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Design and evaluation of a biodegradable implant for improved delivery of oestradiol-17β to steers

P.B. Deasy [a], M.P. Finan [a], P.R. Klatt [b] and T. Hornykiewytsch [b]

[a] *Department of Pharmaceutics, Trinity College, University of Dublin, Shrewsbury Road, Dublin 4 (Ireland)* and [b] *Hoechst AG, Frankfurt am Main 80 (Germany)*

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Summary

Novel biodegradable implants were designed for extended delivery of effective levels of the growth promoting agent, oestradiol-17β, to steers. They were usually composed of five alternating active containing tablets measuring 4 mm diameter × 2 mm thick aligned face on with four inactive spacer tablets of similar dimensions. Both compact types were composed of various copolymers of polylactic-glycolic acid, chosen with a higher glycolide content in the spacer units to promote faster degradation. The resultant controlled increase in surface area of exposed active containing compacts in the composite implant gave increasing release with time, whose rate could be varied by copolymer ratio in the active compacts, their drug loading and by the composition and thickness of spacer compacts. The initial burst of release from poorly entrapped drug on the surface of an implant was minimized by reducing the drug loading in the terminal active compacts, which tend to contribute disproportionately to the initial surface area of the device. Prolonged dissolution testing on single compacts and composite implants facilitated selection of implant designs which showed the desired increase in active release from about 0.25 mg to 0.5 mg daily over 3–6 months implantation in limited numbers of steers with associated favourable weight gain, compared to untreated controls. The novel implants described have potential for the improved delivery of other classes of drugs.

Introduction

Various anabolic agents such as the natural hormones oestradiol-17β, progesterone and testosterone and the synthetic hormones zeranol and trenbolone acetate, are used outside the European Economic Community to enhance growth and improve carcass quality in beef cattle. These products are normally in the form of implants, composed of a variable number of aligned small tablets, which are administered by trochar injection to the base of the ear of treated animals. The injection site should be discarded at the time of slaughter to prevent possible residues entering the food chain. A disadvantage of existing conventional implants is that they tend to produce a declining release of growth promoter over about 1 month, which necessitates multiple reimplantation and does not mimic the requirement for increasing dose of growth promoter as the animal rapidly gains weight. The only prolonged release implant is Compudose® (Elanco), containing 24 or 45 mg oestradiol-17β in a silicone matrix designed for 200 or 365 day treatment, respectively, which has inadequate loading and tends to release active in a pseudo-zero manner. This cylin-

Correspondence to: P.B. Deasy, Dept. of Pharmaceutics, Trinity College, University of Dublin, Shrewsbury Road, Dublin 4, Ireland.

drical implant measures 5 mm × 25.4 mm and tends to be excessively bulky in comparison to other implants. Recently, Yamakawa et al. (1990) have reviewed the limitations of existing drug implants for various classes of drugs and have described a novel double-layered implant based on the use of biodegradable polymer for the extended delivery of insulin.

This project is concerned with the development of a prototype implant intended to deliver oestradiol-17β at an initial rate of about 0.25 mg day^{-1}, increasing to about 0.5 mg day^{-1} over 3–6 months of implantation. This release profile was considered to be more ideal than is provided by existing implants, the new product being particularly suitable for use in veal calves or finishing steers. The in vitro development of the product and its preliminary evaluation in limited numbers of steers are described. The drug delivery system employed is based on the use of homo- and copolymers of polylactic acid (PLA) and polyglycolic acid (PGA), which are reported to cause no localized tissue response, have regulatory approval for use in human and veterinary medicines and unlike silicone polymers are biodegradable. The rate of hydrolysis of these polyesters, which critically influences drug release, decreases in accordance with the series d-PLA < dl-PLA < PLA : PGA < PGA and with increasing molecular weight of each polymer type (Brophy and Deasy, 1990). Because of the lack of commercial availability of an adequate range of these polymers for formulation development work, it is necessary to synthesize and properly characterize them with regard to composition and molecular weight. Use of PGA and high content PGA copolymers is constrained by their lack of solubility in all solvents acceptable for the manufacture of dosage forms, necessitating their incorporation by physical mixing.

