J. Pharm. Pharmacol. 1991, 43: 762–765
Received November 27, 1990

In-vivo Release of a GnRH Agonist from a Slow-release Poly(lactide-glycolide) Copolymer Preparation: Comparison in Rat, Rabbit and Guinea-Pig

NADJA HEINRICH, KLAUS FECHNER, HARTMUT BERGER, DOROTHEA LORENZ, ERIKA ALBRECHT, GERALD RAFLER*, HEINZ SCHÄFER† AND BURKHARD MEHLIS

*Institute of Drug Research, Alfred-Kowalke-Str. 4, 0-1136 Berlin, *Institute of Polymer Chemistry, 0-1530 Teltow-Seehof, and †Institute for Isotope and Radiation Research, Robert-Rössle-Strasse 10, 0-1115 Berlin-Buch, Germany*

Abstract—Different batches of 50:50 poly((±)-lactide-glycolide) copolymer (PLG) were used as biodegradable carriers for D-Phe^6-gonadotropin-releasing hormone (GnRHa) in the form of injectable long-acting implants loaded with 10% GnRHa and tracer amounts of [^{125}I]GnRHa. After their injection subcutaneously into rats, rabbits, and guinea-pigs, the release kinetics of the peptide were determined by counting the radioactivity remaining in the implants (i) after recovery from the rats after death or (ii) directly on the skin above the injection site of rabbits and guinea-pigs in-vivo. No significant differences in the release pattern of the peptide amongst the three species whether the release process was controlled by diffusion or by degradation of the polymeric matrix were found. It is concluded that the results of in-vivo release tests using laboratory animals are valid for man and that enzymes are not involved in the degradation of the polymeric matrix. The results may be of general importance for the use of long-term release PLG formulations of highly active drugs, especially peptides and proteins.

Copolymers of (±)-lactide and glycolide (PLG) have been found to be very useful in forming matrices for parenteral, prolonged controlled-release (depot) formulations of agonists of gonadotropin-releasing hormone (GnRH). These compounds effectively suppress the blood level of the steroid hormones and, therefore, are being used in the therapy of steroid hormone-dependent cancers such as prostatic cancer and other steroid-dependent diseases (e.g. endometriosis). For this purpose it is necessary that the peptides should be continuously present in the blood over months or even years. This can be conveniently achieved by means of injectable drug-loaded implants or microcapsules of PLG (for reviews see Dutta 1988; Filicori & Flamigni 1988; Waxman 1988).

These biocompatible formulations are biodegradable, i.e. in the body the polymeric matrix is degraded to form non-toxic monomers (Hollinger & Battistone 1986). The peptides are thought to be released by two processes: (i) diffusion-controlled, which leads to a rapid release of part of the peptide immediately after injection of the formulation (burst effect) and (ii) degradation-controlled, determining the duration of the release process (Sanders et al 1984; Hutchinson & Furr 1985; Mason-Garcia et al 1985; Fraser et al 1987; Gonzalez-Barcena et al 1989).

We have found that the pattern of the release kinetics of the peptides from PLG implants as investigated in-vitro and in-vivo in rat is strongly dependent on the properties of the polymers and peptides used (Sanders et al 1984, 1986; Hutchinson & Furr 1985; Mason-Garcia et al 1985; Asano et al 1989). Therefore, reliable tests are required with any new batch of a PLG formulation or after changing the formulation.

Of special importance for the use of these formulations in human medicine is the problem that the peptide release kinetics in man should be the same as observed in in-vivo tests using laboratory animals. We thought it reasonable to draw such a conclusion if the release kinetics in different laboratory animals were identical.

Correspondence: N. Heinrich, Institute of Drug Research, Alfred-Kowalke-Str. 4, 0-1136 Berlin, Germany.

Materials and Methods

Formulations

Six batches of 50:50 poly((±)-lactide-glycolide) copolymer (PLG I-VI), obtained by ring opening polymerization of glycolide/lactide mixtures with tinoctoate as initiator (Rafler et al 1990), were used as biodegradable carriers for D-Phe^6-GnRH (Berlin-Chemie AG, Germany), a superactive agonist of gonadotropin-releasing hormone (GnRHa). The polymers differed in their intrinsic viscosities, varying from 10 to 33 mL g^{-1} as measured in dimethylformamide at 20 °C. The wetted copolymer and peptide (10% w/w) together with ^{125}I-labelled peptide, obtained according to Berger et al (1982), were thoroughly mixed by grinding. After drying under vacuum and grinding, cylindrical rods (ca. 18 mg, 2·4 mm diam., 2–3 mm long) were prepared with a spindle press. The rods contained about 40000 counts min^{-1} of [^{125}I]GnRHa as measured in a scintillation well counter.

