of a new class of drugs, the LH-RH agonists. The amino acid compositions [5] of these drugs and the natural peptide LH-RH are reported as in **Figure 1**. These drugs are characterized by a high affinity for the GnRH receptor in the pituitary; moreover, they present a better stability to systemic degradation making possible their clinical use, unlike the natural LH-RH which presents a half-life of only 2–5 minutes [6]. Principal LH-RH agonists are summarized in the **Table 1**. As showed, they are all







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**Article highlights.**

- The scientific advancements of the last decades have shown the therapeutical potentials of peptides and proteins as innovative drug substances
- Leuprolide is a synthetic nonapeptide agonist to the luteinizing hormone-releasing hormone (LH-RH) receptor with main clinical applications for prostate cancer, endometriosis and precocious puberty
- Despite its large use, only implant or depot formulations are currently available. These formulations present different limitations mainly related to patient compliance and safety
- Although a great effort has been taken in the direction of non-parenteral administration of leuprolide, an effective and efficient formulation is still missing, which makes this drug still an interesting challenge in protein delivery

This box summarizes key points contained in the article.



characterized by a relatively high stability, with a half-life of about 3 h, and a relatively small molecular weight, characteristics which make all these molecules interesting candidates for protein delivery in non-parenteral administration.

The clinical importance for these drugs is particularly evident considering the number of therapeutical applications and formulations available on the market, particularly for leuprolide [7]. These formulations consist in general of implantable devices or intramuscular depots, allowing a chronic administration necessary for their therapeutical effect.

The objective of this review is to critically discuss the formulations currently available on the market for leuprolide acetate discussing where drug delivery could play an important role improving the bioavailability of this compound.

### 1.1 Leuprolide

Leuprolide (or leuprorelin) (**Figure 2**) is a synthetic nonapeptide analogous of the natural gonadotropin-releasing hormone (GnRH or LH-RH) with receptor agonist activity; when given continuously, it inhibits the pituitary gonadotropin secretion and suppresses the testicular and ovarian steroid genesis [8], hormones involved in cancer cells growth. Leuprolide is currently commercialized as acetate salt.

Thanks to the structural analogy that leuprolide has toward the natural LH-RH that leuprolide presents a strong affinity for the pituitary receptor with agonist activity, so it is included in the class of LH-RH super agonist. It has a D-amino acid in its chemical structure (**Figure 1**), which contributes to increase its biological stability.

#### *1.1.1 Mechanism of action*

The LH-RH (**Figure 3**) is produced in the hypothalamus, and when released it stimulates the production of the LH in the pituitary gland [9]. In men, LH is then released in the circulation and in the testes it stimulates the production of testosterone in the Leydig cells. Testosterone is then involved in a signal cascade which stimulates the cell growth; interfering with any of these different steps causes interference in the prostate cell proliferation.

LH-RH agonists like leuprolide, buserelin, goserelin, histrelin, nafarelin and triptorelin are capable of preventing the production of testosterone by the testes and consequently the proliferation of prostatic cells. In fact, the chronic administration of an LH-RH agonist induces a reduction in the number of LH-RH receptors (down-regulation) and a suppression of the gonadotropin synthesis and secretion (desensitization) [6]. This activity is exploited in the use of LH-RH agonist in the palliative treatment of prostate cancer. However, due to the agonist activity, after the administration there is an initial stimulation; this phase known as "flare" generally lasts for 10 – 20 days and can be associated to a 10-fold rise in luteinizing hormone [6]. During this time an accelerated tumor growth may be observed causing a worsening of the disease and quality of life [10].

In premenopausal women, there is a similar regulation of the hormonal production: the pituitary gland is stimulated by pulses of LH-RH, producing pulsatile secretion of gonadotropins and maintaining the cyclical activity of the gonads [11]. The chronic administration of an LH-RH agonist, after an initial augmentation of the gonadotropin secretion, provokes a subsequent decrease of the circulating estrogen concentrations to postmenopausal levels. This mechanism is exploited in the treatment of breast cancer.

The use of LH-RH agonist for the breast cancer is limited to premenopausal women. In fact in postmenopausal women the ovarian functions are ceased, and the estrogens are primarily produced in peripheral tissues such as fat and muscle [12].

In recent years, different evidences demonstrate that LH-RH probably affects directly a variety of extrapituitary tissues [13]. An autocrine/paracrine function for LH-RH has been suggested to exist, for example in the placenta, granulosa cells, myometrium and lymphoid cells. Moreover, it is probable that such LH-RH-based autocrine systems are present in a number of human malignant tumours, including breast, ovary, endometrium and prostate cancer. Anyhow, this mechanism of action is not yet well known.