Materials and Methods

Materials

Acetone, methanol (HPLC grade, Riedel-de-Haen), butylcyanoacrylate (Loctite), citric acid, cocktail T, disodium hydrogen orthophosphate, magnesium carbonate, (British Drug Houses), oestradiol-17β (Sigma, micronized grade), [2,4,6,7,16,17-^{3}H]oestradiol-17β toluene/ethanol solution (Amersham), polylactic acid (M_n = 49 000, Polysciences) and water purified by ion exchange resin were used. All materials were reagent grade unless otherwise indicated. The wide range of polymers and copolymers of PLA/PGA required were synthesized from their respective dimers, lactide and glycolide (Boehringer Ingelheim), using stannous octanoate and lauryl alcohol (Sigma) as catalyst and accelerator, respectively, and characterized by intrinsic viscosity, gel permeation chromatography, laser light scattering, DSC, infrared spectroscopy and NMR as described previously (Deasy et al., 1989). The molecular weight of the PGA homopolymer used could not be reliably determined because of lack of solubility, but all the other polymers synthesised for the project had a M_n in the range 15 000–22 500.

Methods

Preparation of compacts and implants

Typical 2 mm thick compacts containing variable percentage of drug, both unlabelled and radiolabelled, and polymer were prepared from feed material formed either by physical mixing or a granulation procedure with acetone, and compressed at a compression weight of 28 mg using 4 mm diameter flat-faced tooling in an IR press under an applied force of 244 kg m^{-2}. Inactive compacts comprised of 60% polymer with 40% added magnesium carbonate to improve binding were prepared likewise, the compression weight being adjusted to achieve compacts of 1, 1.5 or 2 mm thickness as required. Table 1 shows the composition of some active compacts examined in order to study the effect of method of preparation, drug loading, polymer type and the relationship between rate of in vitro and in vivo release. Compacts were drilled centrally with a 0.5 mm hole before being threaded in the required number and order onto a nylon filament with a recessed knot between closely adjacent immobilized compacts to form composite implants radiolabelled with 1.85 MBq activity for in vitro dissolu-

tion studies. Table 2 lists the composition of some implants, whose compacts were prepared by granulation and which were examined by in vitro dissolution testing or by in vivo studies in cattle as indicated.

Dissolution studies

Individual compacts or their composite implants were suspended on nylon filament in 2.5 or 10 l, respectively, McIlvaine's buffer pH 7.4 or water at 37°C in sealed containers which were shaken twice daily. The rate of drug release up to 12 weeks was followed by liquid scintillation counting using cocktail T as scintillant and a Tri-Carb 200 liquid scintillation counter (Packard). The dissolution medium was replaced three times weekly to ensure that adequate sink conditions were maintained for the poorly soluble hormone. Results were based on duplicate determinations and the release profile presented are for the use of water as the dissolution medium because preliminary experiments showed that use of the buffer did not affect in vitro release rate.

TABLE 1

Composition of some active compacts prepared for in vitro and in vivo studies

Compact	Composition	Method of preparation	Studied	
			In vitro	In vivo
1	40% oestradiol-17β +60% l-PLA (1)	PM	yes	no
2	60% oestradiol-17β +40% l-PLA (1)	G	yes	no
3	40% oestradiol-17β +60% l-PLA (2)	PM	yes	yes
4	40% oestradiol-17β +60% dl-PLA	PM	yes	yes
5	40% oestradiol-17β +60% PLA:PGA 80:20	G	yes	yes
6	40% oestradiol-17β +60% PLA:PGA 50:50	G	yes	yes
7	40% oestradiol-17β +60% PGA	PM	yes	no

l-PLA (1) prepared in-house, l-PLA (2) from Polysciences; PM, physical mixing; G, granulation.

