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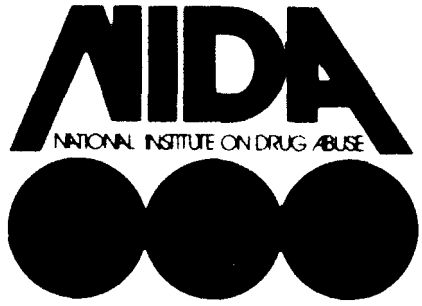
Narcotic Antagonists: The Search for Long-Acting Preparations



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Narcotic Antagonists: The Search for Long-Acting Preparations

Editor
Robert Willette, Ph.D.
Division of Research
National Institute on Drug Abuse
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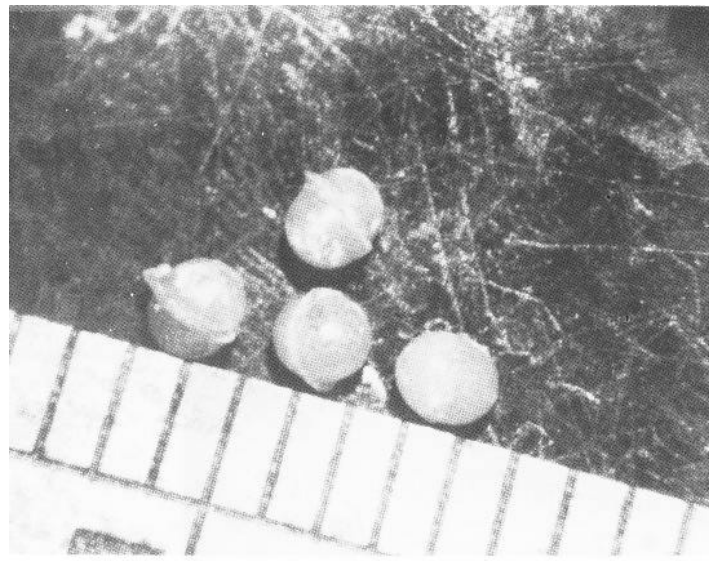
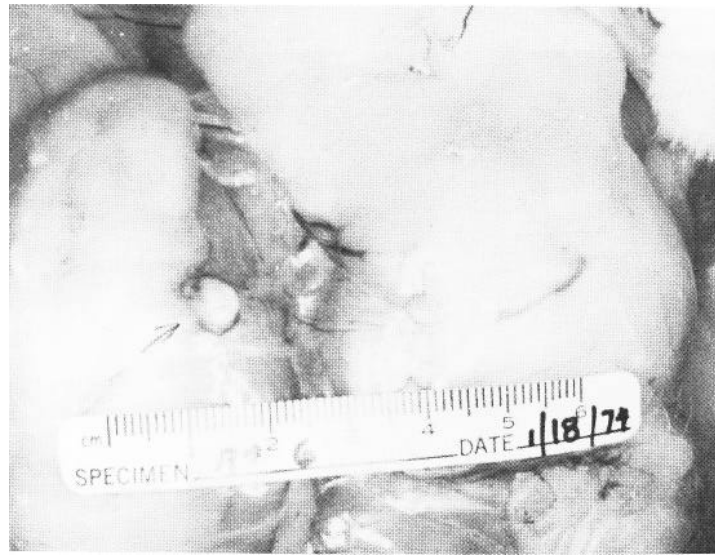
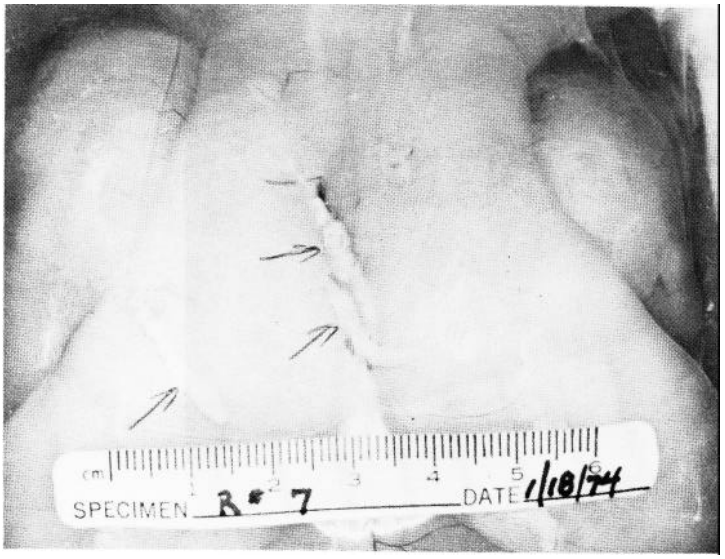