Both leuprolide activities, on the pituitary or directly on target cells like tumor tissues, have led to the use of this molecule in various hormone-dependent pathologies, like prostate cancer [6], central precocious puberty [14], endometrial cancer [15], breast cancer [11] and ovarian cancer [16]. Luteinizing hormone seems to be implicated also in Alzheimer disease [17,18]; so the use of leuprolide in this pathology is currently under study.

## 2. Available formulations

### 2.1 Parenteral administration (injections and implants)

As already shown, leuprolide acetate is currently commercialized in different formulations; the principals are as reported in **Table 2**.

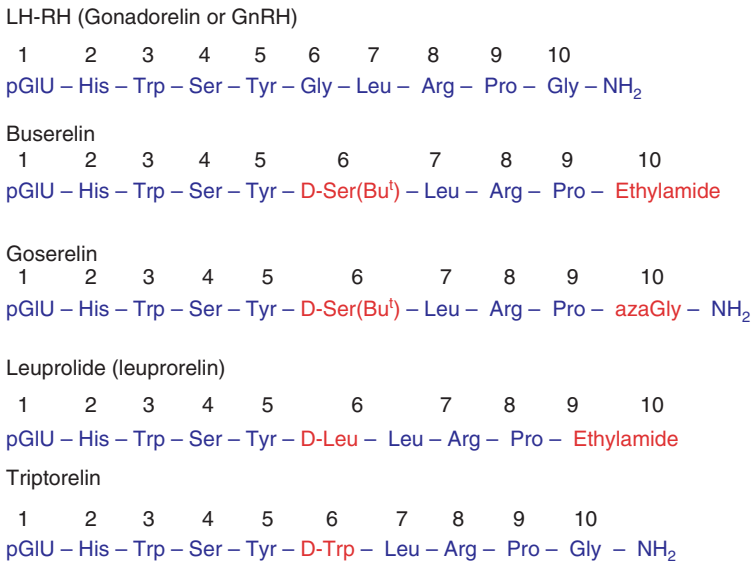


**Figure 1. Amino acid composition of the principal luteinizing hormone-releasing hormone LH-RH agonists.** Modifications from the natural hormone are marked in red. [5].

**Table 1. Principal commercialized LH-RH agonists.**

| | Company | Commercial name | Administration | Half-life | Molecular weight | Therapeutical use |
|---|---|---|---|---|---|---|
| Leuprolide [21,66,67,75-79] | TAP Pharmaceuticals/ Takeda/Abbott<br>Sanofi-Aventis<br>Abbott<br>Bayer<br>Eriochem | Lupron/Enantone/ Leuplin<br>Eligard<br>Lucrin<br>Viadur<br>Lectrum | Injection or implant | 3 h | 1209 | Prostate cancer<br>Endometriosis<br>Uterine fibroids<br>Precocious puberty<br>Breast cancer |
| Buserelin [80] | Sanofi-Aventis | Suprefact | Injection or nasal | 50 – 80 min (inj.)<br>1 – 2 h (nasal) | 1240 | Prostatic cancer<br>Endometriosis |
| Goserelin [81,82] | AstraZeneca | Zoladex | Injection | 2 – 4 h | 1269 | Prostate cancer<br>Breast cancer |
| Histrelin [83,84] | Indevus Pharmaceuticals | Vantas (prostate cancer)<br>Supprelin LA (precocious puberty) | Implant | 3.9 h | 1323 | Prostate cancer<br>Precocious puberty |
| Nafarelin [85,86] | Pfizer | Synarel | Nasal | ~3 h | 1321 | Precocious puberty<br>Endometriosis |
| Triptorelin [87-89] | Ferring Pharmaceuticals | Decapeptyl (infertility, endometriosis)<br>Gonapeptyl (prostate cancer) | Injection | ~3 h | 1311 | Infertility<br>Endometriosis<br>Prostate cancer |
| | Ipsen | Diphereline (prostate cancer, endometriosis) | | | | |
| | Watson Pharma | Trelstar (prostate cancer) | | | | |

Due to the chronic administration of leuprolide, all the formulations are conceived to possibly assure a constant release of drug with the lower number of administrations; at present this is achieved mainly with depot formulations or medical devices.

The implantable device, commercialized by Bayer, consists in a small cylinder containing the drug dissolved in dimethyl sulfoxide, a rate-controlling membrane and a piston. In a separate part of the device, osmotic tablets are present that by absorbing water presses the piston with a

