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PHARMACOKINETICS AND ENDOCRINE EFFECTS OF SLOW RELEASE FORMULATIONS OF LHRH ANALOGUES

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Summary—The LHRH agonists are antigonadotropic agents for reversible ovarian suppression in gynaecology and in oncology. In oncology, pituitary inhibition is maintained with high release rates preferably by implant or microcapsule injection. The pharmacokinetics of buserelin after injection, infusion, and during implant treatment (controlled release) are described. The release rate is monitored by urinary buserelin excretion (fractional excretion of 30% of the daily dose). During therapy, LHRH agonists in serum are measured by specific radioimmunoassays, with or without extraction. A more convenient non-invasive procedure is to measure the amount of buserelin in 24-h urine samples (during injections or nasal spray), or the urinary buserelin/creatinine ratio in morning urine samples (during infusions or implants). After high dose injection, buserelin has a half-life of 80 min, therapeutic plasma concentrations are maintained for 8–12 h. In long-term maintenance with buserelin implants (polylactide–glycolide, 75:25), serum concentrations and urinary excretion showed an extended plateau phase indicating a suitable dose interval of 2–3 months. In endometriosis and leiomyoma, the minimum release rate (urinary buserelin) required for maintenance of steroid suppression was established (buserelin excretion of about 0.5 μg/g creatinine). Buserelin implants in prostate carcinoma are effective for 2 or 3 months, after a single dose of 6.6 or 10 mg buserelin, respectively. A consistent suppression of serum testosterone secretion was confirmed for more than 2 yr. Buserelin microparticles are effective in rhesus monkeys to completely suppress follicular maturation and oestrogen secretion during 4–6 weeks after a single dose of 3.6 mg buserelin. Recent results on the controlled release of an LHRH antagonist (Hoe 013) from biodegradable microparticles in rats with DMBA-induced mammary tumours indicate that tumour suppression by LHRH antagonists is well tolerated and highly effective. The local tolerance at the injection site of antagonist microparticles is excellent as in the case of LHRH agonists like buserelin.

INTRODUCTION

In pharmacotherapy of hormone-dependent tumours, appropriate controlled release preaparations of therapeutic peptides can improve drug efficacy, patient convenience and compliance. When a peptide is administered by conventional injection the pharmacokinetics are less appropriate than if a steady release rate is achieved which is more appropriate to avoid exposure to high drug concentrations, and at the same time ensures a minimum therapeutic concentration for a longer dose interval.

The work presented here is collaborative effort where our own laboratory investigated the preclinical aspects of drug delivery by controlled release, in close relation with clinical investigation of implants in oncology. In the development of injectable preparations for peptides, polylactide glycolide copolymers [1, 2] have played a major role, especially because their toxicity has been carefully evaluated. The tissue tolerance is excellent, the rates of degradation can be controlled by various modifications to the polymer.

METHODS

The investigations on implants and microparticles were performed in rats and monkeys, as described previously [1–5]. The agonist buserelin and the antagonist Hoe 013 [6] were measured by specific HPLC/RIA methods [6]. Several of the clinical studies have been reported in detail [6–11] or reviewed [12, 13].

