Long Acting Delivery Systems for Narcotic Antagonists II: Release Rates of Naltrexone from Poly(lactic Acid) Composites

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Abstract □ Parallel *in vitro* and *in vivo* release rates of tritiated naltrexone from poly(lactic acid) composites were studied. The *in vitro* release of naltrexone was 67% of the dose over a 35-day test period, while the *in vivo* release was only 24% within 70 days. Apparently, an exchange of the tritium for the hydrogen of the body water takes place, indicating that urinary excretion radioactivity is not a reliable measure for estimating the naltrexone released. Naltrexone–poly(lactic acid) composites showed effective blocking action to morphine in rats (24 days), dogs (29 days), monkeys (20 days), and mice (21 days).

Keyphrases □ Naltrexone—release rates from poly(lactic acid) composites, long acting delivery systems for narcotic antagonists □ Narcotic antagonists—long acting delivery systems, naltrexone–poly(lactic acid) formulation, release rates □ Dosage forms—long acting delivery systems for narcotic antagonists, release rates of naltrexone from poly(lactic acid) composites □ Formulations—naltrexone–poly(lactic acid) composites, release rates

The development of systems for controlled release of narcotic antagonists from polymeric matrixes, such as polyethylene and poly(lactic acid), has been the objective of research by this laboratory since 1970. The results of the behavior of cyclazocine[1] (3-cyclopropylmethyl - 1,2,3,4,5,6-hexahydro-6,11-dimethyl-2,6-methano-3-benzazocin-8-ol)–poly(lactic acid) composites were published recently (1). The present paper reports on the release rates of naltrexone[2] (17-cyclopropylmethyl - 4,5α - epoxy - 3,14 - dihydroxymorphinan-6-one), which was described (2, 3) to be a useful narcotic antagonist with less agonistic activity than cyclazocine, from poly(lactic acid) composites.

EXPERIMENTAL

All countings were performed with liquid scintillation spectrometers[3]. The counting solution consisted of a mixture of 2,5-diphenyloxazole (22.0 g), 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (0.4 g), and a nonionic wetting agent[4] (1000 ml) diluted to 4000 ml with toluene in the *in vivo* experiments. A scintillation liquid[5] was used in the *in vitro* experiments.

Preparation of Naltrexone–Poly(lactic Acid) Composites—A chloroform solution of tritiated naltrexone[6] [specific activity 83.9×10^9 dpm/ml (1.0 ml)] was diluted to 1000 ml with methylene chloride. To this solution (30 ml) were added, for the preparation of composites containing 20% naltrexone, unlabeled naltrexone base[2] (4.0 g) and tributyl citrate (1.0 g) in methylene chloride (200 ml) and then 15 g of poly(lactic acid) (1). For the preparation of composites containing 35% naltrexone, unlabeled naltrexone (10.5 g) and tributyl citrate (1.5 g) in methylene chloride (200 ml) and then 18.0 g of poly(lactic acid) (1) were added to the original solution of labeled naltrexone (45 ml).

In the preparation of both types of composites, the solvent was flashed off under reduced pressure and the residue, wrapped in aluminum foil, was melt-pressed[7] at 170° under a total load of 3 metric tons for 30 sec (shims 0.91 mm thick were used) to produce films of uniform thickness in which no imperfection due to air or gas was observed. The films were ground in a blender[8].

The particles obtained were screened, and fractions of an average size of 500–710 μm were collected. The specific radioactivity, determined by combustion of the polymer matrixes and measurement of the radioactivity in the water trapped in a scintillation spectrometer[9], was 107×10^6 dpm/g for the sample containing 20% naltrexone and 61.4×10^6 dpm/g for the sample containing 35% naltrexone.

***In Vitro* Tests**—A sample of the composite containing 20% naltrexone (650 mg) was sewn into a cheesecloth sack and anchored under the water level of the sample holder of the modified extractor (Raab) described in a previous paper (1). The drug was extracted with tepid (29 ± 3°) water as the solvent. Samples of the aqueous extract were collected periodically, and the radioactivity was measured. The values of naltrexone extracted in each interval of time are reported as percent of dose initially present in the sample, calculated on the basis of disintegrations per minute.

At the end of the experiment, the sample of composite left in the extractor was dissolved in methylene chloride and the radioactivity of the obtained solution was measured. The total radioactivity of the extracted aqueous solution plus the radioactivity found in the sample after extraction checked with that present in the sample before extraction.

