J. Pharm. Pharmacol. 1980, 32: 399–403 Received October 8, 1979

Long-term controlled delivery of levonorgestrel in rats by means of small biodegradable cylinders

DONALD L. WISE*, HARRIS ROSENKRANTZ†, JOHN B. GREGORY, HENRY J. ESBER†

Dynatech R/D Company Cambridge, Massachusetts, †EG & G Mason Research Institute, Worcester, Massachusetts

Hormone release rates from biodegradable cylindrical implants consisting of a physical matrix of [^{14}C]levonorgestrel and copolymers of [^{3}H]lactic and glycolic acids have been monitored in rats. Two copolymers were evaluated: one consisted of 90 parts L-lactide/10 parts glycolide (90L/10G) containing 33 or 50% hormone by weight, and the other of 50 parts DL-lactide/50 parts L-lactide (50DL/50L) containing 50% hormone. For each system, 4–6 rods (0·8 × 16 mm) providing 19 mg of steroid per rat were subcutaneously implanted into the scapular regions of 5 rats, and ^{14}C and ^{3}H in faeces and urine were determined weekly for 90–724 days. An initial burst of hormone release, peaking at approximately 90 μg day^{-1}, occurred in the first two weeks. This was followed by an approximately zero-order release for ^{14}C from all systems. Longer-term release rates were approximately 10–30 μg day^{-1} for the 90L/10G system containing 33% hormone, a more uniform rate of approximately 25 μg day^{-1} for the 50DL/50L system and the highest rate of approximately 40 μg day^{-1} for the 90L/10G system containing 50% hormone. ^{3}H and ^{14}C in residual implants of 90L/10G with 33% hormone removed from animals dying of natural causes during the test were assayed. For this system ^{3}H activity decreased by over 50% within 250 days, compared with <25% loss in ^{14}C activity. The amounts of ^{3}H and ^{14}C released were similar over much of the subsequent test period. At the end of the test both polymer and drug were essentially depleted. All animals with the 50DL/50L system died late in the test period. Between days 609 and 724 from 13·7–27·2% initial ^{14}C and from 10·0–22·1% initial ^{3}H was measured in recovered rods. Microscopic inspection of recovered rods showed a loss of core material and tissue encapsulation. There were no signs of local tissue irritation or systemic toxicity.

The application of poly-L-lactic acid as a biodegradable carrier for contraceptive steroids was first demonstrated using thin polymer/drug films implanted subcutaneously in rats (Jackanicz et al 1973). The use of polymer/drug matrices in fertility control has since been reported for finely divided injectable systems (Anderson et al 1976), and for implantable cylinders (Wise et al 1976), the latter work using a wide range of lactic/gylcolic acid copolymers. Sustained release of biologically active agents from similar lactic/glycolic acid polymers has been reported for narcotic antagonists (Schwope et al 1975; Wise et al 1976) and for antimalarials (Wise et al 1976, 1978, 1979).

In studies such as those mentioned above, in vivo release rates were measured over the short term, e.g. up to 100 days. In the present investigation, release rates were monitored for up to two years, and a mass balance was achieved for ^{14}C by analysis of both excreta and recovered residual implants. Preliminary data have been reported (Wise & Rosenkrantz 1976; Rosenkrantz 1976), and ancillary biological information including photomicrographs of recovered implants has been documented elsewhere (Rosenkrantz et al 1977).

* Correspondence.

MATERIALS AND METHODS

Preparation of implantable biodegradable cylinders

Two copolymers were prepared from the purified lactides and glycolide as given in Table 1. The molecular weights were determined viscometrically, based upon light scattering techniques (Billmeyer 1962). Films of the 90L/10G containing 33 and 50% by weight [^{14}C]levonorgestrel (courtesy of Wyeth) and of the 50DL/50L containing 50% by weight levonorgestrel were prepared following the procedure described earlier (Jackanicz et al 1973; Wise et al 1976). Levonorgestrel (spec. act. 147 μCi ^{14}C g^{-1} before incorporation into rods), was labelled on the ethynyl group. ^{3}H was nominally on the carbon in the 2-position of the L-lactide. Pieces of copolymer/drug films were loaded into specially designed extrusion apparatus with a 0·8 mm diameter orifice; uniform cylindrical rods of copolymer/steroid were then extruded and cut into 16 mm lengths (see Gresser et al 1978).

Table 1. Summary of polymer characteristics.