Administration

The rods of PLG containing 10% GnRHa/[^{125}I]GnRHa were injected through a needle (2·5 mm i.d.) subcutaneously into (i) the nuchal region of Wistar rats (~250 g) anaesthetized by pentobarbitone sodium (Nembutal, Serva), (ii) the flank of guinea-pigs (~300 g) under narcosis by Nembutal, and (iii) the flank of rabbits (~2·5 kg) under local anaesthesia by 2% lignocaine (Ursocain, Serum-Werk Bernburg, Germany). Rats received one rod each while rabbits and guinea-pigs received three rods at a single injection site to improve the detection of radioactivity by direct in-vivo counting at the skin.

Release profiles of GnRH

The kinetics of release of GnRHa were determined by radioactivity measurements of [^{125}I]GnRHa in the implants. Rats were, at the indicated times, killed by carbon dioxide and the remaining implants were excised from the injection site in the neck. The radioactivity was determined in a scintillation well counter and compared with that of the rods before their injection (zero time value).

In rabbits and guinea-pigs, the radioactivity was monitored by placing a scintillation counter directly onto the shaved skin above the injection site. With the help of a pointer instrument, the optimum localization of the counter was searched out and the ^{125}I-activity was counted for 1 min. The procedure was repeated several times and the maximum value of the single measurements was used for calculation of the release.

The zero time value was determined within 12 min of injection. Within this time, as control experiments with rats showed, no loss of radioactivity from the injection site occurred. It was also shown that there was at no time any accumulation of radioactivity in the tissue surrounding the implant, i.e. all radioactivity monitored in-vivo originated from tracer bound in the formulation.

The radioactivity measurements were corrected for the natural decay of the ^{125}I-isotope and for possible changes in the counting device during the course of the experiments by using ^{125}I-standards. From the time-dependent decrease of radioactivity following the injection of the formulations, the release of GnRHa was calculated and is shown here as plots of the percent peptide remaining at the injection site vs time.

Comparative experiments

The following six experiments were performed to compare the release of peptide from the injected implants in rats, rabbits, and guinea-pigs, using six different batches, I-VI, of PLG: I, 1 rabbit, 25 rats; II, 1 rabbit, 10 rats; III, 1 rabbit, 10 rats; IV, 1 guinea-pig, 10 rats; V, 6 rabbits, 34 rats; VI, 1 rabbit, 5 guinea-pigs, 29 rats.

Results

There were no problems in monitoring ^{125}I of the implants with the counter on the skin above the subcutaneous injection site of rabbits and guinea-pigs. The efficiency of the counting of the implants in-vivo for rabbits and guinea-pigs was $20{\cdot}0 \pm 0{\cdot}46$ and $20{\cdot}1 \pm 0{\cdot}62\%$, respectively, when compared with the counting in the well counter before the injection. This shows that the implants were uniformly localized in the animals, resulting in good conditions for monitoring their radioactivity.

Control experiments on the direct in-vivo counting of implants in narcotized rats showed the counting efficiency to be of the same value ($20{\cdot}0 \pm 1{\cdot}3\%$). Furthermore, it was observed that the counting efficiency reached a stable value and did not change within 10 min immediately after the injection of the formulations, suggesting that they reached a fixed parition very rapidly.

Fig. 1 shows plots of the time-dependent ^{125}I remaining in the implants at the subcutaneous injection sites of the animals. The 6 batches of copolymers, although all obtained from 50% (±)-lactide and 50% glycolide, released