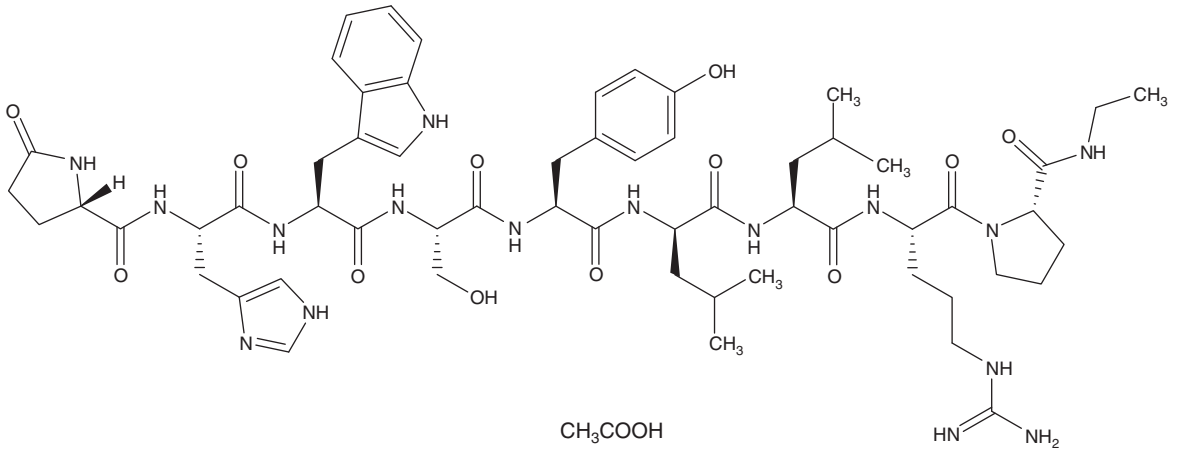


**Figure 2. Leuprolide acetate.**

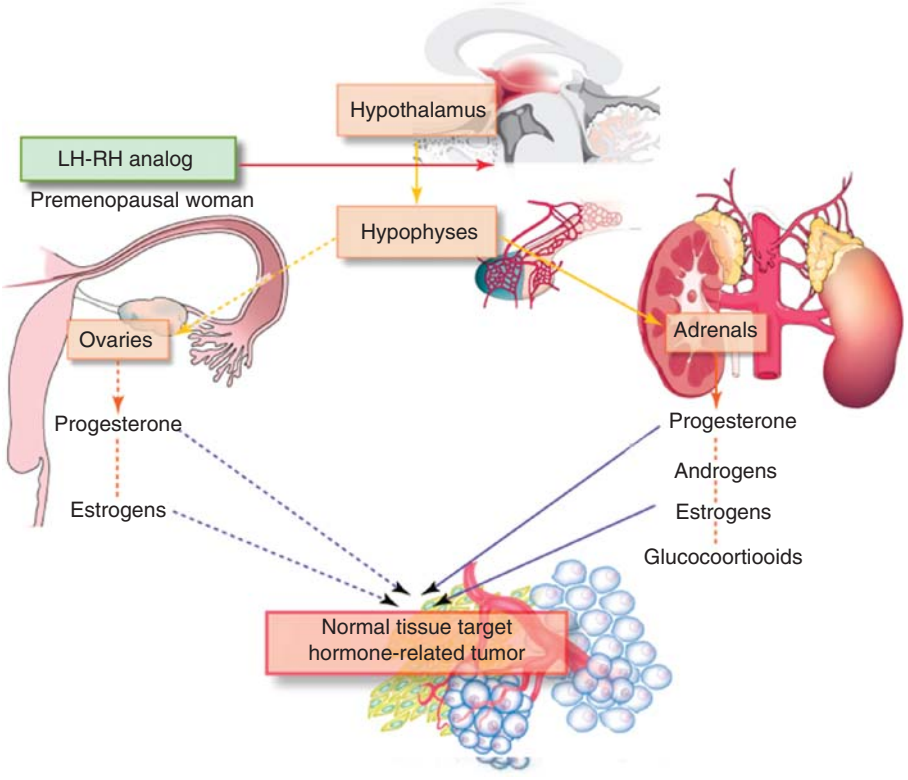


**Figure 3. Schematic representation of the hypothalamus–hypophysis axis and hormonal stimulation of ovaries and adrenal glands.**

Adapted from [95].

progressive release of the drug. Leuprolide showed an increased stability with the increase of the concentration in dimethyl sulfoxide, making this drug a good candidate for implantable devices [19]. *In vitro* performance of this device demonstrates a zero-order delivery for periods up to 1 year [20]. The first day after the implant placement there is a high release of drug due to thermal effects during system start-up, while from the day 14, the system delivers at values a steady state, which continues for the remainder of the 1-year delivery period. This implant must be inserted and removed with a small surgical intervention since the materials are not biodegradable; this aspect is also a major

