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Levodopa Delivery from Controlled-Release Polymer Matrix: Delivery of More than 600 Days *in vitro* and 225 Days of Elevated Plasma Levels after Subcutaneous Implantation in Rats

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ABSTRACT

Parkinson's disease is commonly treated with orally applied levodopa (*l*-dopa). However, because this treatment modality is associated with a number of undesirable side effects, some due to plasma fluctuations, we have developed a slow-release polymer system that can be used to deliver *l*-dopa continuously for extended periods of time *in vitro* (>600 days) and *in vivo* (at least 225 days) in rats. *In vitro* *l*-dopa release was evaluated using polymer matrices with appropriately selected parameters (loading and geometry), and zero-order (linear) release of *l*-dopa was observed for more than 600 days (in highly loaded, noncoated material first-order kinetics), in some instances in mg quantities per day. This was achieved even in polymer matrices, which did not possess a dissolution limiting barrier. Scanning electron-microscopic analysis suggests that the mechanism of release is dissolution through channels and pores within the polymer matrix. To assess *in vivo* release, *l*-dopa was quantified in plasma from rats given s.c. implants of *l*-dopa polymer matrices using high-performance liquid chromatography. We observed release of *l*-dopa for a period of at least 225 days after an initial burst of release. Continuous release of *l*-dopa from s.c. implanted slow-release polymer matrices has several advantages over oral delivery: 1) *l*-dopa plasma fluctuations are eliminated, 2) patient compliance issues are reduced and 3) the gastrointestinal tract is circumvented, thus requiring a lower dose. We suggest that the peripheral implantation of polymer systems containing *l*-dopa, dopamine agonists or other agents constitutes an improved method of drug delivery and may therefore be an alternative treatment modality for the care of patients with Parkinson's disease.

The treatment of Parkinson's disease patients with the dopamine biosynthetic precursor, *l*-dopa (in conjunction with a decarboxylase inhibitor) (Birkmayer, 1969) has enjoyed wide acceptance as an effective approach for the reduction of extrapyramidal symptoms in Parkinson's disease. Nevertheless, a number of problems remain that are of particular concern to patients in advanced stages of the disease (Barbeau, 1974; Fahn, 1974; Mouradian *et al.*, 1987; Shoulson *et al.*, 1975; Yahr and Bergman, 1986; Tolosa *et al.*, 1975).

In the first few years of the disease, the majority of parkinsonian patients show a rather stable clinical response to orally ingested *l*-dopa therapy despite plasma *l*-dopa fluctuations (Mouradian *et al.*, 1987; Hardie *et al.*, 1986). However, after several years of therapy, the central nervous system fails to act as a smooth transformer to compensate amplitude swings of plasma *l*-dopa into a sustained biologic response (Hardie *et al.*, 1986) and patients begin to fluctuate in their clinical response. Symptom reduction, for example in motor performance ("on"-period), alternates, sometimes abruptly, with periods during which *l*-dopa treatment seems ineffective and symptoms reappear ("off"-period) (Shoulson *et al.*, 1975; Hardie *et al.*, 1986; Albani *et al.*, 1986; Chase *et al.*, 1986; Barbeau *et al.*, 1974; Fahn, 1974; Mouradian *et al.*, 1987). The emergence of such clinical fluctuations is often referred to as the "wearing-off" effect and, with further progression of the disease, this becomes a serious problem for the patient.

In many patients, these fluctuations in clinical response appear to be synchronized with fluctuations in plasma levels of *l*-dopa (Shoulson *et al.*, 1975; Albani *et al.*, 1986; Hardie *et al.*, 1984; Muenter *et al.*, 1977; Rossor *et al.*, 1980; Tolosa *et al.*, 1975) associated with the timing and dose of oral ingestion of *l*-dopa combined with a dopa-decarboxylase inhibitor, *e.g.*, levodopa/carbidopa (Sinemet®) or levodopa/benserazid (Madopar®). This observation suggests that fluctuations in plasma

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ABBREVIATIONS: *l*-dopa, levodopa; EVA, ethylene vinyl acetate; SEM, scanning electron microscopy; HPLC, high-performance liquid chromatography.

levels of *l*-dopa may be directly responsible for the unstable clinical response and improved modes of *l*-dopa delivery are therefore desirable.

In 1969, Birkmayer already noted that i.v. infusion of *l*-dopa is the most effective treatment for Parkinson's disease, producing the least side effects; yet this mode of delivery is not practicable. In order to alleviate the problem of plasma and brain fluctuations associated with oral therapy, a variety of other methods for the continuous delivery of *l*-dopa or dopamine receptor agonists has therefore been tested, including i.v. infusion (Hardie *et al.*, 1984; Quinn *et al.*, 1982, 1984), implantable or external reservoir pump systems (Bittkau and Przuntek, 1988; Obeso *et al.*, 1986, 1988) and oral slow-release preparations, such as Sinemet CR 3-CR 5 (Cedarbaum *et al.*, 1987a,b; Juncos *et al.*, 1987a,b; Nutt *et al.*, 1986) and Madopar HBS (Ernie and Held, 1987; Fischer and Baas, 1987; Jansen *et al.*, 1987; Jensen *et al.*, 1987; Ludin, 1987; Marion *et al.*, 1986; Nordera *et al.*, 1987; Poewe *et al.*, 1987; Quinn *et al.*, 1987; Siegfried, 1987), as well as others (Curzon *et al.*, 1973; Gerlach *et al.*, 1988; Lataste *et al.*, 1987; Saarinen *et al.*, 1978). Although the application of i.v. infusion or pump systems results in optimal or near-optimal release kinetics, these approaches have proven to be unsatisfactory with respect to practicability, reliability or safety for the patients. Oral slow-release preparations, in contrast, are relatively practical and reliable, but kinetic studies reveal that they do not provide constant ("controlled") delivery of *l*-dopa but rather simply retard the release for a short period of time ("sustained"-release) (see fig. 1). This transient sustained release is due not only to gastric emptying, but also to the release kinetics of the oral preparation itself (Gerlach *et al.*, 1988; Crevoisier *et al.*, 1987; Malcolm *et al.*, 1987). Although such oral slow-release preparations do appear to improve the clinical condition of patients in some cases (Jensen *et al.*, 1987; Nordera *et al.*, 1987; Quinn *et al.*, 1987; Siegfried, 1987), in others they appear to be ineffective (Fischer and Baas, 1987; Jansen *et al.*, 1987; Ludin, 1987) or have even been reported to be detrimental (Fischer and Baas, 1987; Jansen *et al.*, 1987). Nevertheless, taken together with earlier i.v. infusion studies (Birkmayer, 1969; Hardie *et al.*, 1984; Quinn *et al.*, 1982), these results provide evidence that controlled delivery of *l*-dopa is a superior treatment, effectively reducing the clinical fluctuations in Parkinson's patients.