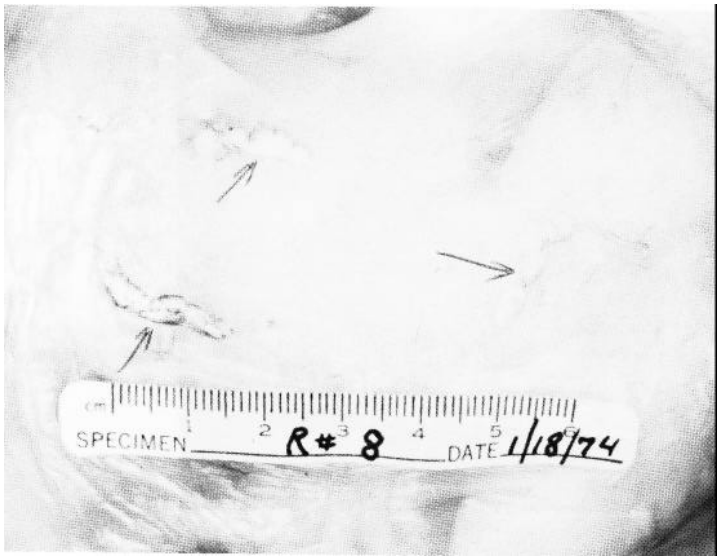
Molded beads of 25/75 lactic/glycolic copolymer 50wt% naltrexone pamoate mixture. (mm) Dynatech R/D. (See first article).



Subcutaneous scapular implant site of Dl-6 five weeks after implantation in rabbit. Note the nodular mass (arrow).



Subcutaneous scapular implant site of D2-7 five weeks after implantation.
Note rod-shaped residues (arrow).



Subcutaneous scapular implant site of D2-8 five weeks after implantation.
Note rod-shaped residues (arrows).

CONTENTS

INTRODUCTION

The use of narcotic antagonists in the treatment of opiate addiction is based on the concept of a pharmaceutical agent capable of blocking the reinforcing properties of a dose of opiate taken during an addicts rehabilitation. The rationale for use is that the antagonist blocks the opiate "high" and makes it pleasureless, thus removing the addict's incentive for continued use. Earlier successful therapy with cyclazocine and naloxone prompted the full-scale development of new and superior antagonists. Presently naltrexone is the drug under the most intensive clinical evaluation and appears to be a promising antagonist candidate.

It was felt from the outset that a most desirable component of antagonist therapy would be a long-acting drug, so that the need for an addict to decide to take his medication would be minimized. Naltrexone in oral doses of 70 mg. will provide adequate blocking protection for at least 48 hours, or perhaps 72 hours in certain individuals. This is not felt to be a long enough interval between dosages to aid the addict in becoming dissociated from his drug-taking behavior.

It was recognized very early that in order to achieve the desired one week, one month or longer duration between dosages, it would be necessary to develop a long-acting drug delivery system or a sustained-release preparation of an acceptable but short-acting antagonist. A "drug-delivery system" is the unwieldy but currently favored expression describing any pharmaceutical preparation capable of providing a sustained or long-acting antagonistic effect. This effect may be achieved mechanically (e.g., by implanted discs with timed release capacity) or chemically (e.g.,

microcapsules, tubes, solid balls, gelatinous masses injected intramuscularly). Distinct from the problem not considered here, of finding an optimum antagonist, is the problem of inventing suitable carriers for the antagonist, carriers that will deliver the antagonist, releasing it uniformly bit by bit over a period of time. Hence the barbarism, a "drug delivery system."

Efforts to achieve satisfactory drug delivery systems were launched in the early 1970's by the City of New York Public Health Department and by the Division of Narcotic Addiction and Drug Abuse, now the National Institute on Drug Abuse (NIDA).

During this early period, the pioneering efforts of Dr. Seymour Yolles, University of Delaware, demonstrated for the first time that a sustained-release of an antagonist could be obtained from a biodegradable polymer, i.e., polylactic acid. This success generated expanded and intensified efforts, a summary of which is the topic of this monograph.

At the present time, the program supported by NIDA includes six contracts that are concerned with the development of new delivery systems and three contracts that have the responsibility of evaluating them for potential clinical trials.

Delivery Systems Development

Borrowing on its experience in the antifertility area supported by the Center for Population Research, at the National Institute for Child Health and Human Development, Dynatech Corporation has had the major goal of developing biodegradable devices from polyactic and polyglycolic acids. This effort is now centered on refining copolymer preparations with naltrexone that are implantable and have either a one or six month duration.