**Table 2. Schematic representation of the principal formulations commercialized of leuprolide acetate.**

| Commercialized preparation | Route of administration | Dosage and pharmaceutical formulation | Description | Sales |
|---|---|---|---|---|
| | | **Leuprolide acetate** | | |
| Eligard [67] | Subcutaneous injection | 7.5 mg/1 month depot<br>22.5 mg/3 months depot<br>30 mg/4 months depot<br>45 mg/6 months depot | Supplied in two separate syringes whose content is mixed immediately before the administration: one contains the ATRIGEL delivery system (poly(DL-lactide-co-glycolide) polymer dissolved in N-methyl-2-pyrrolidone), the other contains leuprolide acetate powder | €58 million in 2004 (increase 76%) [90] |
| Lupron depot [66] | Intramuscular injection | 7.5 mg/1 month depot<br>11.25 or 22.5 mg/3 months depot<br>30 mg/4 months depot | Supplied in a prefilled dual-chamber syringe: one with leuprolide acetate, gelatin, DL-lactic and glycolic acid copolymer; the second with a diluent solution | ¥122 billion (2009) (€1 billion)<br>Product portfolio for: Leuplin (Japan), Lupron Depot (USA), Enantone/ Trenantone (Europe and Asia) [77] |
| Lucrin injection [91] | Subcutaneous injection | 1 mg/daily injection | Supplied as colorless solution with a concentration of 5 mg/ml of leuprolide acetate in an injectable aqueous solution | |
| Lucrin depot [91] | Intramuscular injection | 7.5 mg/1 month depot<br>22.5 mg/3 months depot<br>30 mg/4 months depot | Supplied in two separate depot vials or in a prefilled dual-chamber syringe: one contains leuprolide acetate, polylactic acid and mannitol in powder and the other a diluent solution | |
| Enantone [92,93] | Intramuscular or subcutaneous injection | 3.75 mg/28 days injection<br>11.25 mg/3 months depot | Supplied in two vials, one containing lyophilized micro-capsules of DL-lactic and glycolic acid copolymer and the other containing the solvent. The micro-capsules must be reconstituted just before the administration | |
| Lectrum [79] | Intramuscular or subcutaneous injection | 3.75 or 7.5 mg/28 days injection<br>11.25 mg/3 months<br>22.5 mg/3 months | Supplied in two vials, one contains lyophilized micro-spheres and the other the solvent. The micro-spheres must be reconstituted just before the administration | |
| Viadur [94] | Subcutaneous implant | Is a non-biodegradable osmotically driven implant containing 72 mg dissolved in dimethyl sulfoxide for 12 months | The titanium reservoir (DUROS®) measures 4 × 45 mm and contains a polyurethane rate-controlling membrane, an elastomeric piston, a diffusion moderator and the osmotic tablets, not released with the drug. The device must be removed after 12 months | Discontinuation of marketing in December 2007 [21] |

inconvenience of this delivery system. In 2007, Bayer Healthcare Pharmaceutical announced the discontinuation of marketing Viadur® with Duros technology due to a diminished market demand and growing manufacturing costs [21].

More recently a new patented technology was proposed by MicroCHIPS, Inc. [22]. This technology consists in a microfabricated reservoir composed of a great number of small individual chambers. These reservoirs are realized with microfabrication techniques and are then covered with a metal membrane. When this membrane is removed, the drug, contained in the reservoirs, diffuses. Initially, the mechanism used for the opening was an electrochemical reaction [23]. Successively, the use of an electrothermal mechanism was proposed, in which instead of the solubilization, the metal membrane was removed by a local resistive heating produced by an applied current [24]. This method presents the advantage of being independent from the chemistry of the environment surrounding the device and it is many times faster than the electrochemical device.

Any combination of drugs can be stored within these reservoirs, thereby protecting the sensitive reservoir contents from the environment until they are needed. Pre-programmed microprocessors, wireless telemetry or sensor feedback loops can provide *active control* of the opening of the reservoirs to initiate drug release or expose enclosed biosensors, thus giving the physician or patient a greater control over therapy. Alternatively, layers or combinations of controlled release polymeric matrices can provide *passive control* of the release or exposure of the reservoir contents, a mechanism which does not require microprocessors or power sources.

This delivery system with active controlled reservoir was used for leuprolide delivery. It was shown that this device, loaded with leuprolide, could provide pulsatile on demand delivery *in vivo* for 6 months [25], and consequently different studies were conducted in order to evaluate the *in vitro* performance of the multi-reservoir device with a range of solution phase, solid phase and semi-solid phase leuprolide formulations leading to the development of a novel solid phase formulation that was tested *in vivo* [26].

The main alternative to implant device is represented by the depot formulations. They consist generally in biodegradable and biocompatible polymers that after the injection produce a depot which releases the drug progressively. The administration must be repeated, according to the formulation considered, every 1, 3, 4 and 6 months by a qualified person. The components of the formulation must be mixed together right before the administration in order to obtain the solution that will be injected.

The main injectable leuprolide formulations were realized with poly(dl-lactide-co-glycolide) (PLGA) copolymer, like the already commercialized Lupron®, Enantone® and Eligard®. The drug release from PLGA microparticles is often characterized by a tri-phased release *in vitro* and *in vivo* [27]. A fast initial release phase (burst), followed by a second slow release phase (during days or weeks) and a third rapid release phase, is seen particularly with peptide and protein drugs.