Figure 1 shows theoretical and experimental curves of different types of release kinetics, indicating the difference in plasma drug concentrations between standard oral therapy (*e.g.*, levodopa/benserazide; Madopar HBS®), sustained-release preparations (such as levodopa/benserazide sustained-release formulation (Madopar HBS®) and controlled-release devices (such as controlled-release polymers, see below).

The search for controlled-release devices, *i.e.*, those that deliver drugs to the nervous system in a truly linear fashion ("zero-order" kinetics) has led to a number of systems (see Stahl, 1988, for review). The most widely known device is the ALZET osmotic minipump, a reservoir-type system that can deliver a solution containing a drug, for example dopamine or dopamine receptor agonists (Hargraves and Freed, 1987; de Yebenes *et al.*, 1988), for up to 4 weeks continuously. Because of the relatively short period of release time, these pumps cannot be considered clinically practicable for the delivery of neuroactive substances. In addition, concerns over safety for reservoir-based pump systems in the case of damage or malfunction and relative instability of drug suspended in the vehicle solution have tempered enthusiasm for this technique.

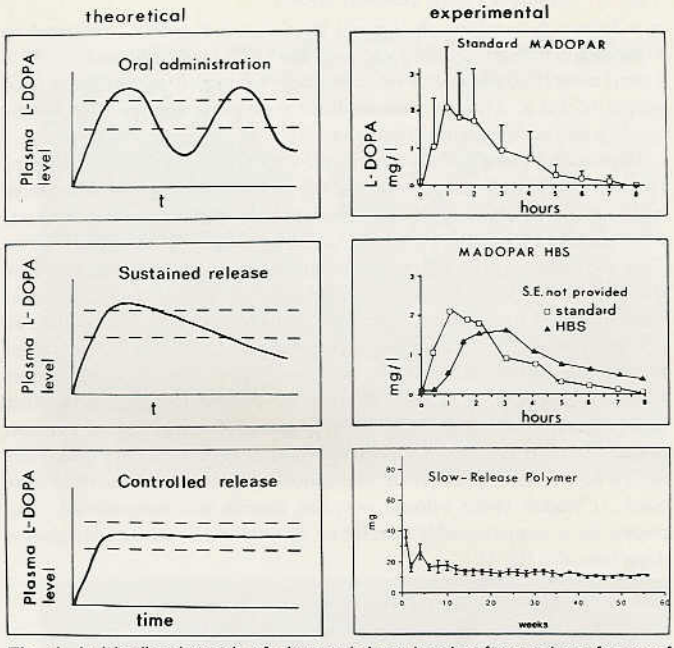

Fig. 1. A: Idealized graph of plasma *l*-dopa levels after various forms of drug delivery. Upper panel: after oral ingestion of a standard tablet, the plasma level soon reaches the "therapeutic window" and then "overshoots" into toxic levels. It then falls gradually below the minimum dose until the next tablet is taken. Middle panel: in the case of "sustained-release" methods, the toxic peak is reduced and the time in the "therapeutic window" becomes greater. Lower panel: in the ideal case, plasma drug levels are continuously kept in the therapeutic range by controlled-release drug delivery systems. Here, there are no toxic peaks or ineffective valleys. B: Pharmacokinetics in the case of various drug delivery methods. Upper panel: standard oral levodopa/benserazide (Madopar®), plasma level of *l*-dopa after single oral application in humans (adapted from Malcolm *et al.*, 1987). Middle panel: Single application of the sustained-release preparation of levodopa/benserazide (Madopar HBS®) in humans. Note the somewhat reduced peak and elevated plasma level beyond 2.5 hr of the sustained-release preparation (▲) as compared with standard Madopar (□; data also adapted from Malcolm *et al.*, 1987). Lower panel: mean plasma level of *l*-dopa release from controlled-release polymers described in this article. *In vivo* data were found to be similar (see also fig. 8).

We have already suggested previously (During *et al.*, 1988, 1989) that continuous delivery of dopamine from brain implants may be of benefit for parkinsonian patients. However, in order to develop a method that is less invasive, *i.e.*, clinically more feasible, we have developed peripherally implantable controlled-release polymer systems for the continuous and controlled delivery of *l*-dopa. We now demonstrate that controlled release can be achieved in the mg range per day for a period of more than 600 days *in vitro*, and that micromolar concentrations can be achieved *in vivo*. Since these polymer matrices can be implanted s.c. our approach might provide a novel method to eliminate *l*-dopa plasma swings permanently and thus the clinical response fluctuations seen in many parkinsonian patients.

Methods

Polymer matrix fabrication. EVA copolymer (DuPont, Wilmington DE) was washed and slabs containing 50, 55, 60, 65, 70 or 80% (w/w) *l*-dopa (Sigma Chemical Co., St. Louis, MO) were prepared according to the following procedure. The polymer was first dissolved in organic solvent (methylene chloride) at 37°C for 24 h and *l*-dopa was added to the liquid polymer. After both were thoroughly mixed, they were cast

in a frozen rectangular glass mold (5 × 7 cm) at −60°C, followed by evaporation at −20°C under vacuum. Rectangular samples (15 × 30 × 2 mm) were then cut out of the raw slabs, having an approximate final weight of 1.3 g. This process leads to a polymer matrix with *l*-dopa crystals embedded within (figs. 2 and 3).