Another project receiving early support has been that of Dr. curt Thies, Washington University, an effort to develop microencapsulated dosage forms. Working primarily with the polylactic and glycolic acids, Dr. Thies achieved early success of multiple-week release with the antagonist cyclazocine. As naltrexone became the drug of choice, it was substituted for cyclazocine. The highly experimental nature of these systems was revealed when it was quickly found that the physicochemical properties of naltrexone posed new problems. Among these was increased water solubility. Notable progress has now been made in improving capsule quality to achieve longer release times.

One of the approaches supported originally by New York City that has yielded very promising sustained-release candidates has been directed by Dr. Alan Gray, Illinois Institute of Technology Research. He has found that zinc and aluminum tannate complexes of naltrexone, injected in 2% aluminum monostearate in sesame oil gives sustained release from three to four weeks. Advanced studies are now underway to evaluate its safety in order to initiate clinical trials.

Another early project that is now approaching final evaluation for clinical trials is based on implantable pellets of naltrexone in polyglycerides. This work has been conducted by Drs. Maurice Sullivan and Donald Kalkwarf, of Battelle Pacific Northwest Laboratories. The natural polyglycerides used have shown excellent biocompatibility, that is, negligible inflammation on insertion and little tissue reaction overall.

A newer contract with Arthur D. Little Inc. is concentrating on the develooment of new biodegradable polymers based on polypeptides. Tubes of polyleucine-polyglutamate filled with naltrexone appear to offer a promising new lead.

One of the more unique approaches is that being pursued by Alza Research. In contrast to the other polymer based release systems, which diffuse drugs, the Alza chronomer erodes at a rate equal to drug release. Interesting induction periods and solubility effects are currently being studied to perfect a desirable release rate.

Evaluation

The evaluation of leading candidate devices developed by these contractors is initiated by a group headed by Dr. Richard Reuning at Ohio State University. The first series of tests consists of following the blocking of morphine-analgesia in mice by the test device to confirm any original test results. Next, the release of radiolabeled drug from the device is followed in rats and finally in monkeys. In order to carry out this type of evaluation, this group has conducted extensive pharmacokinetic studies on naltrexone. This information permits a determination of the actual rate of release of drug from the device under testing.

Candidate devices that pass the first two tests at Ohio State are then submitted to Industrial Bio-Test, Inc., for evaluation of their tissue compatability, and to Parke, Davis and Company for a rigorous final pharmacological evaluation. For the toxicity studies, mice, rats, rabbits and monkeys receive various loadings of the test device and tissue reactions are checked, usually at 7 and 28 days. The pharmacological studies are carried out in a group of monkeys trained to lever-press for injections of morphine. A successful device will block this behavior as long as drug is being released. Simultaneously, blood specimens are collected for the Ohio State group to analyze for drug levels.

As a result of these efforts to date, two or possibly three candidates are being readied for clinical trials. The final preclinical work necessary includes sterility checks, more detailed toxicity studies, and quality control standards. It is our hope to be able to evaluate at least one of these devices in man within a year.

Robert E. Willette, Ph.D.
Division of Research
National Institute on Drug Abuse

September, 1975

ABSTRACT

RESEARCH MONOGRAPH #4, NATIONAL INSTITUTE ON DRUG ABUSE. Narcotic Antagonists: The Search for Long-Acting Preparations.

DEVELOPMENT OF POLYLACTIC/GLYCOLIC ACID DELIVERY SYSTEMS FOR USE IN TREATMENT OF NARCOTIC ADDICTION

Schwope, A.D., D.L. Wise, J.F. Howes

Implantable polylactic/glycolic acid matrix systems have successfully provided the sustained release of naltrexone to mice for periods of up to 200 days. In vitro and in vivo release rates have been determined by measuring chemical concentrations in pH 7 buffer solution and urine, respectively, and in vivo efficacy has been-measured by direct challenge with morphine (Dewey-Harris mouse tail-flick test). Dosage forms of small implantable cylinders, 1/16" diameter, (25 mg/rod, one rod/mouse) containing 33% by weight naltrexone pamoate in 90 L(+)/10 polylactic/glycolic acid have sustained the delivery of chemical for 200 days. Delivery of chemical from dosage forms of 1/16" diameter spherical beads (3 mg/bead, 3 beads/mouse) containing 33% by weight naltrexone base in 90 L(+)/10 polylactic/glycolic acid was sustained for 60 days. Earlier a similar bead type dosage form of 75 L(+)/25 polylactic/glycolic acid containing 50% by weight naltrexone base and coated with the pure polymer provided controlled release for 25 days.
which incorporate the use of pharmacologically suitable catalysts and yield products reproducibly have been delineated. Techniques for sterilization of the final implant have been screened.