The burst release is generally attributed to drug release from the surface of microparticles, while the slow release is a diffusion controlled phase. When the PLGA molecular weight becomes lower, the weight of the microparticles decreases rapidly, causing a rapid erosion-controlled release phase. In order to obtain a more continuous, and not a tri-phased, release the effect of different parameters on the preparation of the formulation was evaluated (type of PLGA, drying process and additives) [27]. The inclusion in the formulation of medium chain triglycerides modifies the release profile for leuprolide-loaded microparticles to a more continuous release *in vitro*. In addition, in order to modify leuprolide release it was also proposed that the blending of PLGA or PLGA-microspheres was obtained using polymers of two molecular weights [28].

*In situ* forming biodegradable drug delivery systems represent an attractive alternative to biodegradable implants and microparticles [29]. In fact, compared with preform delivery systems these formulations present the advantage of an easier administration, a less complicated fabrication process and simpler manufacturing conditions for sensitive drugs. One of the main methods used to obtain an in situ forming drug delivery system is represented by the solvent removal precipitation [30]. This injectable implant system consists of a water insoluble biodegradable polymer, like poly(lactic acid) (PLA), PLGA or Polycaprolactone, dissolved in water miscible, physiologically compatible solvent. After the injection into an aqueous environment, the solvent diffuses in the surrounding aqueous environment while water diffuses into the polymer matrix. Since the polymer is water insoluble, it precipitates upon contact with water and results in a solid polymeric implant. These kinds of drug delivery systems were also applied to leuprolide delivery. PLA was used for the realization of a polymer-based leuprolide delivery showing the possibility to obtain a release of leuprolide acetate for 3 months maintaining testosterone to castration level *in vivo* [31].

A similar formulation was also realized with PLGA. This delivery technology (Atrigel®) developed by Atrix Laboratories (now QLT, Inc.) [32] is at present commercialized in Eligard® (leuprolide acetate) pharmaceutical formulation.

Different disadvantages were associated with in situ forming implant, such as high injection force leading to a painful injection; local irritation at the injection site; the surface area of the resulting implant, controlling the drug release, may be variable depending on the injection technique and site; in addition, a high initial release may occur because of the formation of highly porous implants and the lag time of solidification of the polymer [30,33].

In order to overcome some of these problems a novel in situ forming microparticle system was proposed. This consists of an internal phase (drug-containing polymer solutions or suspensions) and an external phase (oil or aqueous solutions

with an emulsifier). The two phases are stored separately in two syringes and mixed through a connector before administration. The myotoxicity of different formulations was studied for this delivery system [34]. Leuprolide administration with this delivery technology was also considered. The stability of PLGA and leuprolide acetate was studied for in situ systems in various biocompatible solvents, oily and aqueous solutions [35]. Due to the potential instability of the polymer and drug in organic solvents, the formulation was realized as solid PLGA sponges, developed by dissolving PLGA and drugs (leuprolide acetate and lidocaine) in acetic acid or dioxane and then lyophilized. The sponges could be reconstituted in a solvent and formulated into in situ forming systems before parenteral administration.

Studies about leuprolide injectable formulation were also realized with a new lipid-based delivery technology, the DepoFoam® by Pacira Pharmaceuticals [36]. In the DepoFoam® system, each particle contains insight that discontinues internal aqueous chambers bounded by a continuous, non-concentric network of lipid membranes that confer a higher aqueous volume-to-lipid ratio and much larger particle diameters compared with classical multilamellar vesicle. This formulation may be applied to various routes of administration, like skin, intramuscular or intraocular and for the delivery of different proteins and peptides, like insulin, leuprolide, metenkefalin and octreotide (somatostatin analog) [37]. All these molecules showed a drug loading appropriate to their potential therapeutical applications and the leuprolide formulation showed an *in vitro* release profile in human plasma over a period of several weeks. Leuprolide formulation was also tested *in vivo* in rats, where the therapeutical effect was evaluated measuring the serum testosterone levels. An identical dose of leuprolide DepoFoam® or Lupron Depot® was injected intramuscular, and both showed a comparable suppression in testosterone levels.

The use of hetero–stereo complexes has been proposed as an alternative to the use of particulate systems. While homo–stereo complexes are formed by stereoselective polymers of identical chemical composition but of opposite enantiomeric configuration, hetero–stereo complexes may be formed between L-peptides and D-configured poly(lactic acid) (D-PLA). The main advantages of these delivery systems, compared with the use of biodegradable polymer matrices consist in the presence of a molecular interaction, which is only dependent little on diffusion, and in particles which are spontaneously generated, without need for surfactants or other additives. The use of hetero–stereo complexes between leuprolide and D-PLA has been proposed by Slager *et al.* [38]. Leuprolide release was studied in rats showing a burst effect in testosterone production followed by a decrease in the testosterone blood concentration.