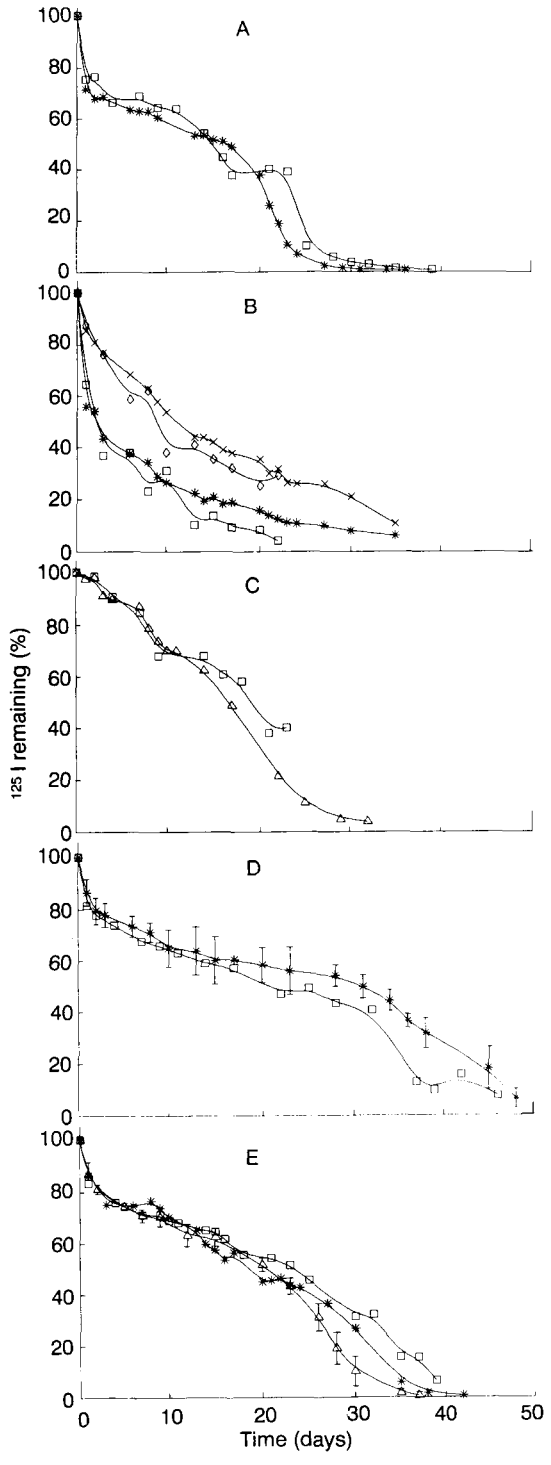

FIG. 1. Time course of the percentage of ^{125}I remaining in the implants at the subcutaneous injection site of rats, rabbits, and guinea-pigs after administration of rods of 6 different batches (I–VI) of biodegradable 50:50 poly((±)-lactide-glycolide) copolymers containing 10% (w/w) D-Phe^6-GnRH and about 40 000 counts min^{-1} ^{125}I-labelled D-Phe^6-GnRH. ^{125}I was monitored in rabbits and guinea-pigs in-vivo and in rats after killing the animals and recovering the implants. A: implants of polymer I in 1 rabbit (*) and 25 rats (□). B: implants of polymer II (*, □) and III (X, ◇) each in 1 rabbit (*, X) and 10 rats (□, ◇). C: implants of polymer IV in 1 guinea-pig (△) and 10 rats (□). D: implants of polymer V in 6 rabbits (*) and 34 rats (□), s.d. is given for rabbits. E: implants of polymer VI in 1 rabbit (*), 5 guinea-pigs (△), and 29 rats (□), s.d. is given for guinea-pigs.

[^{125}I]GnRHa at different rates when used as matrices for GnRHa at 10% (w/w).

Whereas with implants from polymer III (Fig. 1B) and IV (Fig. 1C) no, or only a slight, burst effect was observed, the implants of polymer II released about 50% of the peptide as rapidly as within 3 days, the rest being released very slowly within about 5 weeks (Fig. 1B). Implants of polymer I gave a polyphasic release pattern (Fig. 1A) with an extremely short burst of peptide and a second increase in the release 3 weeks after injection. Generally, the release of peptide was complete within 4 to 7 weeks.

In no case was a significant difference in the release of [^{125}I]GnRHa observed, when rats, rabbits and guinea-pigs were compared.

Discussion

After the injection of implants of poly(($\pm$)-lactide-glycolide) copolymer (PLG) containing D-Phe^6-GnRH (GnRHa) and ^{125}I-labelled GnRHa subcutaneously into rabbits and guinea-pigs, it was possible to pursue the complete time course of the release of the peptide from the long-acting formulation using a single animal (Fig. 1).

For rats the method is not so convenient because they have to be anaesthetized during every measurement to keep them inactive. Furthermore, in this case the implants could not be injected into the nuchal region because a part of the released radioactivity, presumably [^{125}I]iodide, was found to be accumulated in the thyroid gland, interferring with the monitoring on the neck. These drawbacks were not observed with rabbits or guinea-pigs; they also accumulated some radioactivity in the gland, but this did not disturb the measurement over the injection site in the flank.