Description	90L/10G	50DL/50L
Melting point after purification, C°	Components (by % weight	
L-Lactide (1) 96·5–98·0	90	50
DL-Lactide (2) 125–126	×	50
Glycolide (3) 82·5–83·5	10	×
Molecular weight (mean no.)	220000	180000
Specific activity of polymer before incorporation of drug, $\mu Ci^3H\ g^{-1}$	14	4

Purchased from: (1) Henley and Company; (2) Clinton Corn Products and (3) Matheson, Coleman and Bell

Implantation procedure

The 0·8 mm diameter copolymer/steroid cylinders were implanted subcutaneously in the scapular region of adult female Sprague-Dawley rats (Sprague-Dawley, Inc.) 250–300 g. Five rats were implanted with each of the matrices tested. Each rat implanted with the 50% matrices received four rods, two on each side. Those implanted with the 33% matrix received six rods, three on each side. Thus, an equal amount of [^{14}C]levonorgestrel ($\approx$19 mg) was implanted in each rat.

The duration of the experiments varied by design or because of deaths. For the 90L/10G system containing 50% drug, urinary and faecal ^{14}C was measured for 13 weeks (Study 1). Urinary and faecal excretions of ^{14}C from the 50DL/50L system levonorgestrel (Study 2) and from the 90L/10G containing 33% hormone (Study 3) were monitored for 104 and 100 weeks, respectively.

Excreta collection and analyses

Each rat was housed in an individual metabolism cage where multiday pools of both urine and faeces were collected separately. Initially, 3-day or 4-day urine pools were measured for radioactivity. Eventually, weekly urine pools and finally biweekly urine pools were measured for radioactivity. All the 3-day or 4-day faecal pools collected throughout the experiments were measured for radioactivity. Samples to determine the amount of labelled material excreted in the urine were prepared by adding 1·0 ml of urine to 14·0 ml of liquid scintillation cocktail (Riafluor New England Nuclear). The radioactivity was measured using a Packard liquid scintillator to 1% relative error or for 20 min, whichever came first. Homogenized faecal pools were submitted for combustion analysis to the New England Nuclear Corporation for liquid scintillation counting. Narrow band isosets were used on the scintillation counter in all cases for dual counting of ^{14}C and ^{3}H.

Study 1 was conducted to evaluate selected rods in animals over three months. From the results, longer term (studies 2 and 3) tests were made using rods of modified composition.

Residual implants were recovered and analysed for the hormone label from all the animals. In addition, for the 90L/10G/33% system (Study 3) and the 50DL/50L % system (Study 2) rods were recovered from the sites of implantation and analysed for both ^{14}C and ^{3}H; also, a few measurements of urinary and faecal ^{3}H levels were made in rats surviving beyond 75 weeks. Because of deaths (not attributable to implants), there were 4 rats at week 36, 3 at 52, 2 at 81 and 1 at 97 for the 90L/10G 33% system (Study 3). The only survivor was killed on day 715. Similarly, for the 50DL/50L % system (Study 2) there were 4 rats at week 88, 3 at 90, 2 at 102, and 1 at 103; the latter died on day 724.

RESULTS AND DISCUSSION

Figures 1 and 2 illustrate the average daily release rates in $\mu g\ day^{-1}$ of ^{14}C-labelled materials in the urine and faeces for studies 1, 2, and 3. The results for the 90L/10G 50% system (Study 1) (Fig. 1) show an initial release rate of up to over 90 $\mu g\ day^{-1}$ of ^{14}C material in the faeces during the first three weeks;

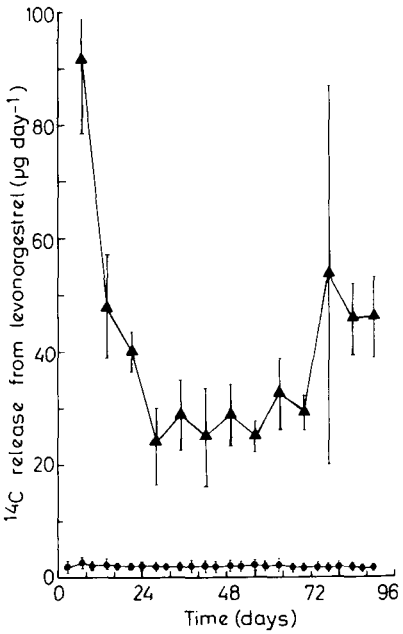

FIG. 1. Rat urinary (●) and faecal (▲) release of ^{14}C from levonorgestrel as $\mu g\ day^{-1}$ versus day of study. Implant was 0·8 mm (1/32″) diameter cylinder, 90L-lactide/10 glycolide copolymer of 220 000 mol. wt 50% levonorgestrel by weight (Study 1).