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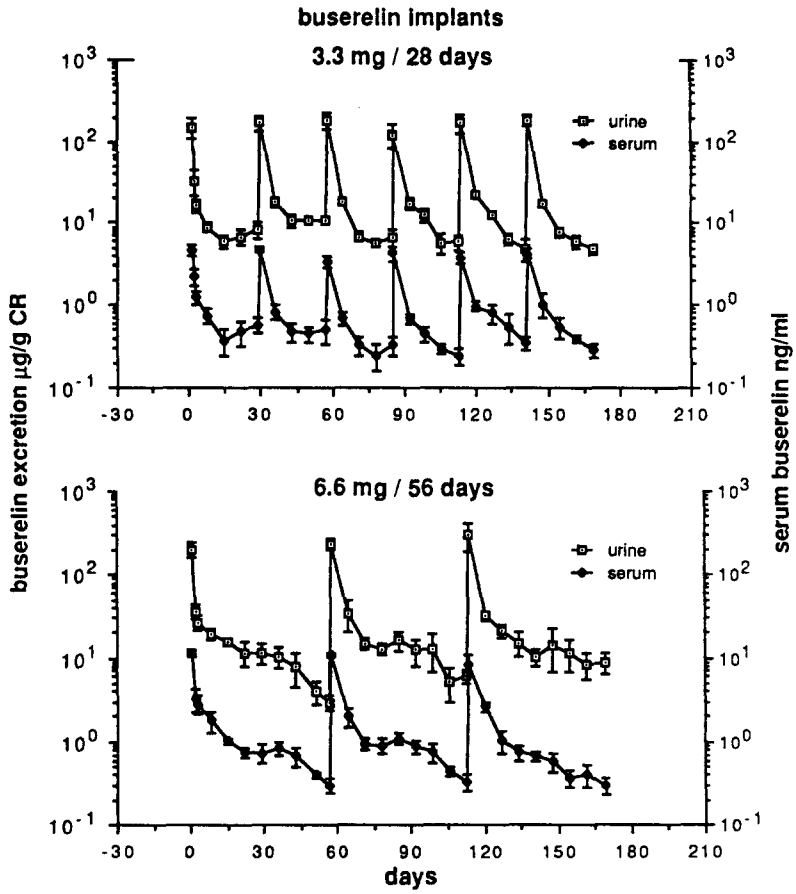

Fig. 1. Buserelin release from implants of polylactide–glycolide, 75:25 in patients with prostate carcinoma estimated by the urinary buserelin excretion. Two doses sizes of 3.3 and 6.6 mg were administered at dose intervals of 28 or 56 days during a treatment period of six months. Note the close correlation of serum concentrations and urinary buserelin/creatinine ratio.

RESULTS

Biodegradable implants

In many of the studies on prostate carcinoma treatment, implants of biodegradable copolymer of lactide/glycolide have been used [11]. In the buserelin implants of slowly degrading polylactide–glycolide, 75:25 there are two pieces of 1 cm, each contains a dose of 3.3 mg buserelin. The degradation rate of the polymers is really the important factor is giving a predictable duration of action. Studies with a dose of 3.3 or 6.6 mg per implant in a long-lasting polymer have shown that the degradation of this polymer starts around 8 weeks after injection and is completed after 14–16 weeks after injection [1, 2, 5, 14]. With the two dose sizes, a dose interval of either 4 or 8 weeks can be selected. For the 8-week interval the dose size was doubled.

In pharmacokinetic and pharmacodynamic studies with buserelin implants PLG 75:25 (dose size 3.3 and 6.6 mg) in prostate carcinoma, a comparison with the previous dosage form (buserelin nasal spray/Suprefact™) shows consistent testosterone suppression when implants are injected at 2-month intervals, and favourable pharmacokinetic release properties (Fig. 1). The release of buserelin had an extended plateau phase with a clinical dose interval of 56 days at the dose size of 6.6 mg buserelin. The maximum therapeutic serum concentration (C_{max}) on the first day of implantation was 8.24 (5.74–10.1) ng/ml serum (mean and range of two studies with six treatment periods), and the minimum therapeutic concentration on day 56 was 0.41 (0.33–0.62) ng/ml serum. The maximum therapeutic concentration (C_{max}) in the urine was 233 (199–303) µg/g creatinine, and the minimum therapeutic concentration (C_{min}) at the end of 56 days was 6.77 (3.02–9.74) µg/g creatinine. Serum testosterone concentrations were consistently suppressed to the low castrate range in each of the four studies with long-acting buserelin implants [6, 11]. In biopharmaceutical studies it was shown that more than 70% of the dose

