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* On leave of absence from the Department of Pharmacy, The University, Manchester M13 9PL, England.

ˣ To whom inquiries should be directed.

In Vivo and *In Vitro* Evaluation of a Microencapsulated Narcotic Antagonist

NORBERT MASON *, CURT THIES *ˣ, and THEODORE J. CICERO ‡§

Abstract □ Injectable microcapsules containing 75% (w/w) cyclazocine, a narcotic antagonist, were prepared with *dl*-poly(lactic acid) as the coating material. Capsule fractions falling between 105 and 295 μm released about 90% of their cyclazocine in 8 days of rotating-bottle extraction at 37° in pH 7.4 phosphate buffer. Although larger capsules released the drug somewhat more slowly, all capsules released cyclazocine far more rapidly than an ideal capsule should. This rapid release is attributed to macroscopic defects located in the capsule walls. The ability of the capsules to block the action of morphine *in vivo* was assessed by injection of a sesame seed oil suspension into Holtzman rats. A hot-plate test procedure was used to evaluate animal behavior. Capsule doses of 100–250 mg/kg to rats caused significant antagonism of morphine's analgesic effect for 14 days after injection. By Day 17, no antagonism occurred, indicating that the capsules completely released the drug *in vivo* between 14 and 17 days after injection.

Keyphrases □ Cyclazocine—injectable microcapsules, *in vivo* and *in vitro* release evaluated □ Microcapsules, injectable—*in vivo* and *in vitro* release of cyclazocine evaluated □ Dosage forms—injectable microcapsules, *in vivo* and *in vitro* release of cyclazocine evaluated □ Narcotic antagonists—cyclazocine, *in vivo* and *in vitro* release from injectable microcapsules evaluated

A significant problem in the long-term treatment and rehabilitation of heroin addicts is their pronounced tendency to undergo drug relapse. One possible means of circumventing this problem would be the injection or implantation of sustained-release preparations of narcotic antagonists, which would, in effect, inoculate the patient against heroin for a prescribed period, preferably 1 month or more. Theoretically, the repeated administration of such a preparation could provide a therapeutically effective level of antagonist for an indefinite treatment period.

An injectable sustained-release formulation must satisfy these requirements:

1. It must be biocompatible and provide uniform sustained release of the antagonist for a specified time.
2. Once the drug has been released, the delivery system must be biodegradable within a relatively brief time.
3. The microcapsules must be easily injected through hypodermic needles of sufficiently small size to be tolerated by patients.
4. The total amount of drug administered to the patient must not approach toxic levels to minimize any risk associated with accidental failure of the delivery system.

Narcotic antagonist formulations that meet several, but not necessarily all, of these requirements were reported recently. Insoluble salts and salt complexes of cyclazocine and naloxone were formulated (1), and several preparations significantly increased the duration of narcotic antagonist activity in mice. Prepared particulate cyclazocine–poly(lactic acid) composites provided sustained release *in vivo* and *in vitro* (2). However, the particles contained only 21% (w/w) of drug and required a 12-gauge needle for injection.

The technique of microencapsulation may provide

a means of preparing narcotic antagonist formulations that are effective longer than those prepared to date, carry a higher drug content, and are capable of being injected through small needles. Microencapsulation is a process whereby individual drug particles are coated to retard the rate of drug dissolution (3). Although few polymers have characteristics that make them acceptable for injectable microcapsule-coating applications, a polymer that appears to be suitable is *dl*-poly(lactic acid). Accordingly, *dl*-poly(lactic acid) was used to prepare microcapsules containing cyclazocine, a narcotic antagonist. This paper describes the *in vivo* and *in vitro* performance of these capsules.

EXPERIMENTAL

Microencapsulation—Microcapsules containing cyclazocine were prepared with additive-free *dl*-poly(lactic acid)[1]. The capsules contained, on the average, 75% (w/w) cyclazocine and 25% (w/w) *dl*-poly(lactic acid). Several capsule sizes were isolated: 105–177, 177–295, and 295–595 μm. For each fraction, the smaller number given represents the size of the rectangular screen opening that retained the microcapsule, whereas the larger number represents the size of the rectangular screen opening through which the capsules passed. All capsule fractions used were isolated from the same capsule batch.

***In Vitro* Evaluation**—The *in vitro* release properties of the capsules were evaluated in a commercially available rotating-bottle apparatus[2] (40 rpm) at 37° in pH 7.4 phosphate buffer. The concentration of cyclazocine in the extracting solution was established spectrophotometrically by using the 278-nm band characteristic of cyclazocine. Release results are plotted as percent cyclazocine extracted from the capsule *versus* time.