Because polymer systems frequently require a dissolution limiting barrier to achieve linear release, the raw slabs were subsequently coated with an impermeable polymer barrier such that various geometries were obtained (fig. 4; see also During *et al.*, 1989; Freese *et al.*, 1989). This helped to identify polymer systems with preferred release kinetics, thus extending the window of linear release. The following samples were prepared in quadruplicate from the same batch: 1) a noncoated polymer matrix, 2) a coated polymer matrix with all sides sealed except for one face of the slab and 3) a polymer matrix that was fully coated with the exception of a pore through which the incubation medium could gain access to the loaded core. Quadruplicates of coated polymer matrices containing a single pore were fabricated with pore diameters of 2, 4 and 6 mm. As controls, the following polymer geometries were used: 1) loaded (with *l*-dopa) polymer matrix was surrounded completely by a nonpermeable barrier or 2) unloaded matrices without *l*-dopa (see also fig. 4).

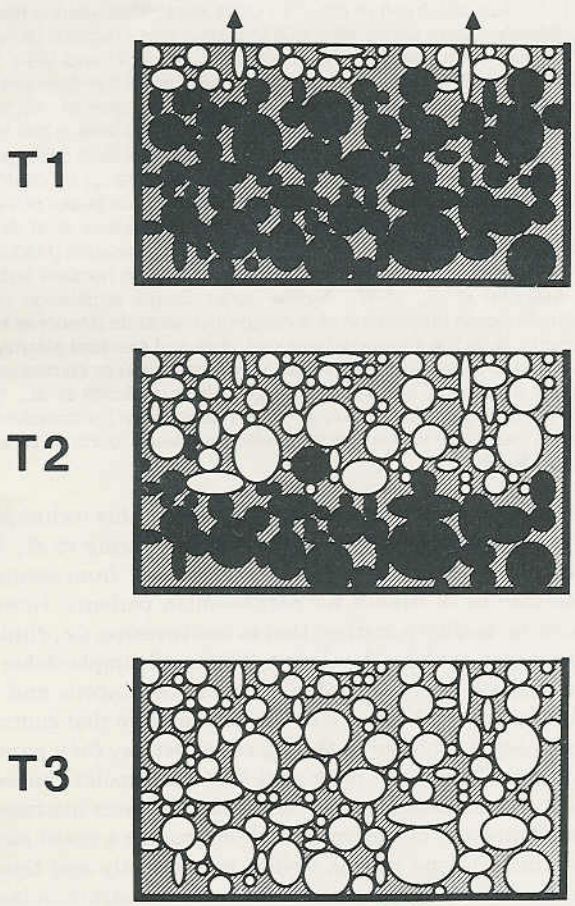

Fig. 2. Mechanism of drug release from slow-release "matrix"-type polymer. A slow-release polymer consists of a continuous phase of polymer carrier (grey area) with a dispersed phase of drug powder particles (black circles). The polymer displayed in this graph has an impermeable barrier (solid black line) around all but one side through which the drug can diffuse out. Upper panel: after the polymer is exposed to an aqueous environment (*e.g.*, saline), the outermost drug particle layer dissolves and the drug enters the environment (arrows) by passive dissolution, leaving behind open pores and interconnecting channels. Middle panel: as time progresses, the medium dissolves further layers of drug particles, until, as seen in the lower panel, the polymer is empty.

SEM evaluation of polymer matrices. Polymer samples were evaluated before and after long-term release using a scanning electron microscope. After small samples of polymer were cut at −20°C on a cryostat, they were thoroughly dried at room temperature and subsequently sputtered with gold for 10 to 15 min. The surface and the core of the polymer matrices were then visualized at a magnification of ×60 or ×1800.

Evaluation of *l*-dopa release from polymer matrices *in vitro*. Quadruplicates of *l*-dopa-containing polymer matrices and controls were individually immersed in glass scintillation vials containing 20 ml of 150 mM NaCl, 0.2% EDTA solution (as antioxidant) and then incubated at 37°C on a light-protected rotating platform. *l*-dopa release was determined in bi-weekly intervals by spectrophotometric analysis (wavelength: 280 nm), replacing the bathing solution each time measurements were taken to avert saturation. At later time points, samples were only measured weekly or every other week. Quantitation of release was performed by comparison of spectrophotometric values of samples to known standards. Authenticity of *l*-dopa was confirmed at various time points using HPLC (Waters) with electrochemical detection as described below. Controls resulted in some, but insignificantly small absorption at 280 nm after long time periods.

Evaluation of *l*-dopa release from polymer matrices *in vivo*. Three male Sprague-Dawley rats (290–360 g) were anesthetized with ether and an *l*-dopa-containing implant was inserted s.c. through a small surgical incision on the back of the rats. The implant had the same dimensions as those used for *in vitro* testing, with 70% loading and no rate-limiting membrane. A control rat received an unloaded implant (without *l*-dopa). On the day after implantation and at various time points thereafter, 700 to 800-μl blood samples were drawn from the tail vein (under ether anesthesia), collected in vials containing 20 μl of a glutathione-EDTA solution (pH 6–7), vortexed and centrifuged at 4°C for 10 min at 4000 rpm. Plasma was then stored at −70°C until samples were assayed.