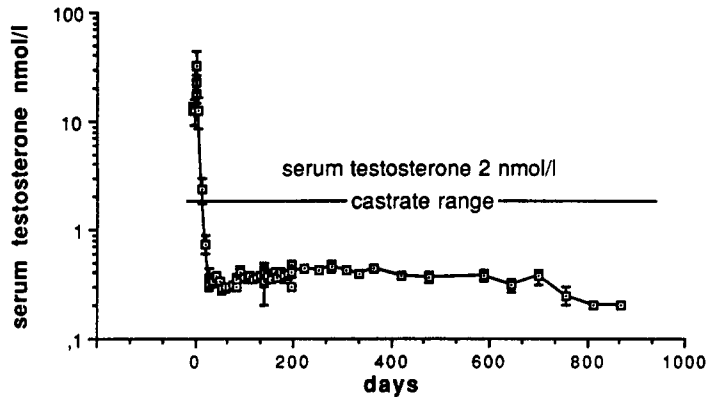

Fig. 2. Long-term follow-up in 17 patients with prostate carcinoma. The treatment period was more than 800 days. Serum testosterone during treatment with buserelin implants 6.6. mg at dose intervals of 56 days. Study by Waxman and coworkers [11].

was released after 56 days, and biodegradation of the implant material was complete after 180–240 days [9, 13].

The buserelin monitored by a specific radioimmunoassay [2, 5, 10, 14] showed that concentrations in the urine were higher than the corresponding serum concentrations [6, 11]. For long-term monitoring, the urinary buserelin/creatinine ratio was preferred (urinary excretion data). The urinary buserelin/creatinine ratio was closely correlated with the serum concentration in all studies. Two studies were based on a dose size of 3.3 mg and a dose interval of 28 days, and two other studies were based on a dose size of 6.6 mg and a dose interval of 56 days [6, 11]. A follow up of more than 800 days in one study (Fig. 2) showed consistent testosterone suppression for the entire treatment period [11]. In this follow up (dose size 6.6 mg and dose interval 56 days), a highly reproducible release was found.

The average conversion factor from serum concentration to urinary buserelin/creatinine ratio was 20. The C_{min} at the end of each treatment period during full testosterone suppression measured in the serum was 0.41 (0.33–0.62) ng/ml. The corresponding calculated C_{min} estimated from the urinary excretion data on day 56 of each treatment period was 0.31 (0.15–0.49) ng/ml serum equivalent to a urinary excretion rate of 6.78 (3.02–9.74) μg/g creatinine (mean and range).

For a comparison of nasal spray and implant treatment, the minimum therapeutic concentration (C_{min}) required for testosterone suppression in prostate carcinoma patients was estimated from urinary excretion data treatment with the buserelin nasal spray (Suprefact™, daily dose 1200 μg [7]). This nasal spray dose is equivalent to the s.c. injection of 30 μg buserelin (corrected for nasal absorption). The average buserelin/creatinine ratio during treatment with the nasal spray formulation was 5.89 μg/g creatinine, and the corresponding cumulative excretion was 6.62 μg buserelin within 24 h [7]. During implant treatment, the average buserelin/creatinine ratio on day 56 (the last day of each treatment period) was 6.77 μg/g, equivalent to a cumulative excretion of 7.60 μg buserelin within 24 h. The bioequivalence study with buserelin PLG 75:25 implants shows that the pharmacokinetics after consecutive implant injection are comparable with those during nasal spray maintenance therapy. The dose size of 3.3 mg (dose interval 28 days) and the dose size 6.6 mg (dose interval 56 days) provide therapeutic release rates which suppress testosterone secretion consistently and reliably [6, 11]. For patient convenience and compliance, the dose interval of 56 days (dose size 6.6 mg buserelin) is preferable. In all patients, serum testosterone was suppressed below the castrate range of 1 ng/ml (2 nmol/l).

The clinical efficacy of a buserelin nasal spray (Suprefact™) was confirmed in numerous studies (400 μg three times per day). The average daily buserelin excretion was 6.62 μg/24 h and the corresponding buserelin/creatinine ratio was 5.89 μg/g creatinine. At this urinary buserelin/creatinine ratio, consistent testosterone suppression is maintained by buserelin absorbed from the nasal spray or released s.c. on the last day of each implant treatment period [3, 12].