Each data point reported was obtained with a separate test sample. The test samples consisted of 15 mg of capsules suspended in 75 ml of buffer. The 15-mg capsule weight was selected to minimize weighing errors, whereas the 75-ml volume of buffer filled the sample reservoirs. When a 15-mg capsule was used with 75 ml of buffer, complete cyclazocine extraction from the capsules yielded a concentration of 14.4 mg of drug/100 ml of buffer. This value is well below the saturation solubility of cyclazocine in pH 7.4 phosphate buffer at 37° (46 mg/100 ml). However, drug concentration in the extracting solution exceeded 15% of the saturation value once the capsules released 50% or more of their active contents.

The permeability of cyclazocine through *dl*-poly(lactic acid) films at 37° was measured by a procedure similar to one published previously (4). Membranes of 0.5–1-mil thickness were cast from solution onto a glass surface and placed in glass diffusion cells. The cells contained 12 ml of liquid on each side of the membrane and were rotated (~10 rpm) in a vertical plane at 37°. A saturated solution of cyclazocine was maintained on one side of the membrane (Side 1); only buffer was present initially on the other side (Side 2). The permeability of cyclazocine through the *dl*-poly(lactic acid) membrane was calculated from the rate of cyclazocine buildup on Side 2 of the dialysis cell, which was established spectroscopically (278 nm).

***In Vivo* Evaluation**—Pharmacological *in vivo* evaluations of the cyclazocine microcapsules were carried out in male Holtzman rats weighing between 125 and 150 g at the time of injection. Microcapsules were suspended in sesame seed oil at a constant drug to suspending medium ratio of 25 mg/ml. An 18-gauge needle was used to inject 177–295-μm capsules; 295–595-μm capsules were injected with a 16-gauge needle. The capsules were injected subcutaneously between the shoulder blades.

Different groups of rats ($n = 7$) were used for each test day following the injection. Rats were never tested more than once. In addition to the rats receiving microencapsulated cyclazocine, groups of rats ($n = 7$) were also injected with an equivalent amount of unencapsulated cyclazocine (dose calculated as 75% of the microcapsule dosage) suspended in sesame seed oil. Other rats ($n = 7$) were injected with the suspending medium free of drug.

At known intervals after injection of the microcapsules, unencapsulated cyclazocine, or sesame seed oil vehicle, the degree of antagonism of an ED_{80} dose of morphine (10 mg/kg) was evaluated using the hot-plate method described previously (5). The degree of analgesia produced by morphine in the three groups was established 45 min after the subcutaneous injection of the narcotic.

[1] Supplied by Dr. Donald Wise, Dynatech R/D Co., Cambridge, Mass.
[2] Manufactured by Ernest Menhold, Lester, Pa.

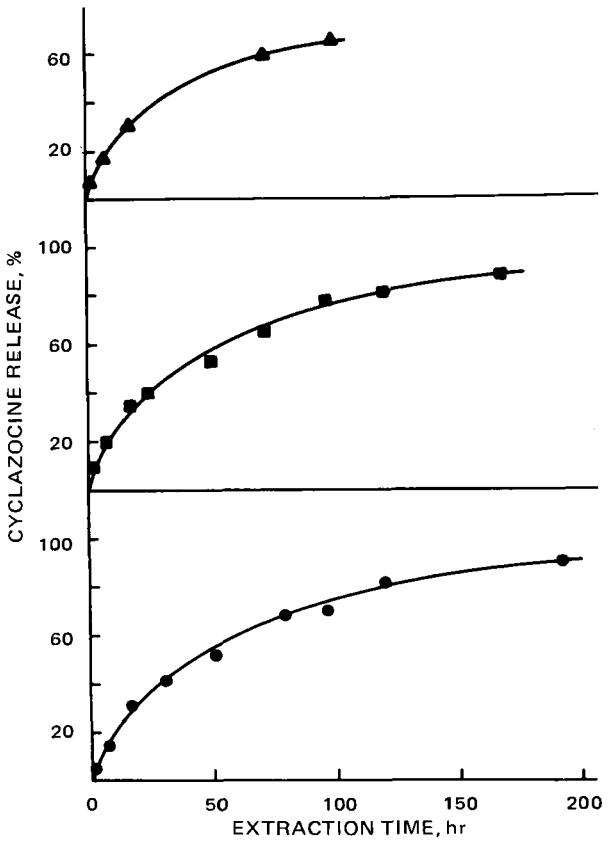

Figure 1—*Rate of cyclazocine extraction from* dl-*poly(lactic acid) microcapsules at 37° and pH 7.4. Key:* ●, *105–177-μm capsules;* ■, *177–295-μm capsules; and* ▲, *295–595-μm capsules.*