Catecholamine assays were initiated by thawing the samples and adding 10 μl of 5 M $HClO_4$ for 2 min to precipitate proteins. After additional centrifuging, the supernatant was placed into Eppendorf tubes containing 20 ng of activated Al_2O_3 and 100 μl of GSH/EDTA. By adding Tris buffer (pH 8.7), plasma catecholamines were absorbed by Al_2O_3. The saturated Al_2O_3 was then washed three times (1 ml Tris buffer, 1 ml GSH-EDTA solution, 98 ml HPLC-water), centrifuged and the supernatant was discarded. Desorption was performed by adding 100 μl of 0.2 m $HClO_4$, and aliquots were injected into a HPLC system (Waters) using a Macherey & Nagel column (Nucleosil $5C_{18}$, 20 cm) and electrochemical detection (Waters M 460). The mobile phase was citrate buffer with 1% methanol and octanesulfone acid, 60 mg/liter, to yield a final pH of 3.2 (flow rate of 0.8 ml/min, 2800 psi). The content of noradrenaline, adrenaline, dopamine and dopa was calculated by comparing the samples to an internal standard of the synthetic catecholamine dihydroxybenzylamine and to the analytical standards of the respective amines (20 ng/ml). The analytical detection limits were about as follows: dopa, 10 pg; plasma-dopa, 20 pg; dopamine, 5 pg; plasma dopamine, 20 pg; noradrenaline and adrenaline, 5 and 10 pg. The sensitivity of each catechol reached 2.5 pg, the variability of reproducibility was 10% (20% in plasma). Recovery rates after alumina adsorption were as follows: dopa, 45 and 55%; dopamine, noradrenaline and adrenaline, 55 and 65%.

Results

Scanning electron microscopy. Figure 3 shows scanning electron micrographs of polymer matrices loaded with *l*-dopa. Figure 3A shows *l*-dopa crystals embedded in a polymer matrix at low magnification (×60), distributed evenly throughout the core of the device. At higher magnification (×1800), *l*-dopa crystals are found to be attached to the matrix which, under standard fabrication procedures, proves to be porous even before release commences (Fig. 3C). After a release period of approximately 1 year, polymer matrices with high loading and

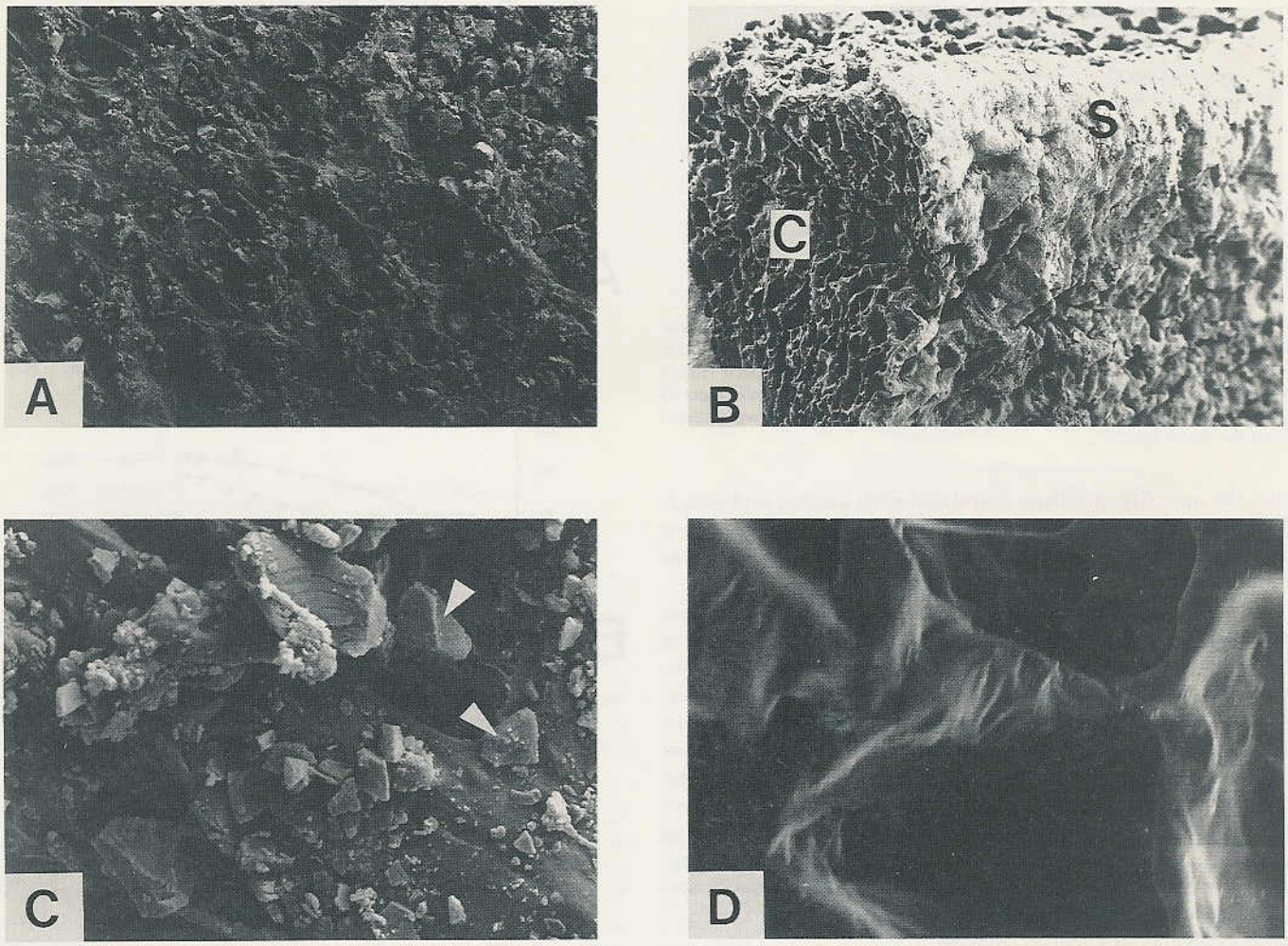

Fig. 3. Scanning electron micrographs of *l*-dopa containing polymer matrices before (A and C) and after (B and D) long-term release at low magnification in the upper panels (×60) or high magnification (×1800) in the lower panels. C, *l*-dopa crystals (arrows) can clearly be distinguished from the polymer matrix (P) before release commences. B, The empty matrix can be seen from the surface (S) or the core (C), clearly displaying pores and channels.