The pharmacokinetics of buserelin release from implants showed a maximum therapeutic serum concentration (C_{max}) of 6.77–9.71 ng/ml on the first day of each implication. In comparison, after a buserelin injection of 500 μg s.c.,

Table 1. Therapeutic concentrations of buserelin during implant treatment

Dose size	Serum conc. (ng/ml)	Urinary excretion (μg/g creatinine)	Dose interval
3.3 mg	C_{max} 4.96	C_{max} 138.3	(28 days)
	C_{min} 0.47	C_{min} 7.4	
6.6 mg	C_{max} 8.24	C_{max} 233.7	(56 days)
	C_{min} 0.41	C_{min} 6.8	

the C_{max} is 7.8–10.3 ng/ml serum. The urinary buserelin/creatinine ratio on the first day of implantation (C_{max}) was 234 μg/g creatinine (range 199–303), and the corresponding excretion during injection of 1500 μg buserelin per day was 295 μg within 24 h (equivalent to 263 μg/g creatinine). It was concluded that the C_{max} concentrations on day 1 of implant treatment are similar to those during s.c. injections of 500 μg buserelin, and the C_{max} excretion of buserelin on the last day of implant treatment is similar to the average daily excretion during nasal spray treatment [12].

The pharmacokinetic data for the two dose sizes of implants indicate a dose-proportional release and similar C_{min} values at the appropriate dose interval.

In one study by Waxman [15] it was also possible to extend the treatment period to 3 months using a polylactide–glycolide copolymer (75:25 molar ratio) by increasing the dose from 6.6 to 10 mg. The critical limit of the drug release required for testosterone suppression was maintained throughout the study. Implants maintain testosterone suppression much more approximately than daily injections and the maximum and minimum values found in patients remain in the low castrate range.

Similar implants have also been used in studies on treatment of premenopausal mammary carcinoma [12], and again there was a very reproducible release pattern.

Minimum therapeutic release rates

In many instances, the effective concentrations and release rates of LHRH agonists and antagonists can be measured using urinary excretion of intact drug or the sum of intact drug of metabolites. This parameter of urinary peptide excretion is more useful than the closely related serum concentrations, because it is non-invasive and the patient can provide frequent morning urine samples for a long-term follow up.

In patients with benign disease conditions responsive to transient suppression of gonadal steroid secretion we were able to assess what one cannot do in oncology, i.e. to look at the duration of suppression and the related pharmacokinetics to establish the minimum therapeutic drug concentration (C_{min}), which is required for consistent inhibition of gonadal steroid secretion. The minimum therapeutic concentration (C_{min}) required for ovarian steroid suppression was estimated from studies in women of reproductive age treated for endometriosis and other gynaecological disorders [9,12]. In women with endometriosis [9], four consecutive implants were administered. After the last implant, the release rate decreased steadily and reached the point where the therapeutic effects were no longer maintained. Oestrogen secretion was consistently suppressed until a urinary buserelin/creatinine ratio of 0.4–0.6 μg/g was reached. At this release rate, the return of ovarian activity was observed in all patients. The minimum therapeutic serum concentration (C_{min}) for oestrogen suppression calculated from the urinary excretion data was 20–60 pg/ml serum. An average conversion factor of 20 is applicable for calculation of serum buserelin (ng/ml) from data of urinary buserelin excretion (μg/g creatinine).

One preclinical study was performed in male rhesus monkeys [16] starting treatment before the onset of puberty, designed as an experiment to predict the effects in children with precocious puberty because concern had been expressed about their fertility when reaching adult age after prolonged suppression. In 8 prepubertal rhesus monkeys about 3 years of age maturation was suppressed for 20 months by buserelin implants of polylactide–glycolide injected s.c. every 4 weeks. During treatment, testosterone levels remained low (0.25–0.5 ng/ml) in all monkeys. Serum testosterone and testicular volume started to rise 10 weeks after the last implant in all animals, when buserelin excretion had decreased below 1.5 μg/g creatinine. Pubertal maturation proceeded normally, maximal serum testosterone concentrations were measured 28 weeks after the last injection. Spermatogenesis was detectable in all monkeys 32 weeks after the last implant. It was concluded that buserelin treatment delayed pubertal maturation for 20 months. Sexual development was completed in all animals within one year after treatment.