***In Vivo* Tests**—These tests were performed on groups of three male Sprague–Dawley rats, 550–600 g, using the following injection method. Into a 5-ml plastic syringe, whose opening at the tip was first enlarged with a 0.21 cm (0.081-in.) drill and then plugged, were added, while stirring, carboxymethylcellulose[10] (210 mg), the composite containing 20% naltrexone (400 mg), and normal saline (portionwise, 0.5-ml portions) until the total volume was 3 ml. The time of mixing and stirring was about 1 min.

An intimate mixture of particles in nearly clear, viscous carboxymethylcellulose resulted. The plug at the tip of the barrel was then replaced with a 12-gauge, thin wall needle. The injection site on the rat was shaved, the area having been selected by measuring the length of the needle from the nape of the neck to a lower section of the back. The animal was lightly anesthetized with ether to

Table I—Blocking Action of Naltrexone–Poly(lactic Acid) Composites

Animal	Number of Animals	Dose, mg/kg
Rats[a]	70	240
Dogs[b]	5	17
Monkeys[a]	4	30
Mice[c]	10	39

[a] D. A. McCarthy, Parke, Davis and Co., Ann Arbor, Mich., personal communication. [b] W. R. Martin, Addiction Research Center, Lexington, Ky., personal communication. [c] R. H. Reuning, College of Pharmacy, Ohio State University, personal communication.

[1] Sterling-Winthrop, New York, N.Y.
[2] Endo Laboratories, Garden City, Long Island, N.Y.
[3] Packard Tri-Carb model 3003 (Packard Instruments, Downers Grove, Ill.) was used in the *in vivo* experiments, and a Beckman LS-100 (Beckman Instruments, Fullerton, Calif.) was used in the *in vitro* experiments.
[4] Triton X-100, Rohm and Haas, Philadelphia, Pa.
[5] Aquasol, New England Nuclear Corp., Boston, Mass.
[6] Tritiated by the catalytic exchange method by New England Nuclear Corp., Boston, Mass.
[7] Carver laboratory press model D.
[8] Waring.
[9] Packard Tri-Carb model 3003.
[10] 7LF, Hercules, Inc., Wilmington, Del.

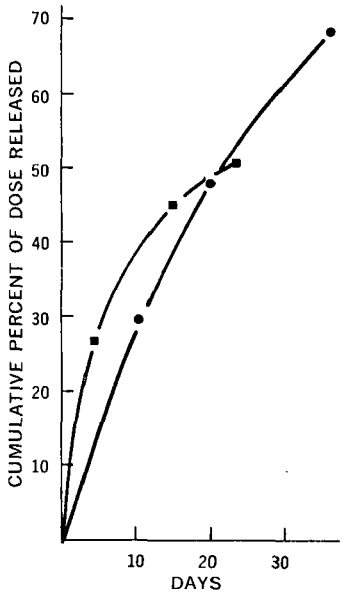

Figure 1—*Cumulative amounts of naltrexone and cyclazocine released* in vitro *from composites in particle form. Key:* ■, *cyclazocine (Ref. 1, Sample F); and* ●, *naltrexone.*

avoid excessive movement. The time required for injection was approximately 15 sec. On removing the needle, the opening was pressed and supported with the finger, painted with antiseptic, and immediately closed with a clip or adhesive tape. The animals were immediately placed into individual metabolism cages designed for the collection of urine.

The collected urine and cage washings were combined and counted on a daily basis over the first 10 days and then every 4 days for the duration of the experiment. The urine samples were diluted to 100 ml with water, and samples of 1 ml were pipetted into 15 ml of scintillation solution and radioassayed by liquid scintillation counting techniques. Internal standardization was used for the calculation of counting efficiency. The values of naltrexone delivered in each interval of time are reported as percent of dose released calculated on the basis of disintegrations per minute.

Determination of Tritiated Naltrexone Remaining in Composites at End of *In Vivo* Tests—A sample of the composite was collected from the sacrificed animal and extracted with chloroform. The radioactivity of the extract was 0.43×10^6 dpm (1% of the original radioactivity).