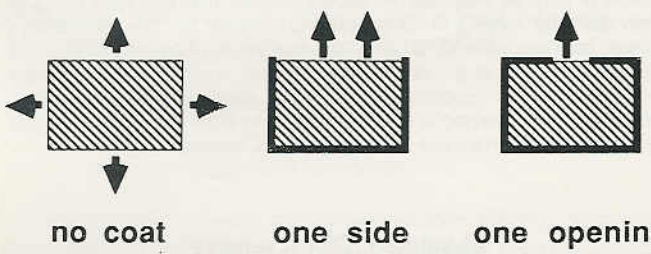

Fig. 4. Variations in polymer design. With an impermeable polymer membrane surrounding the loaded core it is possible to manipulate the rate of release. Left: having no polymer coat around it, the loaded core releases the drug particles in all directions, emptying in a relatively short period of time. Middle: if only one side is left uncoated, the release rate and kinetics are altered and become more linear. Right: if only one opening is left open in the membrane, the drug will diffuse out in a linear rate for a prolonged period of time.

without a rate-limiting coat are empty (see fig. 6C). At this time the SEM evaluation displays the surface and core of the polymer to be comprised of empty channels and pores (fig. 3, B and D).

***l*-dopa release *in vitro*.** To assess whether the "impermeable" coat does indeed prevent the dissolution of *l*-dopa, we measured release from polymer samples that were fully coated. In the 125-day period studied, these controls did not release *l*-dopa. The measurement of *l*-dopa release from unloaded control polymer matrices also did not lead to absorbance at 280 nm, indicating that the absorbance values are not an artifact of polymer breakdown.

Figure 5 shows the cumulative release of *l*-dopa from coated polymer matrices with a single pore of 2, 4 and 6 mm, respectively (60% loading). Matrices with 2- and 4-mm pores result in surprisingly similar release kinetics, which may be attributed to uncontrolled factors during the fabrication process. The observed discoloration of the polymer matrices may offer clues to attest to this speculation. When aqueous solution gains access to the loaded core, *l*-dopa will diffuse out through the pore, leaving behind emptied pores with some residual *l*-dopa trapped inside (for release mechanism, see Discussion). This residual *l*-dopa changes color probably due to polymerization or oxidation, indicating that the aqueous solution had gained access to this portion of the polymer matrix. Polymer matrices with a single hole do display such decolored areas adjacent to the pore.

We also studied the effects of *l*-dopa loading on release kinetics by comparing polymer matrices with different loading (50–70%) and a constant pore size (2 mm). Figure 6A shows

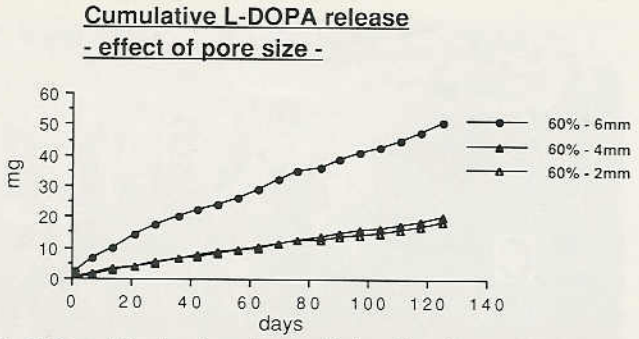

Fig. 5. Cumulative *in vitro* release of *l*-dopa (in milligrams) from quadruplicates of polymer matrices with 60% loading. The polymer matrices were surrounded by an impermeable coat, containing one pore with a diameter of 2, 4 or 6 mm, through which the physiologic medium could gain access to the *l*-dopa-loaded core. Quadruplicates were always taken from the same batch.

that the quantity of release correlated with loading and that *l*-dopa was released in a controlled ("zero-order") fashion for a period of at least 125 days (at which point this experiment was terminated).

In order to increase the absolute amount of release, we assessed the release pattern of *l*-dopa from polymer matrices that had one entire side of the slab exposed to the solution, while all other sides were sealed with an impermeable barrier. Thus, *l*-dopa dissolution could occur only in one direction (see also fig. 4). Zero-order kinetics were achieved in all polymer matrices, irrespective of their loading (50, 60 or 70%). The polymer matrices released *l*-dopa in the mg-range per day and release continued for more than 600 days (fig. 6B). Linearity of release was shorter in polymer matrices with 70% loading, suggesting that the polymer was emptying out after about 300 days. In contrast, release remained linear for longer time periods in 50 and 60% loaded samples.

Due to alterations in the standard fabrication procedures, even noncoated polymer matrices released *l*-dopa with zero-order kinetics. As figure 6C shows, the 50% loaded polymer matrices release linearly for at least 600 days after the initial burst, 60%-loaded polymers approach linearity, but polymers with higher loading had a first-order release kinetics. Figure 7 displays *in vitro* release from 60% samples, indicating absolute amount of release and release variability (S.E.M.) for two selected geometries.

We have also tested some polymeric devices with only 30% loading (data not shown), but no appreciable release of *l*-dopa was found, suggesting inadequate pore formation at lower loading.

***l*-dopa release *in vivo* following s.c. implantation.** Following the s.c. implantation of a noncoated polymer matrix with 70% loading, the mean plasma dopa level was relatively high in the initial period of release, ranging from 220 to 700 ng/ml (mean of 480 ng/ml; see fig. 8A). Within approximately 50 days, this level decreased by approximately 90% and approached linearity thereafter. For example, figure 8B shows a magnified scale of release in one rat to illustrate zero-order release kinetics. We observed at least 8 ng of *l*-dopa/ml from day 84 through at least day 226, at which time the experiment was terminated. The control rat had a mean plasma *l*-dopa level of 1.66 ± 0.16 ng/ml. Thus, the polymer implants resulted in continuous *l*-dopa release to obtain stably elevated plasma *l*-dopa levels *in vivo* for over half a year, which were 4 to 8