To determine the biodegradability of the formulations, rats were killed at different times following implantation. From the decrease of weight of the implants recovered from the injection site, it was found that all batches of polymers used were degraded within 6-7 weeks (data not shown). Generally, combining the use of a small number of rats and one or two rabbits or guinea-pigs should allow the assessment of the biodegradability and release kinetics of peptides that can be labelled with ^{125}I for subcutaneously injected slow release formulations.

Although all PLG samples used here had the same composition with respect to the relative amounts of lactic and glycolic acid residues, they showed different release profiles of GnRHa from the implants. Generally, the release kinetics of peptides from implants or microcapsules of PLG depend on the average molecular weight and the polydisperity of the polymers, and on the arrangement of the monomers within the polymer chain. The procedure for preparation of the implants may also influence the release pattern. This is shown in Fig. 1B where implants III were obtained by brief heating of implants II at 100°C. We found that this procedure generally lowers the release of the peptide (unpublished data). However, the exact mechanisms underlying the different release kinetics are not clear.

The most striking result is that no significant differences in the kinetics of the in-vivo release of GnRHa from the implants were found when compared for rats, rabbits, and guinea-pigs (Fig. 1). This was true for the kinetic phases of rapid release of peptide immediately after injection, assumed to be controlled by diffusion, as well as for those of slow release thereafter, controlled by biodegradation. These results are most important for drawing conclusions about the kinetics of PLG slow release formulations of drugs in man; it is likely that the drug release pattern in man is the same as in any laboratory animal if there are no differences amongst various species. A similar but indirect conclusion may be drawn from Ogawa et al (1989) who found serum levels of leuprolide in dogs showed essentially the same pattern as those in rats, following the injection of a PLG formulation.

Furthermore, our results lead to the conclusion that biodegradation of PLG is by hydrolysis of their ester bonds without participation of enzymes since it seems unlikely that such enzymatic activities would be precisely the same in all species. Similarly, identical release kinetics of a GnRH agonist were found in rats after subcutaneous and intramuscular injection of PLG implants containing the peptide (Ogawa et al 1989). Certain enzymatic activities have been shown to accelerate the hydrolysis of polylactic and polyglycolic acid in-vitro (Williams & Mort 1977; Williams 1981; Fukuzaki et al 1989). However, studies on PLG in the form of rod-like implants (Sanders et al 1986) or microcapsules with incorporated GnRH agonists (Ogawa et al 1988) did not reveal differences in the kinetics of release of the peptides, when in-vivo experiments using rats were compared with in-vitro experiments; thus, participation of enzymes in the in-vivo degradation could be excluded. We have also found the release kinetics in-vivo to correspond with those of in-vitro studies (unpublished data).

Parenteral biodegradable slow-release depot formulations have been studied mainly in combination with agonists of GnRH incorporated into implants or microcapsules of PLG. GnRH agonists are ideal compounds to be used in such dosage forms for several reasons. Like other peptides, their bioavailability after oral and other non-parenteral administration is too low to make use of them with the possible exception of administration by the nasal route (Yamazaki 1984; Chan et al 1988; Dutta 1988; Filicori & Flamigni 1988). For GnRH agonists to be used in the therapy of hormone-dependent diseases, especially cancer, they need to be present in the blood continuously over months and even years, which can only be effectively achieved by injectable depot formulations such as biodegradable PLG implants or microcapsules. Furthermore, the doses of the GnRH agonists necessary for their biological activity are extremely low, which means that they may be easily packed into an injectable depot form, to be effective for weeks or even months (Dutta 1988; Filicori & Flamigni 1988).

However, it is unlikely that biodegradable long-acting depot formulations will be restricted to the GnRH peptide family. The number of biologically active peptides, such as natural regulatory peptides or analogues of such peptides obtained by chemical synthesis, is being increased and new peptide and protein drugs can be expected from developments in biotechnology.