Biodegradable microparticles

The prospects of developing injectable microcapsules or microparticles are particularly

attractive, because thermal inactivation of peptides during manufacturing is avoided and injections are facilitated by using a narrow gauge needle. What about future preparations? In gynaecology, the use of implants is inconvenient, because local anaesthesia may be required. It improves patient convenience to develop injectable microparticles resuspended immediately before use and injected through a 22 gauge needle. The price one has to pay for this increased convenience is a marked change in the release profile. As shown in Figs 1 and 2 for the release profile of implants used in oncology, there is a very steady release rate from slow degrading polymers like polylactide–glycolide (75:25 molar ratio). This is usually not the case with microparticle or microcapsule preparations. There is a more pronounced initial release, a secondary increase and at some point, the minimum therapeutic concentration is reached. Probably the differences are due to the high surface area of the microparticles in relation to the more condensed and more compact implants. With such microparticle preparations one can achieve suppression periods of about 6 weeks, in one example shown above and with a different polymer that is usually employed for the clinical studies. In monkeys, one can achieve a suppression period of about 28–35 days [16]. The onset of suppression and the end of suppression were shown by measurement of serum oestradiol concentrations in two preclinical studies (Fig. 3). The local reaction to the subcutaneous injection of microparticles may be of interest. The usual histology around implants is well known, there is a formation of a thin fibrous capsule with sparse macrophages and a few giant cells which remove the degradation products [1]. The question, of course, is how microparticles affect the local environment of a subcutaneous injection site when an LHRH agonist is gradually released, and especially if other peptides, e.g. LHRH antagonists, are incorporated. The investigation of microparticles loaded with an LHRH antagonist 4 weeks after s.c. injection in the inguinal region of a rat confirmed that there is no inflammatory reaction, but the formation of a thin fibrous capsule as in the case of implants. Microparticle preparations of polylactide–glycolide (50:50 molar ratio) are completely resorbed at the injection site within 6–8 weeks.

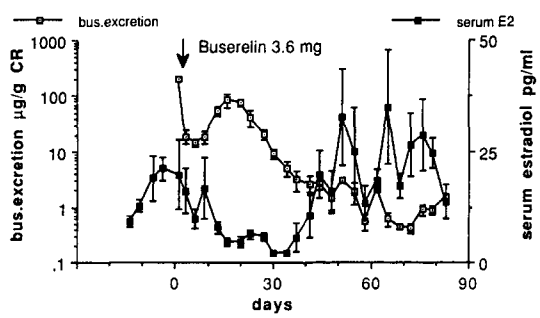

Fig. 3. Establishing the minimum therapeutic release rate of buserelin in monkeys treated with one injection of microparticles (PLG 50:50) at a dose of 3.6 mg buserelin. The busereline release was monitored by measuring the urinary buserelin/creatinine ratio. When the minimum therapeutic concentration had been reached (at a urinary ratio of 3 µg buserelin per gm creatinine), follicular maturation was resumed as shown by the rise in serum estradiol.

Studies with an LHRH antagonist

Recently we have investigated the use of similar preparations for an LHRH antagonist. In this particular case, the release profile was monitored *in vitro* and *in vivo*, by a specific RIA method for the antagonist and by HPLC. With a similar technique of suspending the peptide in microparticles, one can achieve a half-life of release of about 8 days, in contrast to buserelin implants with a half-life of release of more than 40 days and a duration of inhibition of tumour progression of more than 120 days after a single implant (Fig. 4). In studies with tumour bearing animals, DMBA was injected 17 days before preventive treatment to inhibit tumour development, or 76 days after DMBA at the time of full tumour formation. Ten weeks after DMBA induction, there was a fully developed tumor progression in the DMBA-treated rats and all untreated rats had died until day 130 after DMBA. The injection of one buserelin implant or the repeated injection of the antagonist every