In vivo studies

For in vivo studies, unradiolabelled and undrilled single compacts or composite implants were inserted at the base of the ear in veal calves or steers, respectively, by trochar injection, having previously sedated each animal by injection of xylazine (Rompun®, Bayer). To prevent the observed displacement of compacts on implantation, neighbouring faces in implant 9 were bonded together with a thin film of butylcyanoacylate, whereas in implant 10 the inactive spacers were omitted to see if this would affect in vivo release. Drug release was determined by periodic assay of oestradiol-17β content by HPLC in the residue recovered from the implantation site. A 25×0.4 cm C_{18} μ-Bondapak column was used with a mobile phase of water:methanol 5:1, flow rate of 1 ml min^{-1} and UV detection at 220 nm (Hewlett-Packard). In some studies plasma oestradiol-17β levels were also monitored by radioimmunoassay (RIA) using the Serono Diagnostika kit for the steers. The assay has a detection limit of approx. 6 pg ml^{-1} plasma. The implantation site was examined for any signs of histotoxicity. A minimum of five animals was entered into each study. During the steer trial, animals received feed according to their requirement to calculate the feed conversion at the end of the trial period.

Results and Discussion

In vitro studies on compacts and implants

Fig. 1 shows the effect of granulation vs physical mixing on the in vitro release of oestradiol-17β from compacts. The release profile is less variable from compacts produced by granulation as presumably they contain a more uniform distribution of drug. A large initial release of oestradiol-17β (burst-effect) is apparent due to greater ease of dissolution of drug particles exposed on the surface of the compacts. Periodically the matrix randomly eroded to give irregular release, but there was a tendency for release to decline with time due to reduction in area of the active front. Similar irregular in vitro release of progesterone from PLA films was reported by Pitt et al., (1979).

TABLE 2

Composition of some implants for in vitro and in vivo studies

Implant	Active compacts			Inactive compacts			Bonded	Studied	
	No. used × composition	Thickness (mm)	Weight (mg)	No. used × composition	Thickness (mm)	Weight (mg)		In vitro	In vivo
1	5 × 60% oestradiol-17β + 40% PLA : PGA 80 : 20	2	28	4 × 60% PLA : PGA 60 : 40 + 40% $MgCO_3$	1	14	no	yes	no
2	5 × 1 above	2	28	4 × 1 above	1.5	21	no	yes	no
3	5 × 1 above	2	28	4 × 1 above	2	28	no	yes	no
4	5 × 1 above	2	28	4 × 60% PLA : PGA 70 : 30 + 40% $MgCO_3$	2	28	no	yes	no
5	5 × 1 above	2	28	4 × 60% PLA : PGA 50 : 50 + 40% $MgCO_3$	2	28	no	yes	no
6	5 × 60% oestradiol-17β + 40% PLA : PGA 90 : 10	2	28	4 × 1 above	2	28	no	yes	no
7	5 × 60% oestradiol-17β + 40% PLA : PGA 50 : 50	2	28	4 × 1 above	2	28	no	yes	no
8	2 × 50% oestradiol-17β + 50% PLA : PGA 70 : 30 and 3 × 1 above	2	28	4 × 4 above	2	28	no	no	yes
9	as 8 above	2	28	4 × 4 above	2	28	yes	no	yes
10	as 8 above	2	28	omitted	–	–	no	no	yes

Increase in active loading from 40 to 60% in l-PLA (1) containing compacts increased their release from about 0.1 to 0.15 mg $week^{-1}$ after the burst-effect declined. Increase in molecular weight of the l-PLA (1) [M_n = 17 000] to l-PLA (2) [M_n = 49 000] decreased drug release by retarding erosion. Fig. 2 also shows that when the polymer was altered to the more biodegradable dl-PLA, the terminal release becomes greater and more variable due to its faster and more irregular erosion compared to l-PLA (1) at constant loading.

Fig. 2 shows that increasing the content of PGA from 20, 50 and 100% in the polymer used at constant oestradiol-17β loading resulted in greater release at 6 weeks of about 0.1, 0.5 and 0.6 mg $week^{-1}$, respectively. The compacts prepared from PLA : PGA 50 : 50 eroded randomly liberating fragments, whereas those prepared from PGA disintegrated within two weeks of immersion into the dissolution medium.