References

Asano, M., Fukuzaki, H., Yoshida, M., Kumakura, M., Mashimo, T., Yuasa, H., Imai, K., Yamanaka, H. (1989) In vivo characteristics of low molecular weight copoly(D,L-lactic acid) formula-

tions with controlled release of LH-RH agonist. Biomaterials 10: 569–573

Berger, H., Schäfer, H., Klauschenz, E., Albrecht, E., Mehlis, B. (1982) Rapid assay for in vitro degradation of luteinizing hormone releasing hormone. Anal. Biochem. 127: 418–425

Chan, R. L., Henzl, M. R., LePage, M. E., LaFargue, J., Nerenberg, C. A., Anik, S., Chaplin, M. D. (1988) Absorption and metabolism of nafarelin, a potent agonist of gonadotropin-releasing hormone. Clin. Pharmacol. Ther. 44: 275–282

Dutta, A. S. (1988) Luteinizing hormone-releasing hormone (LHRH) agonists. Drugs Fut. 13: 43–57

Filicori, M., Flamigni, C. (1988) GnRH agonists and antagonists. Current clinical status. Drugs 35: 63–82

Fraser, H. M., Sandow, J., Seidel, H., von Rechenberg, W. (1987) An implant of a gonadotropin releasing hormone agonist (buserelin) which suppresses ovarian function in the macaque for 3-5 months. Acta Endocrinol. 115: 521–527

Fukuzaki, H., Yoshida, M., Asano, M., Kumakura, M. (1989) Synthesis of copoly(D,L-lactic acid) with relatively low molecular weight and in vitro degradation. Eur. Polym. J. 25: 1019–1026

Gonzalez-Barcena, D., Perez-Sanchez, P. L., Graef, A., Gomez, A. M., Berea, H., Comaru-Schally, A. M., Schally, A. V. (1989) Inhibition of the pituitary-gonadal axis by a single intramuscular administration of D-Trp-6-LH-RH (decapeptyl) in a sustained-release formulation in patients with prostatic carcinoma. Prostate 14: 291–300

Hollinger, J. O., Battistone, G. C. (1986) Biodegradable bone repair materials. Clin. Orthop. Related Res. 207: 290–305

Hutchinson, F. G., Furr, B. J. A. (1985) Biodegradable polymers for the sustained release of peptides. Biochem. Soc. Trans. 13: 520–523

Mason-Garcia, M., Vigh, S., Comaru-Schally, A. M., Redding, T. W., Somogyvari-Vigh, A., Horvath, J., Schally, A. V. (1985) Radioimmunoassay for 6-D-tryptophan analog of luteinizing hormone-releasing hormone: measurement of serum levels after administration of long-acting microcapsule formulations. Proc. Natl. Acad. Sci. USA 82: 1547–1551

Ogawa, Y., Okada, H., Yamamoto, M., Shimamoto, T. (1988) In vivo release profiles of leuprolide acetate from microcapsules prepared with polylactic acids or copoly(lactic/glycolic) acids and in vivo degradation of these polymers. Chem. Pharm. Bull. 36: 2576–2581

Ogawa, Y., Okada, H., Heya, T., Shimamoto, T. (1989) Controlled release of LHRH agonist, leuprolide acetate, from microcapsules: serum drug level profiles and pharmacological effects in animals. J. Pharm. Pharmacol. 41: 439–444

Rafler, G., Dahlmann, J., Wiener, K. (1990) Biologisch abbaubare Polymere. Acta Polymerica 41: 328–333

Sanders, L. M., Kent, J. S., McRae, G. I., Vickery, B. H., Tice, T. R., Lewis, D. H. (1984) Controlled release of a luteinizing hormone-releasing hormone analogue from poly(d,l-lactide-co-glycolide) microspheres. J. Pharm. Sci. 73: 1294–1297

Sanders, L. M., Kell, B. A., McRae, G. I., Whitehead, G. W. (1986) Prolonged controlled-release of nafarelin, a luteinizing hormone-releasing hormone analogue, from biodegradable polymeric implants: influence of composition and molecular weight of polymer. Ibid. 75: 356–360

Waxman, J. (1988) Gonadotrophin releasing hormone analogues for prostatic cancer: an overview. Semin. Oncol. 15: 366–370

Williams, D. F. (1981) Enzymic hydrolysis of polylactic acid. Engin. Med. 10: 5–7

Williams, D. F., Mort, E. (1977) Enzyme-accelerated hydrolysis of polyglycolic acid. J. Bioengin. 1: 231–238

Yamazaki, I. (1984) Serum concentration patterns of an LHRH agonist, gonadotrophins and sex steroids after subcutaneous, vaginal, rectal and nasal administration of the agonist to pregnant rats. J. Reprod. Fert. 72: 129–136