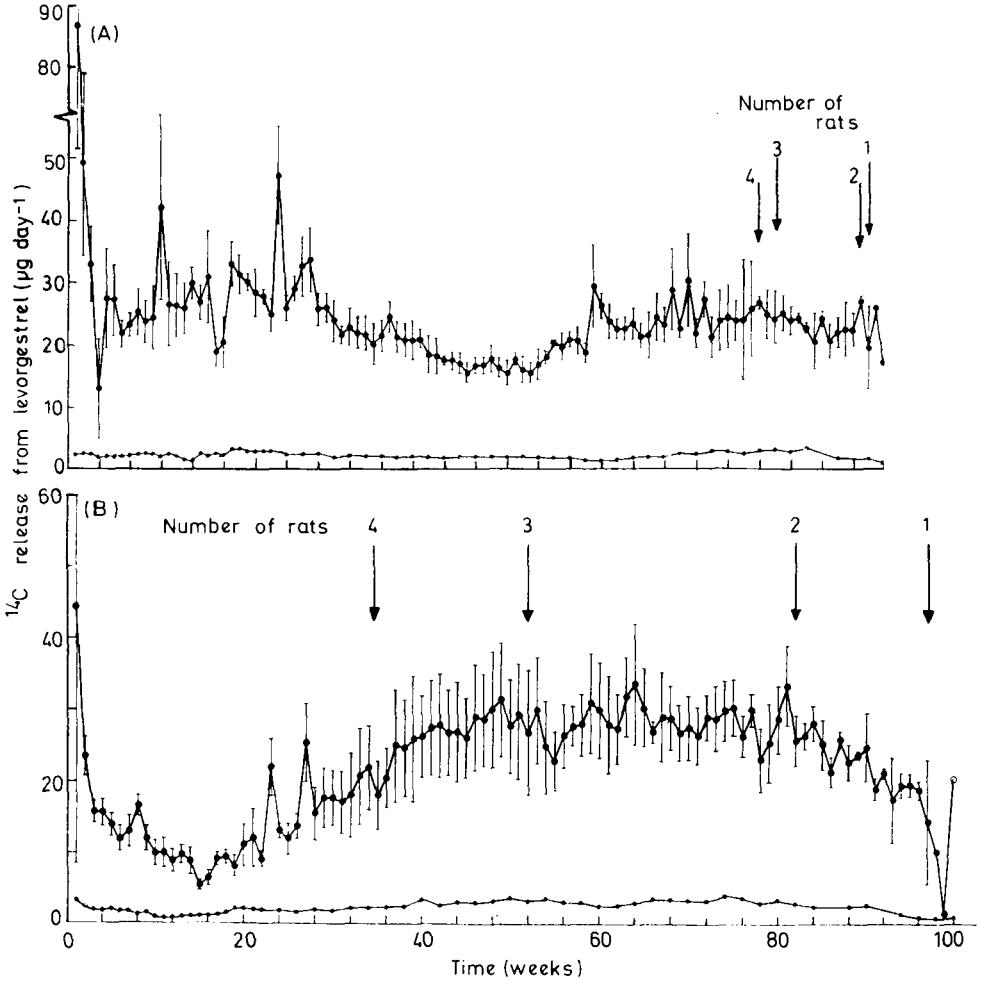

FIG. 2. Rat urinary and faecal release of ^{14}C from levonorgestrel as μg day^{-1}, upper curve faeces, lower curve urine. Implant was 0·8 mm (A) 50DL-lactide/50L-lactide copolymer of 180 000 mol. wt. 50% levonorgestrel by weight (Study 2). (B) 90L-lactide/10 glycolide of 220 000 mol. wt. 33% levonorgestrel by weight (Study 3).

this was followed by a fairly constant rate of 30 μg day^{-1} for seven weeks and a slight increase for the last three weeks of the test. The overall average release rate was approximately 40 μg ^{14}C day^{-1}. The release of ^{14}C in the urine ranged from 1·4 to 2·3 μg day^{-1}. The ratio of ^{14}C excreted in the faeces to that in the urine was approximately 22:1. The recoveries of levonorgestrel from the 90L/10G 50% system (Study 1) after 90 days were from urine 1%, from faeces 19% and from implant 85%, i.e., 105% but this is believed to be within acceptable limits of experimental error.