The composite implants all contained inactive spacer compacts, which were composed of more biodegradable polymer than used in the adjacent active compacts as illustrated in Fig. 3, which shows the design of implant 8. This differential degradation causes a progressive increase in the effective surface area of active compacts, if effectively separated by distance exceeding their boundary layers, resulting in increasing oestradiol-17β release with time. The initial burst of oestradiol-17β from the surface may be reduced by employing two active compacts with reduced loading or less biodegradable polymer at either end of the implant as shown in Fig. 3. Obviously this flexible design permits the total oestradiol-17β dose of the implant and its rate of release to be controlled over a wide range by altering the

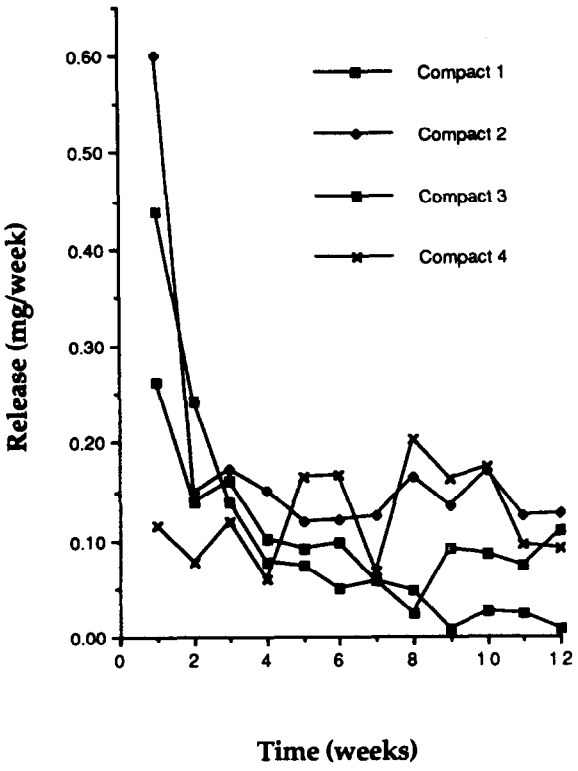

Fig. 1. Plot showing the in vitro release of oestradiol-17β from various compacts.

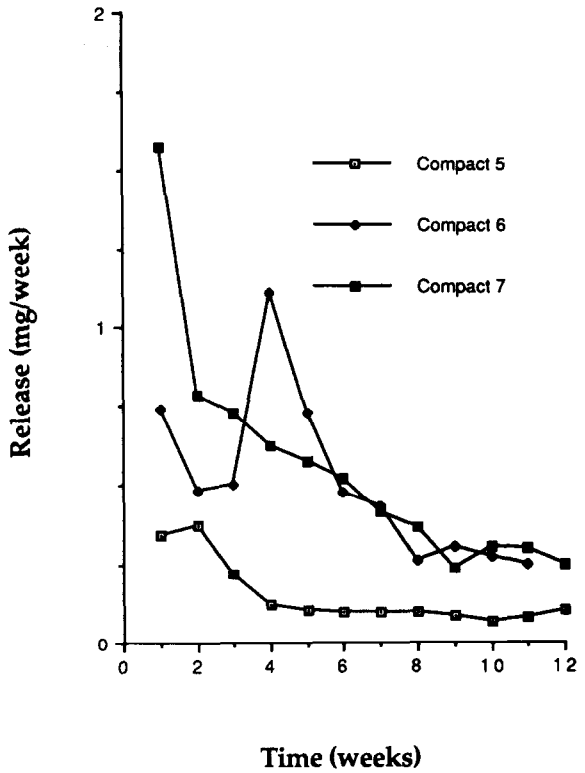

Fig. 2. Plot showing the in vitro release of oestradiol-17β from various compacts.

number and loading of the active compacts and by the relative composition and thickness of the alternating spacers. The knots fitted between each compact ensured that when the inactive units had degraded the remaining active units did not slide together during dissolution testing and reduce drug release from the faces of the active compacts.