In the hot-plate method, analgesia is defined as an increase in reaction time over baseline levels. The response usually consists of licking the paws or, less frequently, jumping out of the restraining cylinder. A cutoff point of 30 sec on the hot plate after morphine injection was established to prevent injury to the paws. The maximum possible analgesic effect, as assayed by the hot-plate method, would thus be 30 sec minus the baseline reaction time. The degree of agonism of a given dose of morphine was determined by:

$$\frac{\text{reaction time after morphine} - \text{baseline reaction time}}{30\text{ sec} - \text{baseline reaction time}} \times 100 = \text{percent agonism} \quad \text{(Eq. 1)}$$

In the present studies, a dose of morphine was used that produced approximately 80% agonism (*i.e.*, ED_{80}) on the hot plate. The degree of antagonism of this dose of morphine was determined by:

$$\left(1 - \frac{\text{percent agonism of morphine plus drug}}{\text{percent agonism of morphine only}}\right) \times 100 = \text{percent antagonism} \quad \text{(Eq. 2)}$$

The percent antagonism of an ED_{80} dose of morphine was determined at 4, 7, 14, 17, and 21 days after the administration of microencapsulated cyclazocine, uncapsulated cyclazocine, or sesame seed oil.

RESULTS AND DISCUSSION

Figure 1 contains *in vitro* release curves for the various capsule fractions evaluated. Cyclazocine release was nonlinear under the extraction conditions used and declined steadily as extraction continued. About 8 days of extraction was needed to remove 90% of the cyclazocine from the 105–177- and 177–295-μm capsules. Capsules in the 295–595-μm fraction released 64% of the drug in 100 hr. They

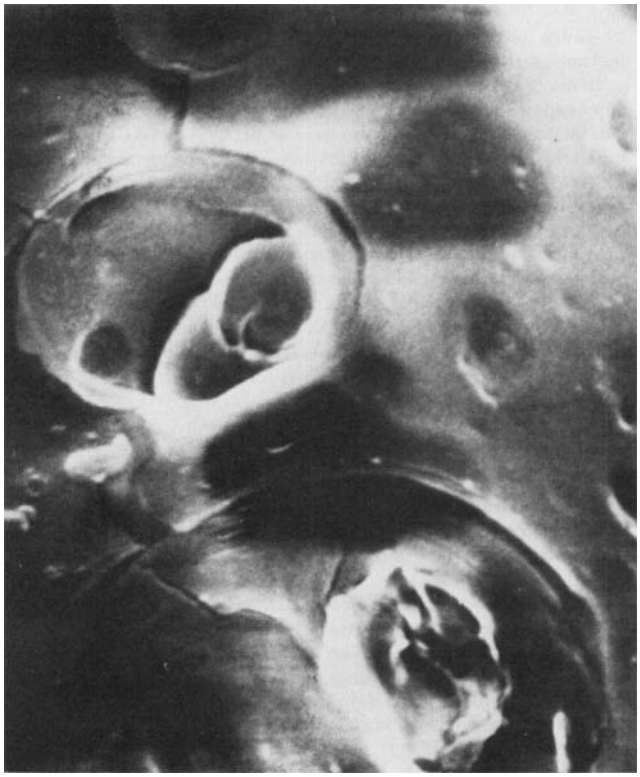

Figure 2—*Scanning electron micrograph of surface of* dl-*poly(lactic acid) microcapsule (105–177-μm fraction) containing cyclazocine (magnification = 2500×).*

appeared to release cyclazocine at a somewhat slower rate than the smaller capsules as extraction progressed, but the difference was not great. Unencapsulated cyclazocine dissolved completely in buffer within 2 hr, so the *dl*-poly(lactic acid) capsule walls clearly retarded cyclazocine release. However, the capsules released their cyclazocine considerably faster than they should have due to defects in the capsule coating.