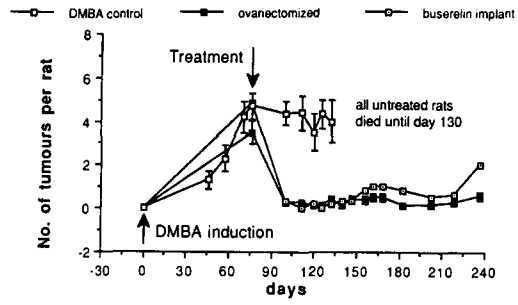

Fig. 4. Effect of treatment with one buserelin implant or ovariectomy on progression of DMBA-induced mammary tumours in rats. After tumour induction by DMBA on day 1, treatment started on day 76. All untreated tumour-bearing rats died by day 130. Ovariectomy prevented tumour progression completely for more than 160 days. A single buserelin implant injection of 3.3 mg prevented tumour progression for 120 days. Means and standard error of 10 rats per group.

second week prevents the development of tumours or induces regression of existing tumours. There is a gradual increase of the number of tumours when the release from the slow release formulations is exhausted and peptide release decreases very quickly by accelerated biodegradation of the polymer.

The effect of the new glycosylated LHRH antagonist (Hoe 013) on DMBA-induced mammary tumours in rats using a microparticle preparation for controlled release confirmed that the immediate onset of gonadal steroid suppression after LHRH antagonists is a distinct clinical advantage on oncology. The glycosylated decapeptide [17], [AcD-Nal(2),D-PCl-Phe,D-Trp,Ser,Tyr,D-Ser(Rha),Leu,Arg, Pro,AzGly-NH2] LHRH, was evaluated for its biological potential and pharmacokinetics in normal female rats and in rats with DMBA tumours. Female rats were treated with controlled release preparations of the antagonist in polylactide–glycolide, 50:50. Pituitary gonadotropin depletion by the agonist and antagonist was established by specific RIA for rat LH. Constant rate infusion of buserelin 5 μg or antagonist Hoe 013 60–120 μg s.c. per day from osmotic minipumps (Alza Corp.) reduced uterine weight in a similar manner as castration. The controlled release formulation (3.6 mg antagonist per rat for 2 weeks) suppressed tumour development as effectively as ovariectomy. Tumour growth was also inhibited after repeated doses of 3.5 mg buserelin microparticles for 4 weeks. The organ distribution of ^{125}I-labelled antagonist Hoe 013 showed long-lasting pituitary accumulation, a serum half-life of 40–60 h, and prolonged urinary and biliary excretion. Receptor affinity for rat pituitary membranes was 10-fold higher than buserelin. No symptoms of histamine release were found in dogs after 0.5 mg/kg i.v., and 0.5–1 mg/kg i.v./s.c. were well tolerated by mice, rats, guinea-pigs and rabbits. From these recent results we concluded that this LHRH antagonist is highly effective and well tolerated, its long duration of action indicates an important clinical potential which is presently being investigated in other experimental tumours.

CONCLUDING REMARKS

First of all, we want to achieve better drug efficacy with controlled release formulation in clinical oncology. At the same dose, the peptide has higher therapeutic activity. If you treat a prostate carcinoma patient with implants instead of injections you will need a 10-fold less dose. Furthermore, we want to improve drug safety. In the case of the treatment with LHRH agonists, this is really a point which is less important due to the absence of severe side effects, but with other more toxic substances incorporated into controlled, release formulations it becomes important, e.g. with cytostatic agents. At lower peptide concentrations, the risk of side effects is reduced. Finally, the most important point to date is that drug acceptance by the patient is improved. There is an effective long-term medication without involuntary errors or compliance problems. Controlled release is an important improvement for therapeutic peptides and examples are provided by several therapeutic peptides in this meeting.

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