Preliminary studies with implants containing eight active compacts separated by seven inactive compacts indicated that the release of oestradiol-17β was excessive to achieve the target dose. Accordingly implants were shortened to five active containing compacts separated by four inactive spacers and had a total loading of 75–80 mg oestradiol-17β, which should be adequate to provide for the increasing dose of 0.25 to 0.5 mg daily up to 6 months desired. Fig. 4 shows the effect of spacer thickness on in vitro release from such implants. The narrower spacers were observed to degrade more slowly and the average release of drug after elapse of the initial 2 week burst period tended to be lower for the implants with the narrowest spacers. As the narrower

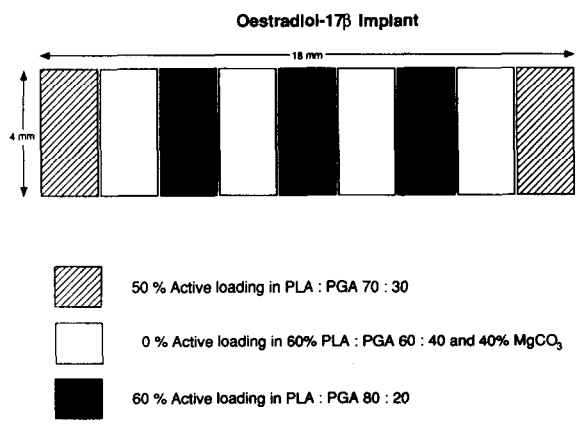

Fig. 3. Design of implant 8.

spacers biodegrade and erode away, they cause separation of faces of active subunits by distances apart which are comparable to the distances that the stagnant boundary layers associated with active compacts are apart. This poorly stirred region between adjoining active compacts has a reduced rate of active release. Wider spacers minimize this effect and give the observed greater average release rates.

Fig. 5 shows the effect of alteration in the polymer composition of the spacers, while maintaining thickness constant at 2 mm. The spacers in implant 4 are composed of a less biodegradable copolymer than used in implant 3 (see Fig. 4) and a desirable progressive increase in drug release over an extended period of time was observed. If however the copolymer used in the spacers of implant 5 was more biodegradable than that used in compacts 3 and 4, release

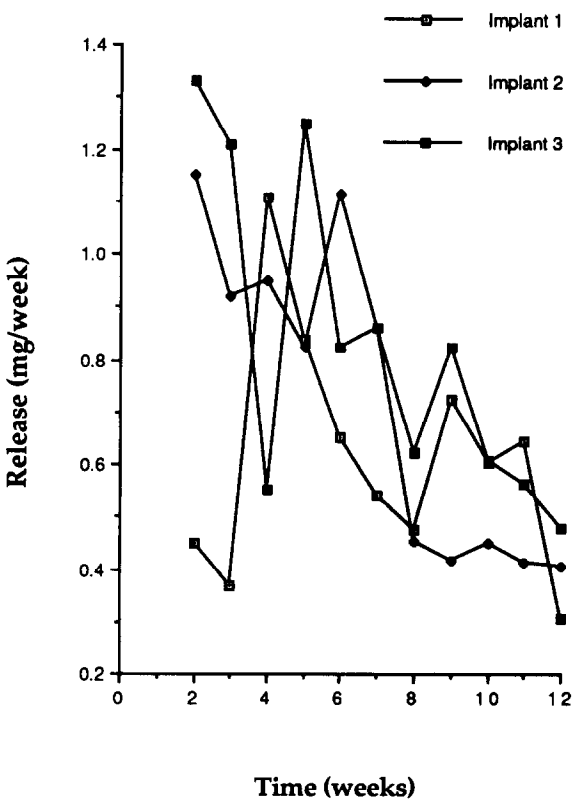

Fig. 4. Plot showing the in vitro release of oestradiol-17β from various implants.