Direct evidence that the capsule coatings contain defects is provided by the scanning electron micrograph shown in Fig. 2. This micrograph is representative of cyclazocine capsule surfaces and shows that such surfaces are irregular and cratered. The irregularities and craters represent weak points in the capsule wall and provide sites for accelerated transport of cyclazocine from the capsules. The craters could extend to the cyclazocine surface and thereby provide direct routes for passage of cyclazocine from the capsules. Although this possibility cannot be completely discounted, the ability of the capsules to provide cyclazocine release over at least a 9-day period suggests that few craters extend completely to the cyclazocine surface.

The permeability of a large drug molecule such as cyclazocine through a defect-free unswollen polymeric film in which it is insoluble would be expected to be very low. Thus, measurements of permeability through solvent-cast films should provide an estimate of permeability against which the performance of microcapsules may be compared. Accordingly, the permeability of cyclazocine through solvent-cast *dl*-poly(lactic acid) films was determined as discussed under *Experimental*. These measurements yielded permeability values of 2.9 and 3.0×10^{-11} cm^2/sec at 37°.

From the average of these values, the time required for a spherical 200-μm diameter capsule of uniform wall thickness (assumed to be 7.2 μm) to lose 50% of its drug [initially 80% (w/w) cyclazocine] was calculated. The interior of the capsules was assumed to be a saturated solution of cyclazocine; the extracting medium outside the capsule was assumed to contain no cyclazocine. Ideal capsules, obeying these boundary conditions, should give zero-order cyclazocine release and require 28.6 months of extraction to remove 50% of the drug. This calculated half-life for ideal capsules is about 300 times greater than that achieved by the cyclazocine capsules used in this study.

Presumably, most of the difference between observed and calculated rates of cyclazocine release was due to macroscopic defects in the polymer membrane like those shown in Fig. 2. One cannot ignore the possibility that nonuniform capsule wall thicknesses will always result when irregularly shaped solids are encapsulated and thereby make it impossible to prepare capsules with the maximum possible lifetime. Shear forces imposed upon the embryo capsules during the encapsulation process also may always contribute to wall nonuniformity and have the same effect. Nevertheless, the measured permeability coefficient for cyclazocine diffusing through a *dl*-poly(lactic acid) membrane was low and indicates that the concept of preparing truly long-acting cyclazocine capsules with *dl*-poly(lactic acid) is a valid one. Minimizing the capsule wall defects (Fig. 2) should lead to capsules with substantially longer lifetimes than the ones prepared to date.

Even though the cyclazocine microcapsules prepared for this study did not have defect-free coatings, they provided *in vitro* release for 8 or more days. Thus, their *in vivo* performance was evaluated. In preliminary studies, 105–177-μm cyclazocine capsules, suspended in 0.1% aqueous methylcellulose, were injected into rats at a dose level of 50 mg/kg. After 2 days, rats were tested on the hot plate after the injection of an ED_{100} dose of morphine (15 mg/kg). Such a high morphine dose was selected to provide a stringent test of the *in vivo* microcapsule performance in these preliminary studies. The analgesic effects of this morphine dose were antagonized by over 88% after the 2-day postinjection interval. Control animals that received only aqueous methylcellulose showed no antagonism of morphine's effect.

These preliminary data established that the cyclazocine capsules provided antagonism of a rather high dose of morphine for at least 2 days. However, these studies also revealed that the aqueous methylcellulose solution was inadequate as a suspending medium. Because this solution was so dilute, the capsules settled rapidly, thereby making it difficult to achieve an even suspension. Instead of using more concentrated aqueous methylcellulose solutions, sesame seed oil was used exclusively as the suspending medium for subsequent *in vivo* microcapsule evaluations. It gave relatively stable capsule dispersions, which were readily injected in a reproducible manner.

Table I summarizes the *in vivo* performance of several cyclazocine capsule fractions injected into rats with sesame seed oil as the suspending agent. These data show that the cyclazocine capsules pro-

Table I—Percent Antagonism of an ED_{80} Dose of Morphine (10 mg/kg) by Sesame Seed Oil, Unencapsulated Cyclazocine, and Microencapsulated Cyclazocine[a]

	Cyclazocine Dose, mg/kg	Days after Injection				
		4	7	14	17	21
Sesame seed oil	0	0	0	0	0	0
Unencapsulated cyclazocine	75	100.0 (0)	2.0 (0—7.8)	0	0	0
	175	100.0 (0)	4.4 (0—11.3)	0	0	0
Microencapsulated cyclazocine	100[b]	100.0 (0)	100 (0)	61.4 (27.8—93.9)	—	—
	250[c]	100.0 (0)	81.9 (65.3—90.9)	95.5 (84.6—100)	0	0