ABSTRACT

RESEARCH MONOGRAPH #4, NATIONAL INSTITUTE ON DRUG ABUSE. Narcotic Antagonists: The Search for Long-Acting Preparations.

DEVELOPMENT OF INJECTABLE MICROCAPSULES FOR USE IN THE TREATMENT OF NARCOTIC ADDICTION

Thies, C.

Injectible microcapsules containing narcotic antagonists have been prepared with dl-poly (lactic acid) as the coating material. The encapsulation technology has developed to the point that high yields of less than 180 μ capsules can be prepared routinely. Such capsules with an initial payload of 50 wt. % naltrexone pwnoate provide 60-90% atagonism to the action of morphine 28 days after injection into mice as a peanut oil/aluminum monostearate suspension at a dose level of 40 miligrams naltrexone pamoate/ kg. mouse.

ABSTRACT

RESEARCH MONOGRAPH #4, NATIONAL INSTITUTE ON DRUG ABUSE. Narcotic Antagonists: The Search for Long-Acting Preparations.

LONG-ACTING NARCOTIC ANTAWNIST COMPLEXES

Gray, A.P., W. J. Guardina

We evaluated the ability of close to 100 organic acids to form water-soluble salts with methadone, cyclazocine, naloxone, naltrexone and, more recently, diprenorphine. About half the acids yielded insoluble salts. Polybasic acids affording insoluble salts were evaluated for their ability to form drug:acid:metal complexes with the polyvalent metal ions, Zn^{++}, Al^{+++}, Mg^{++} and Ca^{++}. Optimum conditions for forming complexes have been developed and the consistency of their composition has been established.

Salts were analyzed spectrophotometrically for drug content, and complexes were analyzed for drug and metal content. The in vitro degree of dissociation at equilibrium measured for the preparations suspended in a simulated physiological buffer, pH 7.3.

Preparations of the narcotic antagonist drugs showing relatively low degrees of dissociation in vitro, since it-early appeared that a high degree of dissociation contraindicated a prolonged duration of pharmacologica1 action, were evaluated in mice after intramuscular administration at several dose 1evels by the mouse tail-flick test for the potency and duration of their morphine antagonist activity.

Our most promising preparations to date, showing the most prolonged durations of action without evidence of gross toxicity, are naltrexone zinc tannate and naltrexone aluminum tannate. These are undergoing detailed evaluation as potential clinical candidates. Thus far, the most useful of several dosage forms studied is a suspension in an aluminum monostearate gel.

The abstracts are provided as summary and may be extracted for reference *and filing convenience.*

ABSTRACT

RESEARCH MONOGRAPH #4, NATIONAL INSTITUTE ON DRUG ABUSE. Narcotic Antagonist's: The Search for Long-Acting Preparations.

SUSTAINED RELEASE OF NALTREXONE FROM GLYCERIDE IMPLANTS

Sullivan, M. F., D. R. Kalkwarf

Solid dispersions of naltrexone in natural glycerides were used to form injectable implants which continuously release narcotic antagonists in vivo. The dispersions were formed and tested either as small cylindrical pellets, e.g. lx3.0 mm in size, or as particles with diameters in size ranges between 125-250 μ, that are suspended in an aqueous methyl cellulose solution. Both types of implants delivered naltrexone to mice at rates that were effective in blocking the antiociceptive action of morphine for at least one month. The rate of naltrexone release was controlled by altering its concentration in the dispersion and by varying the glyceride composition. Degradation and absorption of the implants were found to depend on their composition, dimensions and location in the body. No appreciable tissue incompatibility was seen in mice, rats, rabbits, monkeys and swine, even when long-lasting preparations were removed a year after treatment.

ABSTRACT

RESEARCH MONOGRAPH #4, NATIONAL INSTITUTE ON DRUG ABUSE. Narcotic Antagonists: The Search for Long-Acting Preparations.

USE OF SYNTHETIC POLYPEPTIDES IN THE PREPARATION OF BIODEGRADABLE DELIVERY VEHICLES FOR NARCOTIC ANTAGONISTS

Sidman, K.R., D.L. Arnold, W.D. Steber, L. Nelsen, F.E. Granchelli, P. Strong, S.G. Sheth

Synthetic polypeptides consisting of copolymers of glutamic acid and leucine have been shown to be useful materials for the fabrication of practical, biodegradable delivery vehicles for narcotic antagonists.