There was a sharp initial burst of ^{14}C activity, peaking the first week after implantation at 90 μg day^{-1} (faeces plus urine) from the 50DL/50L system (Fig. 2A). Some four weeks after implantation, the rate became uniform and stayed so over the remaining 104 weeks (number of surviving animals noted on the figure). Overall, there was a release rate of approximately 25 μg day^{-1}.

The release from the 90L/10G 33% system (Study 3) was not as uniform (Fig. 2B). While there was an initial burst of ^{14}C of almost 90 μg day^{-1} (faeces plus urine) in the first week, release rates subsequently declined to about 10 μg day^{-1} and then climbed to approximately 30 μg day^{-1}.

While the prime objective of this study was to prepare implants and evaluate levonorgestrel release

rates, the degree of polymer degradation rate was also examined. Individual measurements varied but mean polymer (or lactate) excretion rates were similar for all rats alive at the time of testing (Table 2).

For the 90L/10G 33% system (Study 3), deaths amongst the rats which died of natural causes were

Table 2. Summary of polymer (or lactate) excreta analysis.

System	Weeks	Polymer (or lactate), $\mu g\ day^{-1}$ Urine	Faeces
90L/10G/33% Study 3	76–80	45 ± 32	265 and 477[1]
	76–96	53 ± 19	×
	76–100	33 ± 20	×
50DL/50L/50% Study 2	80–104	41–203[2]	
	82–86	×	325 and 306[3]

[1] Two determinations or one rat.
[2] Individual rats were 180 ± 71, 41 ± 43, 78 ± 35, 203 ± 84, 183 ± 74 $\mu g\ day^{-1}$. Here the lower values of excretion rates were associated with two rats which succumbed between days 609–620 and the higher values with three rats which survived approximately an additional 100 days.
[3] Single assays for each of two rats.

at almost regular intervals, (Fig. 3). The measurements of residual ^{14}C and ^{3}H activity in recovered rods is shown as a percentage of the original radioactivity in Fig. 2. There was a greater loss of ^{3}H activity early in the test compared with ^{14}C activity. A summary of recovery is given in Table 3. The longer the implants were in situ, the less activity was recovered. ^{14}C recovery appeared to be linear between days 245–725, while the ^{3}H recovery appears to be curvilinear over the same time. There appeared to be an early substantial release of ^{3}H activity but after, both ^{3}H and ^{14}C releases were largely the same. Similar data for the 50DL/50L system (Study 2) is collected in Table 3.

During treatments, no atypical behaviour was observed and no tissue change or tumour could be attributed to the presence of the implants. Histopathological findings were minimal, and the lack of a consistent effect on ovaries and uterus suggested that the dose of levonorgestrel, and/or its metabolites did not exert a significant effect on the reproductive tract. Within the limitations of obtaining any more meaningful test results from the rat, and for the small number of animals tested, there appear to be no adverse effects due to the implanted device.

When the rods were removed from implantation, all were recognized by their gross appearance. There

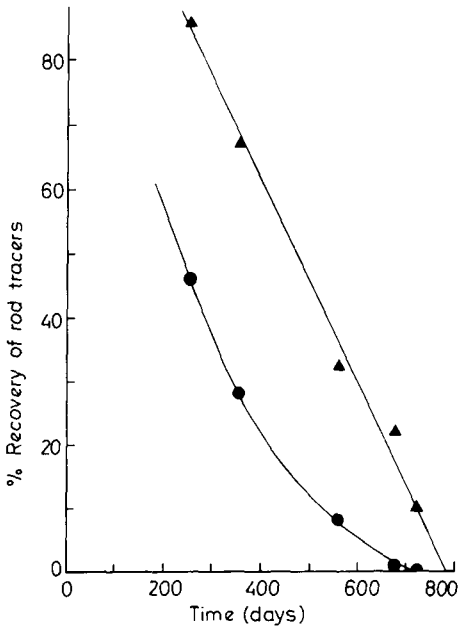

FIG. 3. Recovery of ^{14}C (▲) (levonorgestrel) and ^{3}H (●) (90:10 L-lactide/glycolide polymer) tracers from implanted rods (33% by weight levonorgestrel) removed from rats in Study 3.

was encapsulation by tissue and, in one long-term survivor, individual rods had migrated and formed one clump of material. For both types of polymer devices, the recovered rods were brittle and friable and unlike the rods initially, several fragmented during the cryostat sectioning. Estimates of recovered dry weights of rods were between 21–41% for both types of polymers. There was no noticeable discoloration that might possibly have indicated oxidative and/or

Table 3. Summary of analysis of rods following removal.