Liposomes were also proposed in the intravenous injection of leuprolide with the objective to prolong the biological half-life and reduce the injection frequency [39].

## 2.2 Non-parenteral administration

### 2.2.1 *Transdermal route*

For a large number of drugs the delivery across the skin is limited by the natural barrier properties of the stratum corneum, the most external skin layer. Many approaches have been proposed in order to circumvent this barrier, including both physical and chemical methods but although in some cases they showed good results often they are also characterized by toxicological effects.

Iontophoresis is one of the strategies believed to have a great potential increasing transdermal absorption. It may be defined as the facilitated movement of ions of soluble salts across a membrane under an externally applied potential difference [40]. Drug input rate is controlled by the applied current intensity enabling individualized therapy according to patient needs and disease progression. A limiting aspect in transdermal delivery consists in the limited surface which can be available for the absorption, considering transdermal patches of a reasonable size, so the best candidates for this route of administration are represented by molecules at the same time potent, but with an insufficient, absorption for the other routes of administration, and peptides often present these properties [41]. By modulating the electric impulse it is possible to evaluate different delivery profiles. For example, continuous release of leuprolide induced a suppression of the testosterone levels achieving a chemical castration, while the pulsatile administration of LH-RH was able to mimic the body's natural release profile and promoted secretion of luteinizing hormone [42]. Between the LH-RH agonists, both leuprolide [43,44] and nafarelin [45] were studied for transdermal delivery.

The transdermal administration of leuprolide using patches delivering electrical current was compared with a subcutaneous administration, and the pharmacokinetic parameters found were similar, setting in evidence the interest of this kind of administration [46].

### 2.2.2 *Oral route*

The oral route represents the more interesting alternative to parenteral administration, since oral administration is easier, more economic and with a higher patient compliance. Despite these advantages, the oral administration of peptides and proteins also presents significant drawbacks, like high drug degradation and a low permeability with a consequent extremely low bioavailability. For these reasons, a great effort was applied to the research of formulations for the oral delivery of peptides and proteins.

Different studies have been conducted to assess the intestinal degradation of leuprolide, showing that the degradation is proportional to the amount of protein of intestinal homogenates [47]. The presence of enzymatic inhibitors can reduce the peptide degradation, and the same effect can be obtained with a w/o/w emulsion, although with a smaller degree of protection compared with the use of an inhibitor. Leuprolide permeability was also studied *ex vivo* across the different

intestinal regions in rabbits and rats [48], showing no significant differences in the permeability for ileum and colon but a reduced permeability (six times lower) for the jejunum in rabbits. Also, if the passage mechanism of leuprolide was not investigated, these results suggested that leuprolide may be absorbed not only via paracellular route but also by transcellular route. In fact, generally the paracellular transport of a molecule has a tendency to decrease from the small intestine to the colon due to a decrease in the surface area, cell density and tight junctions between cells. The same absorption tendency was also obtained in anesthetized rats where leuprolide was administered in solution directly into the different intestinal regions with a surgical intervention.

Finally, the oral administration of a leuprolide w/o/w emulsion showed the possibility to obtain a reduction in testosterone serum levels comparable to the one obtained with commercially available Lupron® depot [49]. The study was conducted in rats for 35 days comparing the effect of two doses, 1 and 2 mg/rat/day and a dose of 1 mg/rat administered two times in a day (with a final dose of 2 mg/rat/day). All these different combinations of doses had the same pharmacological effect, reducing the testosterone concentration to the castration level. Plasma testosterone concentrations increased greatly just after the first dose of leuprolide microemulsion and then decreased sharply to below the normal control level after 2 days of treatment. A similar pattern was also observed with the administration of Lupron® depot with a dose of 3.75 mg in a single subcutaneous injection.

Liposomes are often used to encapsulate drugs in order to protect them from degradation and/or obtain a controlled release. However, liposomes present the great inconvenience to be easily destructed by the pH, bile salts and pancreatic lipase if administered via oral route [50].

To minimize this degradation, the introduction of a polymeric membrane around the liposomes has been proposed. Same authors evaluated the use of chitosan, a hydrophilic biocompatible polysaccharide, as coating material [51]. These chitosan-coated liposomes showed interesting properties for controlled delivery purposes, although the choice of the chitosan molecular weight and of the lipids is extremely important because of their interaction with leuprolide [51].