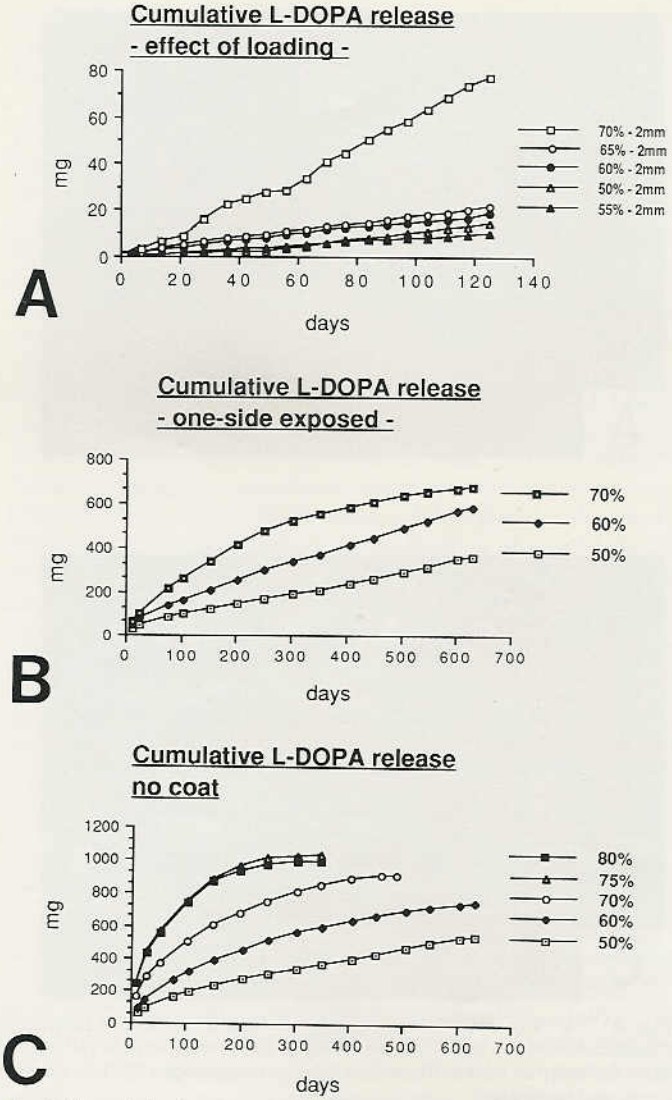

Fig. 6. Cumulative *in vitro* release of *l*-dopa (in milligrams) from quadruplicates of polymer matrices. A: shows polymer matrices with pores of 2-mm diameter varying in *l*-dopa loading from 50 to 70%. B: displays polymer matrices with 50, 60 and 70% loading in which one side of the slab was exposed to the physiologic solution, whereas all other sides were coated by an impermeable barrier. C: displays polymer matrices without any coat, varying in loading from 50 to 80%. Note linear release kinetics in polymer matrices with 50 and 60% loading.

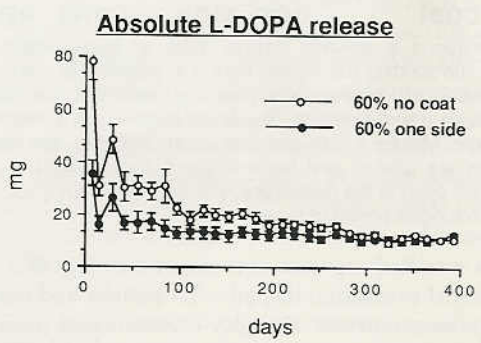

Fig. 7. Absolute *in vitro* release of *l*-dopa (in milligrams) from quadruplicates of polymer matrices (mean ± S.E.M.) either without coat or with one side exposed. In both cases, the matrices were loaded with 60% *l*-dopa.

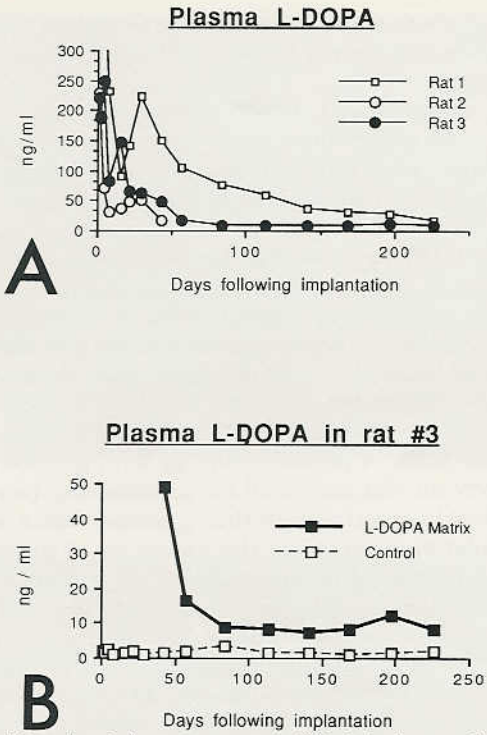

Fig. 8. A: *l*-dopa levels (nanograms per milliliter) in plasma of rats either implanted with *l*-dopa containing polymer matrices (rats 1, 2 and 3) or with blanks (control). B: a plot using a magnified scale to document zero-order release kinetics in rat 3.