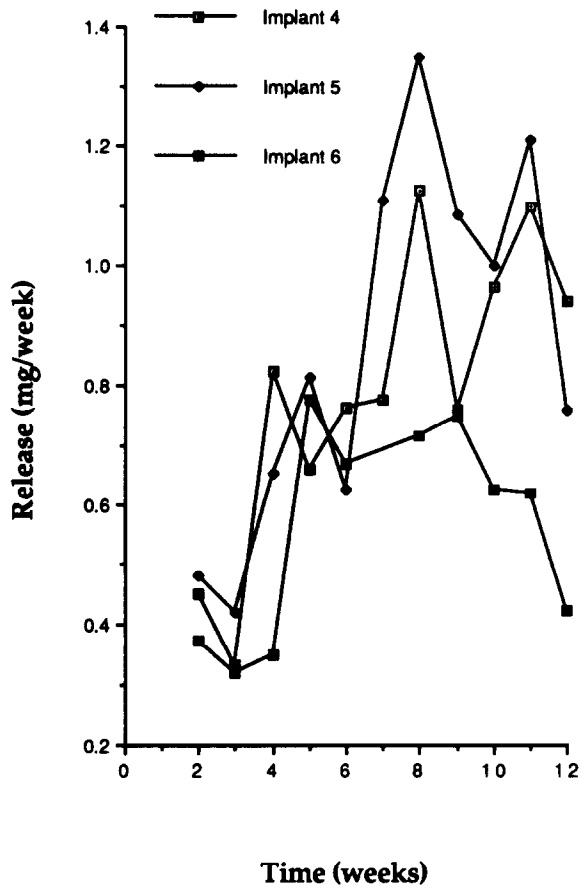

Fig. 5. Plot showing the in vitro release of oestradiol-17β from various implants.

tended to become more irregular as shown in Fig 5 due to the variable erosion of the polymer. Fig 5 also showed the effect of varying the polymer type used in the active compacts. Implant 6 used a less biodegradable copolymer than used in implant 3 and the resultant release rate of oestradiol-17β was reduced. Likewise though not illustrated, use of more biodegradable polymer in the active subunits of implant 7 increased release, which tended to be more irregular.

In vivo studies on compacts and implants

Initially, the release profile in implanted calves of four different types of single compacts (see Table 1) was studied as an aid to establishing a correlation between the rate of in vitro and in

vivo release of oestradiol-17β prior to developing composite implants. As expected, in vivo release data confirmed that single compacts showed a burst-effect followed by declining release, less biodegradable polymers and lower oestradiol-17β loading causing slower active release. The terminal in vivo release rate was approximately twice as fast for the least biodegradable polymer examined (compact 3) and up to 8-times faster for the most biodegradable polymer (compact 6) as the in vitro release rate for comparable compacts. This was probably due to the greater perfusion with a more hydrolytic tissue fluid of the implantation site compared to the relative stagnant and non reactive conditions pertaining during the dissolution experiments performed in buffer or water.

Fig. 6 shows the in vivo release profile from implants 8–10, based on periodic assay of residual oestradiol-17β at the implantation site. The calculated daily release rate over the 12 weeks of the study was usually in the desired range as recorded in Table 3. Implant 8 had a high initial release rate of 0.41 mg day^{-1} for the first 42 days, declining thereafter which was attributed to observed displacement of the active subunits during implantation causing a larger initial surface area than intended. When the adjacent subunits were bonded as in implant 9 or by elimination of the spacers as in implant 10, increasing daily release was observed over the 12 week period. This result questions the need for inactive spacers for the in vivo efficiency of the composite implant. However, the release from implant 9 is lower initially at 0.18 mg day^{-1} than from implant 10 at 0.24 mg day^{-1}. The terminal release rate of implant 9 is slightly higher at 0.47 mg day^{-1} than from implant 10 at 0.43 mg day^{-1}, which may indicate that the spacers slow down