The use of thiolated chitosan was also recently proposed for the intestinal delivery of leuprolide [52]. The use of thiolated polymers, also called thiomers, has appeared as a promising excipient for the delivery of drugs [53]. These polymers present the advantage of forming inter- and intramolecular disulphide bonds within the thiomers itself or with mucus glycoproteins showing mucoadhesive properties, prolonged disintegration time and a comparatively more controlled release of incorporated drugs. Chitosan has been reported in literature to increase leuprolide permeability across Caco-2 cells of a factor greater than 5 [54], and leuprolide delivery with unmodified chitosan particles showed an increase in absolute bioavailability compared with the control solution, from 0.3% to 43% [52]. The use of thiolated chitosan as gel formulation showed an increase in absolute bioavailability, $AUC_{0\text{-}t}$ and $C_{max}$ compared with the same formulation with unmodified chitosan.

Also polyacrylic acid nanoparticles were proposed for the oral delivery of leuprolide [55]. In this formulation, in order to achieve the formation of nanoparticles in a mild, aqueous environment, two different techniques were combined, the hydrophobic ion pairing between leuprolide and sodium dodecyl sulfate in a first step, followed by encapsulation into nanoparticles gained by interpolymer complexation between polyacrylic acid and Pluronic F68. The drug encapsulation in nanoparticles showed an absolute bioavailability in rats of 0.55, with 0.26 for the control solution.

DOR BioPharma, now Soligenix, [56] has developed a new system, the Lipid Polymer Micelle (LPM™) for enhancing the intestinal absorption for water-soluble drugs, like peptides, which are not absorbed or are degraded in the gastrointestinal tract. This new delivery platform has also been applied to leuprolide delivery, and in preclinical study this system has demonstrated significant intestinal absorption.

Also gastrointestinal patches have been proposed in order to improve the intestinal absorption of leuprolide. This drug delivery device was recently proposed in order to protect the drug from degradation, optimize the concentration gradient and localize the effect of enzyme inhibitors or permeation enhancers, reducing the amount administered of these substances and also localizing their side effects [57]. The use of gastrointestinal patches showed an increased intestinal absorption *ex vivo* compared with the control solution, thanks to the high local concentration release obtained with these devices [58].

### 2.2.3 Pulmonary and nasal

Pulmonary route was also proposed for leuprolide administration. This route presents different advantages compared to muscular and intestinal administrations, like large surface area, extensive vasculature, easily permeable membrane, and low intracellular and extracellular enzymatic activity.

Leuprolide for pulmonary administration was studied formulated in liposomes, in order to enhance the drug bioavailability [59]. A preliminary pharmacokinetic study, conducted in rats showed an absorbed fraction increased significantly compared with the leuprolide solution administered intratracheally and to the physical mixture with liposomes. Liposomal encapsulation acted as a biodegradable reservoir which prolonged the pulmonary residence time of the drug. Moreover, controlling the proportions in the hydration during liposomes preparation, it was possible to obtain neutral and negatively charged liposomes, and this last formulation showed a slight increased bioavailability compared with neutral liposomes.

Also an alcohol-based aerosol suspension of leuprolide was administered intratracheally to beagle dogs [60], in order to evaluate the leuprolide absorption after repeated administrations. In this early study a decrease in lung bioavailability for the leuprolide was noticed, when the results obtained on day

1 were compared with those obtained on day 14. This decrease in plasma levels may be related to a tolerance effect and/or an increased enzymatic activity of the lung following chronic administration of this alcohol-based aerosol formulation.

In a different study various leuprolide formulations were tested to evaluate the parameters influencing the nasal and the aerosol absorption of the drug in rats. The information obtained were then used to conduct Phase I studies in healthy male volunteers [61]. The pharmacokinetics of nasally administered leuprolide acetate in humans was not significantly affected by co-administration with EDTA and alpha-cyclodextrin (alpha-CD). Moreover, contrarily to rat data, there was a slight decrease in bioavailability of leuprolide comparing with versus without alpha-CD/EDTA, suggesting a negative effect of this enhancer system on nasal absorption of this peptide in humans. This difference in absorption between rats and human suggests also that the absorption mechanism for this drug is probably different in the two species.

Numerous studies have been conducted in order to optimize the residence time and the release profile in the nasal cavity and to obtain a prolonged release and/or a better absorption [62,63]. The use of lipophilic sugar-based compounds, like oligosaccharide ester derivatives (OED) has been proposed to obtain a modified release profile of leuprolide [64]. The particles realized in this study were not optimized for specific routes of administration, but were administered subcutaneously and intratracheally, and compared with intravenous administration as control. The results showed a controlled release *in vitro* and in both intratracheal and subcutaneous administrations. Improved encapsulation and initial burst of release have been demonstrated using a single o/w emulsion method using hydrophobically ion paired leuprolide. The objective of this study was to reduce the burst release of leuprolide, since this may represent a way to reduce the secondary effects related to the initial increase in testosterone concentration. Hydrophobic ion pairing of peptides has the potential to alter solubility and facilitate stability in organic solvents; in this case the ion pairing technique has been based on spray drying to improve hydrophilic peptide encapsulation in lipophilic OED microparticles.