System	Rat, in order of death	% ^{14}C remaining in rod	Total ^{14}C Acc.	% ^{3}H remaining in rod
90L/10G/33% Study 3 (Re. Fig. 4)	1	86·2	104·7	45·5
	2	66·5	111·2	27·9
	3	31·5	105·7	8·4
	4	22·7	111·7	0·9
	5	10·0	110·9	0·7
50DL/50L Study 2 (Re. Fig. 2)	1	15·0	95·8	10·0
	2	26·3	115·0	22·1
	3	—	125·6	—
	4	—	131·0	—
	5	—	119·1	—

polymerization reactions by endogenous ingredients of surrounding intercellular fluid.

Earlier work on thin films revealed the potential for using a biodegradable carrier to release implanted hormonal steroids (Jackanicz 1973). Subsequent studies defined the regime for polymer molecular weight, copolymer composition, and implant form (Wise et al 1976). The direction provided by this earlier work has now been largely substantiated, and in the present work, the goal of long-term uniform steroid release with a biodegradable carrier has largely been achieved. It appears that for system duration of many months, a lactic/glycolic acid copolymer of suitably high molecular weight will be required. Copolymer compostion would seem to require high L-lactic acid content for the lactic/glycolic acid copolymer, or the alternative of a suitable balance between L-lactic acid and the less crystalline, more biodegradable DL-lactic acid. Drug content should be between one-third to one-half by weight of the final implant. Compared with earlier work (Wise et al 1976) using 1·5 mm diameter rods, the smaller 0·8 mm diameter rods will probably be more desirable for ultimate human application. Based on the present results, it appears that testing in larger animals is merited.

Acknowledgements

Samples were prepared at Dynatech R/D Company under NICHHD Contract No. NO1-HD-3-2736 and tested in rats at Mason Research Institute under NICCHD Contract No. NO1-HD-2-2746. Sincere gratitude is expressed for the excellent technical assistance of Paul Rosenblum and Phyllis Hughes of MRI.

REFERENCES

Anderson, L. C., Wise, D. L., Howes, J. R. (1976) Contraception 13: 375–384

Billmeyer, F. W. (1962) Textbook of Polymer Science, 2nd ed., Wiley, 88

Gresser, J. D., Wise, D. L., Beck, L. R., Howes, J. F. (1978) Contraception 17: 253–266

Jackanicz, T. M., Nash, H. A., Wise, D. L., Gregory, J. B. (1973) Ibid. 8: 227–234

Rosenkrantz, H. (1976) in: H. L. Gabelnick (ed.) Proc. Drug Delivery Systems DHEW Publication No. (NIH) 77–1238, 279–304

Rosenkrantz, H., Esber, J. H., Baker, J. R. (1977). Report No. MRI-CDBO3–77–73 to the National Institute of Child Health and Human Development, 1–89

Schwope, A. D., Wise, D. L., Howes, J. F. (1975) Life Sci. 17: 1877–1886

Wise, D. L., Rosenkrantz, H. (1976) in: Gabelnick, H. L. (ed.) Proc. Drug Delivery Systems DHEW Publication No. (NIH)77–1238, 253–364

Wise, D. L., Gregory, J. B., Newberne, P. M., Bartholow, L. C., Stanbury, J. B. (1976) in: Kostelnik, R. J. (ed.) (1978) Polymeric Delivery Systems, Gordon and Breach Science Publishers, Inc., New York

Wise, D. L., McCormick, G. J., Willet, G. P., Anderson, L. C. (1976) Life Sci. 19: 867–874

Wise, D. L., McCormick, G. H., Willet, G. P., Anderson, L. C., Howes, J. F. (1978) J. Pharm. Pharmacol. 30: 686–689

Wise, D. L., Gresser, J. D., McCormick, G. J. (1979) Ibid. 31: 201–204

Wise, D. L., Schwope, A. D., Harrigan, S. E., McCarthy, D. A., Howes, J. F. (1978) in: Kostelnik, R. J. (ed.) Polymeric Delivery Systems, Gordon and Breach Science Publishers, Inc., New York