[a]Microencapsulated cyclazocine doses are reported as milligrams of capsules per kilogram body weight. Since the capsules contain 75% (w/w) drug, approximately this percentage was given as unencapsulated cyclazocine. An equal volume of sesame seed oil was given to controls. Groups of seven rats were used for each dose and time interval. Numbers in parentheses are the range of antagonism values measured at that point; other numbers are mean percent antagonism. [b]Capsule size of 177–295 μm. [c]Capsule sizes of 177–295 and 295–595 μm. Since there was no difference between capsule sizes in terms of their *in vivo* effectiveness, the data were pooled.

vided a high degree of antagonism toward an ED_{80} dose of morphine (10 mg/kg) for at least 14 days after injection. For comparative purposes, it should be noted that all encapsulated cyclazocine doses administered showed negligible blockade toward morphine 7 days after injection. How long the capsules effectively block the morphine challenge dose does not appear to vary with the capsule dose administered, at least over the dose range examined.

A dose of at least 100-mg capsules (72 mg of encapsulated drug/kg rat) was sufficient to produce a high degree of antagonism for up to 14 days after injection. Increasing the amount of capsules injected to 250 mg/kg also provided a high degree of antagonism for at least 14 days. As was the case with the 100-mg/kg capsule dose, no antagonism occurred 17 or 21 days after injection. The capsules, regardless of the amount injected, apparently completed release of drug sometime between 14 and 17 days after injection. This *in vivo* lifetime is not substantially greater than the *in vitro* lifetime of the capsules as determined by the rotating-bottle technique. Thus, longer acting injectable cyclazocine capsule formations will require capsules with polymer walls with fewer defects.

The *in vivo* performance of the cyclazocine capsules evaluated appeared to be independent of their size. Consistent with the *in vitro* release data shown in Fig. 1, there was little difference *in vivo* between 177–295- and 295–575-μm capsules. Since all capsule fractions had approximately the same cyclazocine content initially (75% w/w), the larger capsules, by virtue of having a thicker polymer coating, should release their payload at a significantly slower rate. The fact that they did not, either *in vitro* or *in vivo*, provides further evidence that the *dl*-poly(lactic acid) capsule coatings are at present defective.

For an injectable drug delivery device, the active ingredient should ideally be released at some uniform rate that provides a therapeutic drug level *in vivo* throughout the entire lifetime of the device. Once empty, the activity of the device should decline rapidly in a predictable manner until it has no ability to block the action of morphine. With such a device, successive injections could be given and overlap of drug release from two injections would be minimal.

The microcapsules utilized here approached this ideal behavior. A 250-mg/kg dose of 177–295- or 295–595-μm capsules provided 88–100% antagonism to morphine 14 days after injection and 0% antagonism 17–21 days after injection. It was not possible to ascertain precisely how fast the capsules declined, but clearly they went from a high degree of activity to zero activity within 3–7 days.

A significant factor in the successful administration of injectable microcapsules is the size of the needle required for injection. Complete delivery of capsules requires a needle with an internal diameter at least three times greater than that of the largest capsule. Otherwise, capsules lodge in the needle itself or at the constriction in the syringe where it joins the needle, and the amount actually injected is less than expected. Thus, an 18-gauge needle is required to administer 177–295-μm capsules, and a 16-gauge needle is needed for 295–595-μm capsules. Therefore, injectable capsules to be administered with a 21-gauge needle, the size used clinically for many intramuscular injections, must be less than 170 μm in diameter.

In conclusion, this study demonstrates that injectable microcapsules capable of providing prolonged effective *in vivo* release of a drug can be prepared. The capsules used so far have defect-filled coatings and release their drug content far more rapidly than predicted. Nevertheless, they can provide at least 2 weeks of activity *in vivo* when administered at a relatively high dose. They fall short of the 1-month or more lifetime desired, but formulation of such capsules should prove useful immediately in animal studies of narcotics and narcotic antagonists. These capsules also should serve as a promising first step in the development of a clinically useful sustained-release preparation. Since the capsules described in this paper release drug at a rate estimated to be 300 times greater than that of ideal *dl*-poly(lactic acid) capsules, there is considerable room for improving capsule quality. Efforts are being made to this end.

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ˣ To whom inquiries should be directed.