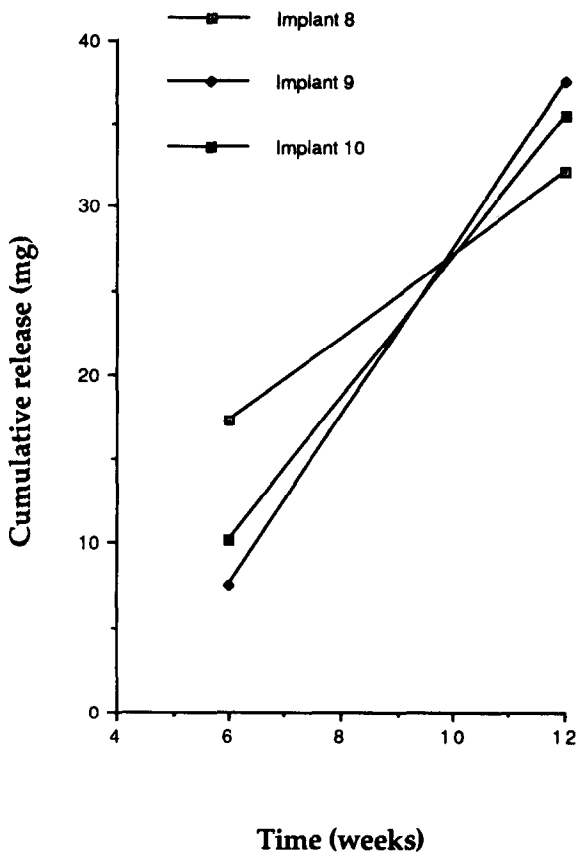

Fig. 6. Plot showing the in vivo release of oestradiol-17β from various implants.

TABLE 3

Oestradiol-17β content of implants before and at 42 or 84 days after implantation

Implant	Average estimated [a] oestradiol-17β content prior to 42 days implantation (mg)	Average oestradiol-17β content of implant recovered after 42 days (mg)	Average estimated [a] oestradiol-17β content prior to 84 days implantation (mg)	Average oestradiol-17β content of implant recovered after 84 days (mg)	Average oestradiol-17β daily release rate over	
					42 days (mg)	84 days (mg)
8	78.52	61.25	78.89	47.77	0.41	0.37
9	77.03	69.45	78.03	39.17	0.18	0.47
10	77.93	68.00	77.21	40.87	0.24	0.43

[a] Based on assay of unused compacts from batches employed in the construction of the different implants studied in vivo, where the theoretical content should be 78.4 mg per implant.

the initial release and so allow for greater release later in the treatment period. In addition, implant 9 appears to have the greatest percentage of active remaining after the 6 week treatment period, indicating that it is more likely to be effective for up to 6 months treatment.

As the residual assay method only involved two time points for estimating drug release per implant type, plasma oestradiol-17β levels were determined more frequently to monitor in vivo release. Despite the fact that the interpretation of such levels is complicated by variable data for plasma level by reason of sampling once a week, overall Fig. 7 shows that in comparison to unimplanted controls, animals treated with implants 8–10 showed elevated oestradiol-17β levels. In all cases there is an initial burst of hormone release from the implant which subsequently drops off at week 2 or 3. This drop is particularly apparent in

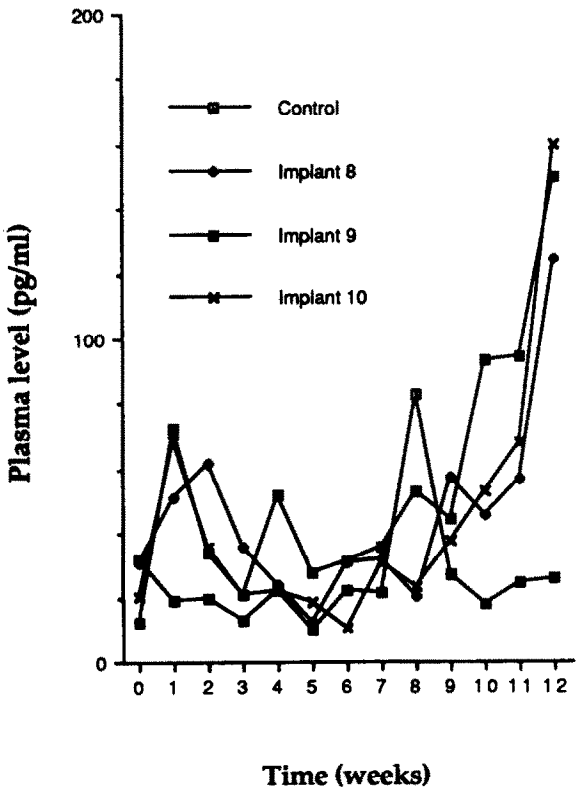

Fig. 7. Plot showing plasma levels of oestradiol-17β in steers treated with various implants.