#### 2.2.4 Sublingual

Leuprolide gel formulations were studied after a sublingual administration in dogs, monkeys and humans [65]. In dogs a higher bioavailability (47%) was found compared with the one found in monkeys (3%) with the same dose. In humans, the bioavailability was similar to that of monkeys (2 – 3%), also if the doses administered were different. The pharmacokinetic data of the intravenous administration were also analyzed in the three species. In human and monkey the data were better described by a two-compartmental model, while in dogs the concentration kinetics were better described by a one-compartmental model, suggesting a different *in vivo* distribution between dogs and humans.

## 3. Conclusions

Leuprolide is an LH-RH superagonist used in clinical practice for various pathologies, like palliative treatment of prostate cancer, breast cancer, endometriosis and precocious puberty. Despite all these applications leuprolide is at present commercialized only in injectable formulation and implantable devices. Although if these formulations improved the clinical applicability of leuprolide treatments, they present also important limitations mainly related not only to the injectable formulation itself but also to the complex therapeutical applications of this drug. A great effort was applied on the research of delivery systems to allow the leuprolide administration for alternative routes of administration, for example, oral, pulmonary, nasal, transdermal and sublingual. Also if the proposed formulations showed an improvement in the leuprolide absorption there is still a need for a completely efficient delivery system which could be safely and effectively applied in clinic to replace or integrate the current available formulations.

## 4. Expert opinion

All the depot formulations previously described introduced an important improvement in leuprolide clinical practice, particularly for the palliative treatment of prostate cancer since they present an administration interval of several months reducing the costs and the discomfort for patients when compared with the daily injections.

On the other hand, these formulations are often complex to administer, needing qualified persons to administration the injection, for example, Lupron Depot® requires the presence of a physician when the two components are mixed [66]. The injectable formulations are characterized by two prefilled syringe, one with the delivery system and one with leuprolide acetate powder (i.e., Eligard®) or by a prefilled dual chamber syringe (i.e., Lupron Depot®). The storage and administration conditions must be carefully verified, for example, in the case of Eligard®, the vials must be stored between 2 and 8°C but must reach room temperature before mixing; once mixed, it must be administered within 30 min or discarded [67].

Owing to leuprolide's relatively small molecular weight and long half-life (**Table 1**), it is a good delivery candidate for other routes of administration, like buccal, nasal or oral. As general improvement in clinical practice these routes will present a better patient compliance in comparison with parenteral administrations (injectable formulations or implant devices). Another important limitation of the formulations currently commercialized is represented by the impossibility of a more accurate adjustment of the dose to the individual patient. A clinical study conducted with Lupron Depot® (3-month depot) showed the influence of the body mass index (BMI) on the serum concentration of testosterone, also after the administration of Lupron Depot® [68]. These analyses showed

that despite lower baseline serum testosterone levels, obese men had significantly higher serum levels of total and free testosterone during treatment than men with normal BMI. In this study the testosterone concentration was measured only at the end of treatment period, 3 months after the injection, so the testosterone concentrations were unknown during this time. A possible explication to this phenomenon was proposed by Agarwal *et al.* [69]. In the Lupron Depot® the leuprolide, a hydrophilic peptide, is entrapped in biodegradable, highly lipophilic synthetic polymer microspheres. After injection into the intramuscular space, the polymer microspheres are slowly degraded releasing the biologically active peptide. It is possible that the lipophilic depot injection forms a larger area of depot in the obese subject, due to the increased amount of adipose tissue in the muscle. Consequently, there will be a greater amount of polymer exposed to the enzymatic degradation and the result will be an altered release profile from the microspheres that probably will be degraded before the 3-month period. Anyhow, there are still different aspects of the leuprolide administration in obese subjects that must be clarified.

The Guidelines on Prostate Cancer 2008 [70] also suggested the possibility to use an intermittent therapy of androgen blockade, offering the possibility to preserve quality of life and reduce the treatment-associated costs. This could be possible only with an intermittent administration of LH-RH agonists. In addition, more frequent, but easily, administrations could offer the possibility to stop the treatment at any time, and this is an important aspect considering the number of allergic and anaphylactic reactions reported for leuprolide and LH-RH agonists in general [71-74].

As previously reported a great effort has already been done in the non-parenteral delivery of leuprolide, but a completely efficient formulation is still missing representing still a challenge in the delivery of this interesting drug. Non-parenteral administration of leuprolide could indeed offer concrete improvement not only in patient compliance, but also in patient safety allowing a better control on the administered dose and making possible a discontinuation of the therapy at any time. On top of that, alternative routes of administration may also offer the opportunity eventually of an intermittent therapy with a consequent reduction of the costs related to the therapy.

## Declaration of interest

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