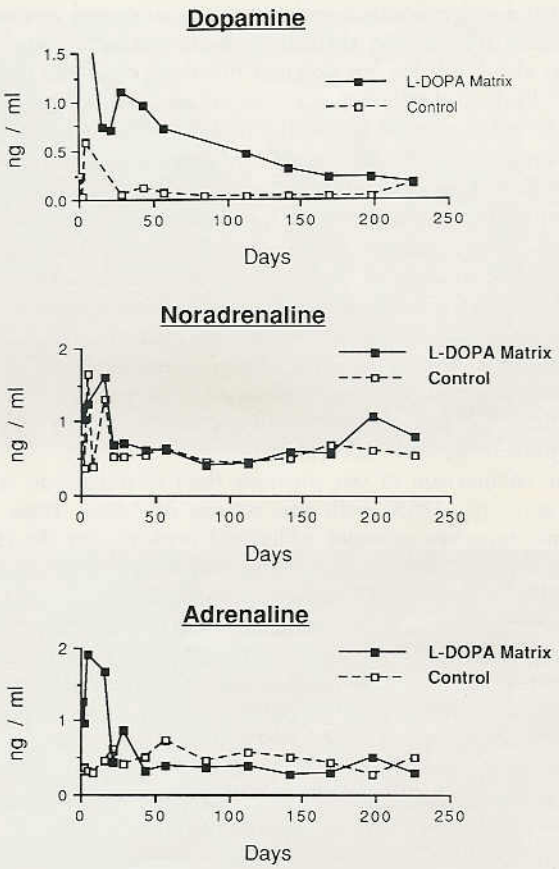

Fig. 9. Levels of dopa metabolites in the three rats from figure 8; dopamine (A), nonadrenaline (B) and adrenaline (C) in plasma (nanograms per milliliter) of rats implanted with *l*-dopa containing polymer matrices. Beyond a few weeks postimplantation, dopa-metabolites are comparable to control levels. However, note a slight rise in NA starting approximately 170 days after implantation.

times higher than levels observed in the control animal. The release kinetics *in vivo* were found to parallel those observed *in vitro*. However, one should note the variability of release in the three rats examined, which may be due to a variable degree of encapsulation of the device by fibrous tissues. It was also interesting that the levels of other catecholamines (dopamine, noradrenaline and adrenaline) parallel those of *l*-dopa only in the first few weeks after implantation (fig. 9). At later time periods, metabolite levels were comparable to those of the control rat, although a slight rise in noradrenaline was noted after about 170 days. In addition, some local irritation was observed and interpreted as a result of the obstructive shape of the polymer. Future improvements of the polymer shapes are expected to alleviate this problem.

Discussion

Following Birkmayer's early observations (1969), numerous investigators have confirmed that continuous delivery constitutes the best mode of *l*-dopa therapy for Parkinson's patients. Because plasma fluctuations resulting from *l*-dopa given orally may be a severe limitation, particularly in patients in progressed stages of the disorder, a variety of approaches for the continuous delivery of *l*-dopa or other dopamine agonists have since been studied, including recently developed sustained-release preparations (Cedarbaum *et al.*, 1987a,b; Curzon *et al.*, 1973; Erni and Held, 1987; Fischer and Baas, 1987; Gerlach *et al.*, 1988; Jansen *et al.*, 1987; Jensen *et al.*, 1987; Juncos *et al.*, 1987a,b; Lataste *et al.*, 1987; Marion *et al.*, 1986; Nordera *et al.*, 1987; Nutt *et al.*, 1986; Poewe *et al.*, 1987; Quinn *et al.*, 1987; Saarinen *et al.*, 1978; Siegfried, 1987).

Although sustained release using such retard preparations (such as sustained-release levodopa/carbidopa (Sinemet CR3-CR5®) or sustained-release levodopa/benserazide (Madopar HBS®) is of advantage in some patients, there are still problems associated with this approach (see Introduction), prompting our search for an alternative means of *l*-dopa delivery.

Since the early 1980s, drug delivery systems have become available that achieve a truly constant (also called "controlled") release (Tanquary and Lacey, 1974) of either low or high molecular weight compounds for extended time periods (Rhine *et al.*, 1980; Siegel and Langer, 1984; see also fig. 1, lower panel). One such drug delivery system is a polymer matrix fabricated with EVA. This matrix consists of a continuous phase of polymer carrier with a dispersed phase of drug powder particles.

The mechanism of drug release from polymer matrices has been proposed to be as follows (see also fig. 2): as soon as the polymer matrix is brought into contact with a physiologic medium such as saline, the medium has access to the outermost particles of the water-soluble phase of the polymer, *i.e.*, the drug particles at the surface of the matrix. The subsequent outward dissolution of drug particles leaves behind pores in the polymer matrix through which the medium gains access to the next layer of the drug. In this way, drug molecules continuously diffuse out through the previously emptied pores and their interconnecting channels into the aqueous environment. A number of fabrication factors influence the kinetics of drug release including drug-particle size, drug loading, matrix coating (Rhine *et al.*, 1980) and drug solubility (Peppas, 1983).

Several observations suggest that the proposed mechanism of release (dissolution through communicating channels and pores) also holds for our polymer matrices. First, we observed that 30%-loaded polymer matrices did not display appreciable release, which may be due to the finding that low loading does not permit sufficient development of pores and communicating channels (Rhine *et al.*, 1980). Second, SEM revealed the fine structure of the *l*-dopa polymer matrices, namely emptied pores after long-term release. It is therefore a reasonable argument that higher loading of the polymer with drug particles will result in greatly increased absolute quantities of release (more pores and communicating channels are formed), which cannot be explained simply by the greater total quantity of drug embedded in matrix. Nevertheless, even in samples with high loading, some particles may still be surrounded by polymer, thus preventing their dissolution.

The comparison of our previous data of dopamine release (Freese *et al.*, 1989) with the release of *l*-dopa from EVA polymer matrices provides additional evidence for the theory that *l*-dopa diffuses through pores and interconnecting channels. There is no linear release of dopamine from noncoated polymer matrices with a loading of 50% (Freese *et al.*, 1989), a loading for which *l*-dopa release was still found to be linear (fig. 5C). Since *l*-dopa and dopamine are comparable with respect to molecular weight and the polymer design was comparable, the differences in release kinetics may in part be due to a greater solubility of dopamine in aqueous solution. Assuming that the mechanism of controlled release from a polymer matrix involves the solubilization and subsequent dissolution of *l*-dopa or dopamine through pores and interconnecting channels, we speculate that the superior release characteristics of *l*-dopa containing polymer matrices are due to the fact that the less hydrophilic *l*-dopa diffused less readily through the pores toward the outside.