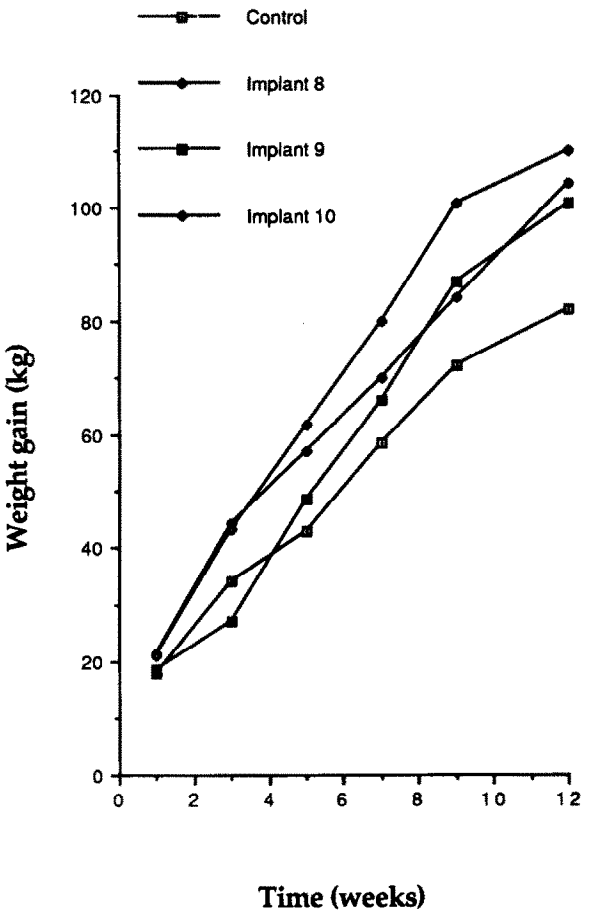

Fig. 8. Plot showing weight gain in steers treated with various implants.

the case of implant 10 where the plasma levels from weeks 3 to 8 are comparable to those of the control. This could be due to lack of spacers which would facilitate greater drug release as they degrade. Implant 9 produced the highest plasma levels from weeks 3 to 8, but its release does not increase as rapidly as implant 8 or 10 from weeks 10 to 12, where all plasma levels are seen to rise.

As the exact relationship between plasma oestradiol-17β level and enhancement of growth promotion is unknown, the weight gains of implanted cattle compared to unimplanted controls were recorded as shown in Fig. 8. In all cases, implanted animals showed greater weight gain than the control, those treated with implant 10

recording the greatest weight gain, which finding again questions the need for spacers in the final design of the implant. This may be due to the observed tendency of compacts in such implants to achieve variable spacing by displacement in vivo following implantation. Compared with the control group, feed conversion was improved 37, 34 and 36% in treated steers with implants 8, 9 and 10, respectively. No signs of local histotoxicity were observed at the site of implantation of any of the animals studied and no side-effects were observed associated with the initial burst of active release.

These in vivo studies were only carried out using limited numbers of animals per set of implants ($n = 5$) and a much larger in vivo study would be required before reliable inference may be drawn in this particular application of the technology described. Also further work will be needed to fine-tune the device when adequate information on the precise dose requirements for growth promotion become available. However, this pilot study does indicate that the novel implants described have potential for the effective increasing long-term delivery of natural and synthetic steroids to cattle and other animal species for growth promotion. The general design of the implant should be also easily amenable for modification to achieve a wide variety of drug loadings and extended release profiles in man or animals for other agents such as for hormone replacement, fertility control, peptides, antimicrobials, analgesics and anticancer drugs.

Acknowledgement

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