Exchange of Tritium from Tritiated Naltrexone for Hydrogen of Water—A solution of radioactive naltrexone in methylene chloride (0.1 ml) (83.1×10^6 dpm/ml, experimentally determined) was evaporated to dryness in a 250-ml beaker. To the residue was added 50 ml of distilled water. The obtained solution (specific radioactivity 2.6×10^4 dpm/ml) was frozen on the inside wall of a 1000-ml, round-bottom, single-necked flask connected to a dry ice-cooled moisture trap and kept under vacuum (<0.01 torr) overnight. The water (49 ml) condensed in the trap showed a radioactivity of 4400 dpm/ml (17% of the radioactive tag was lyophilized).

Blocking Action to Morphine—The blocking action of naltrexone composites to morphine was determined in rats (Sprague–Dawley), dogs, monkeys (*Macaca mulatta*), and mice by injecting suspensions of 35% naltrexone–poly(lactic acid) composites in 7% carboxymethylcellulose gel (Table I). The duration of morphine antagonistic activity was determined in rats and in mice by using the tail pinch test; in dogs by measuring the flexor reflex, the skin twitch reflex, the pulse rate, and the pupillary diameter; and in monkeys by measuring the changes in morphine-induced prolongation of interblinking time.

RESULTS AND DISCUSSION

The results of the *in vitro* tests are shown in Fig. 1 in comparison with those obtained with cyclazocine composites under the same conditions. The total amount of naltrexone release within 35 days was 67% of the dose originally present in the composite. The time at which one-half of the dose had been delivered, $T_{1/2}$, was 22 days, and the average daily delivery of naltrexone was 1.53 mg. This delivery is comparable to that found for cyclazocine.

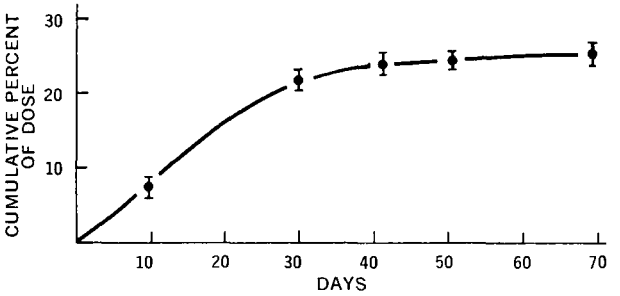

Figure 2—*Cumulative amounts of naltrexone excreted in urine, expressed as percent of dose. Each point represents the mean observation on three animals* ± SE. *In this work, the standard error is equal to the standard deviation divided by the square root of the number of animals tested:* ($SE = \sigma/\sqrt{N}$).

The *in vivo* test results (Fig. 2) show that only 24% of the administered dose was excreted in urine over a 70-day period. However, the determination of the drug in the composite at the end of the test showed that only 1% of the original dose remained in the composite.

The considerable difference in the amounts of naltrexone released in the *in vivo* and *in vitro* tests and the discrepancy between the amount of naltrexone excreted and that left in the composite led to the investigation of the stability of the tritium label on the naltrexone molecule. This investigation was performed by freeze-drying samples of tritiated naltrexone in aqueous solutions and determining the radioactivity of the condensed water. An amount of 17% of the radioactivity originally present in naltrexone was found in the condensed water, indicating exchange of the tritium for the hydrogen of water.

These results show that urinary excretion radioactivity is not a reliable measure for estimating the naltrexone released in *in vivo* tests. At present, other methods are being investigated that are not based on radioactivity, such as chromatography.

Significant blocking action against the effect of morphine was observed in rats throughout the 24-day test period[11]. The tests on dogs showed a highly significant level of blockage for all measures, except lowering of pulse rate, through the 21st day and a significant level of blockage for the flexor reflex through the 29th day (3). Complete antagonism to the depressant effect of morphine on the blinking rate occurred 24 hr after antagonist injection in monkeys. The estimated duration of statistically significant activity was approximately 20 days[11]. In the tests on mice, a 21-day average duration of blocking action was observed.

REFERENCES

(1) J. H. R. Woodland, S. Yolles, D. A. Blake, M. Helrich, and F. J. Meyer, *J. Med. Chem.*, **16**, 897(1973).

(2) *Chem. Eng. News*, **July 3, 1972**, 14.

(3) W. R. Martin and V. L. Sandquist, *Arch. Gen. Psychiat.*, **30**, 31(1974).

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[11] D. A. McCarthy, Parke, Davis and Co., 1973, personal communication.