The main finding of our study, however, is that controlled-release polymer matrices can be used for the delivery of *l*-dopa in a controlled ("zero-order") rate for prolonged periods of time *in vitro* (for at least 600 days) in a mg-range per day and that s.c. implantation in rats results in stable *l*-dopa levels that are at least 4 to 8 times above normal for more than 225 days. This elevation of dopa also resulted in initial increases of the major metabolites, noradrenaline, adrenaline and dopamine in plasma, which, at least for noradrenaline and adrenaline, was compensated for within approximately 1 month, at which time these metabolites were within control range levels (fig. 9).

Using the same polymer-matrix system, we have also previously reported that delivery of dopamine can be obtained *in vivo* (During *et al.*, 1988, 1989). Since our studies in rats have shown that the long-term *in vitro* observations of *l*-dopa release can likewise be demonstrated in the *in vivo* situation, our polymer system can be considered of potential value for the controlled delivery of *l*-dopa in suitable amounts and for a time span suitable for clinical application. Here, *l*-dopa (with a decarboxylase inhibitor) containing polymer matrices could be implanted s.c. into patients with Parkinson's disease. It appears that both the rate of release and the service lifetime of the peripheral implants could prove to be adequate. The established biocompatibility and the fact that this polymer system in conjunction with other drugs has already been approved for human use by the U.S. Food and Drug Administration provides the basis for further *in vivo* testing. It would then be appropriate to assess some of the potential advantages of peripheral implantation over standard oral tablets or recently developed oral slow-release preparations (Sinemet CR 3 to CR5®, or Madopar HBS®).

The question arises as to whether the peripheral implantation of *l*-dopa containing polymer systems is a feasible alternative approach to the oral administration of *l*-dopa to patients. Several points should therefore be considered, especially concerning the dose and the mode of application: 20 years ago, Birkmayer (1969) pointed out that i.v. infusion requires substantially less *l*-dopa than when given orally, up to one order of magnitude. In contrast, oral sustained-release preparations require more *l*-dopa than conventional tablets (Nordera *et al.*, 1987; Poewe *et al.*, 1987). This suggests that the dose recommended by Birkmayer is so low because i.v.-infused *l*-dopa circumvents the gastrointestinal tract and not because of the continuous mode of delivery. This, in turn, provides support for the concept that peripheral *l*-dopa containing polymer implants placed s.c. particularly when combined with a decarboxylase inhibitor, would permit the application of smaller doses, perhaps one order of magnitude below the dose administered orally. Although the plasma *l*-dopa levels provided by our current implants are not sufficient for application in patients, it is possible to significantly increase the size of the polymer matrix devices, thus greatly increasing the mass of drug release per day. It is furthermore possible to embed drugs into the polymer matrixes, which are more potent, requiring smaller absolute quantities of delivery. Such drugs could include dopamine receptor agonists such as apomorphine, bromocriptine or lisuride.

One may argue that polymer implants do not allow flexibility to adjust the dose for an individual patient. However, it is conceivable that one could first determine an appropriate daily dose of *l*-dopa for a particular individual by pretreating the patient with continuous *l*-dopa either by i.v. infusion or by external pumps. Having obtained this information, a polymeric implant with appropriate release characteristics could then be selected and implanted s.c. into the patient in an ambulatory setting. It is reasonable to assume that the minor surgery required for implantation would be tolerated by patients and might even be preferred over the frequent oral ingestion of standard or retard preparations.

Potential disadvantages of our approach include the fact that the residual EVA polymer matrix has to be replaced after linear release has ceased and that no short-term dose adjustments can be made, unless the implant is supplemented with oral low-dose formulations. However, if medical or psychiatric problems arose, one could easily remove the implant on short notice. We have also noted an initial burst of *l*-dopa release that would not be desirable. However, it is possible to introduce alterations in the polymer fabrication technology, and it is conceivable that one incubates the polymer matrices in physiologic solution ("priming") and implant the devices only when a stable and desirable release is obtained *in vitro*. There is potentially another issue that requires attention. As Nutt (1988) has pointed out, continuous dopaminergic stimulation may not necessarily be without complications; it may increase levels of dopamine metabolites or downregulate dopaminergic neurotransmission. However, our initial analysis of some catecholaminergic metabolites in the periphery did not reveal any significant observations confirming such a concern.

The foremost advantage of our approach is, however, the likelihood that the controlled delivery of *l*-dopa (in conjunction

with a decarboxylase inhibitor) will reduce or eliminate the enormous fluctuations of plasma (and brain) *l*-dopa levels and their clinical correlates. It is conceivable that patients who would benefit from such polymer implants are not only those in the later stages of the disease (who suffer from "on-off" or "wearing-off" syndromes), but also those early on. Implantation at early stages of the disorder might, by virtue of keeping the dose constant and relatively low, even retard or preclude the appearance of the "on-off" and "end-of-dose" responses typically found at the more advanced stages of the disease.

Implanted s.c. *l*-dopa containing slow-release polymer matrices would have other potential advantages as well: 1) a functional life time of many months, perhaps over 1 year, 2) biocompatibility and 3) virtual elimination of patient compliance problems, although this is a problem in only few Parkinson patients. In addition, polymer implants 4) circumvent side effects and drug wastage attributable to gastrointestinal absorption and 5) they can be implanted with relatively noninvasive outpatient surgery. In addition, and unlike internal or external reservoir pumps, the possibility of excessive drug release due to mechanical failure or breakage of the device is negligible. Advantages over pumps include the feature that drugs embedded in polymer matrices have a long-term biologic activity, exceeding that of drugs that are suspended in solution (see also Stahl, 1988). It is, of course, now necessary to determine the efficacy of *l*-dopa polymers in combination with a decarboxylase inhibitor in animal experiments and to evaluate the feasibility of matrices loaded with dopamine receptor agonists.

Since the potential advantages of controlled-release *l*-dopa-containing polymer matrices clearly outweigh the potential disadvantages, we believe that peripheral implantation of polymer matrices containing *l*-dopa or dopamine receptor agonists might comprise a viable alternative treatment modality and become a realistic clinical option for improved care of parkinsonian patients.

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