Model delivery vehicles in film form were prepared from copolymers containing 10 mole percent to 40 mole percent glutamic acid, and loaded with 10% to 40% naltrexone by weight. The naltrexone was found to be released by diffusion, exhibiting diffusion coefficients that varied as a function of the glutamic acid content and the initial naltrexone loading. A wide range in diffusion coefficients were achieved (0.31×10^{-7} cm^2/hr to 120×10^{-7} cm^2/hr), leading to release rates within practical ranges of interest for meeting the program goals.

We have demonstrated that the polypeptides can be fabricated into dosage forms that are amenable to administration by trochar. For example, rods 0.4 mm to 0.8 mm in diameter containing as much as 40% naltrexone by weight were extruded using a simple compression mold and die arrangement. An in vitro evaluation of the rods showed that antagonist is released by diffusion at a continuously decreasing rate, a behavior similar to that observed with the film devices that were, nonetheless, capable of blocking an AD_{80} challenge of morphine sulfate in mice for more than 30 days.

One of the most promising delivery vehicles that we have developed to date consists of a polypeptide tube filled with a naltrexone/polypeptide core. Preliminary experiments have shown that these devices may be capable of administering high, constant rates of release for prolonged periods of time. Additional work, however, is required to develop techniques for the preparation of reproducible delivery vehicles.

ABSTRACT

RESEARCH MONOGRAPH #4, NATIONAL INSTITUTE ON DRUG ABUSE. Narcotic Antagonists: The Search for Long-Acting Preparations.

DEVELOPMENT OF CHRONOMERS™ *FOR NARCOTIC ANTAGONISTS*

Capozza, R.C., E.E. Schmitt, L.R. Sendelbeck

The object of this program is to prepare a bioerodable naltrexone deLivery system which can be implanted subcutaneously in humans and which can relieve the narcotic antagonist over 1-6 months at relatively constant and sufficient rates to block the euphoric effect of morphine based drugs. The system is composed of naltrexone uniformly dispersed in a solid hydropholic CHRONOMER™ matrix which undergoes predictable surface erosion when exposed to an aqueous medium. Kinetic studies in vitro have been carried out during the course of the program to determine the best composition for the system.

Toxilogical studies conducted at ALZA during the past 2 years have not revealed limiting adverse effects of either the CHRONOMER™ materials or their hydrolysis products. The tail-flick test procedure was used to measure the effectiveness of naltrexone to antagonize the analgesis of morphine in rats. Naltrexone infused intravenously at doses of 4 and 16 ug/kg/hr resulted in, after 6 hours, 54 and 89% antagonism, respectively, against a 63.5% effective dose of morphine.

Preliminary Sterilization studies; showed that no adverse effects to CHRONOMER™/naltrexone systems occurred after exposure to 2.5 or 5.0 mrads of $_{60}$Co irradiation.

ABSTRACT

RESEARCH MONOGRAPH #4, NATIONAL INSTITUTE ON DRUG ABUSE. Narcotic Antagonists: The Search for Long-Acting Preparations.

TESTING OF DRUG DELIVERY SYSTEMS FOR USE IN TREATMENT OF NARCOTIC ADDICTION

Reuning, R.H., L. Malspeis, S. Frank, R.E. Notari

The evaluation of the drug release characteristic of four naltrexone delivery systems has been carried out together with the development of analytical techniques and an investigation of the metabolic profile of naltrexone. Pharmacologic evaluation of the four delivery systems in the mouse indicated significant analgesic antagonism for a period of from 16-22 days. Further evaluation of one of these systems by measurement of the rate of excretion of radio--activity after administration of radiolabelled naltrexone in the delivery system confirmed that significant release occurs for a time period of about 15 days. Electron capture gas-liquid chromatographic assays for naltrexone and naloxone in plasma or urine have been developed that yield linear calibration curves and are sensitive to one ng/ml. Studies on naltrexone disposition indicate that (a) binding to plasma proteins in several species varies from 20-26%, (b) distribution of drug from blood is extremely rapid and extensive, (c) β-naltrexol is a major metabolite of naltrexone in man, monkey and guinea pig among six species studied, whereas α-naltrexol is a minor metabolite in the monkey and guinea pig only, and (d) metabolic reduction of naltrexone occurs in the 100,000 x g supernatant of guinea pig liver. Pharmacokinetic studies of naltrexone in the dog and monkey indicate that the drug is rapidly distributed and eliminated, has a very large apparent volume of distribution and a total body clearance greater than the rate of *liver blood flow.*

DEVELOPMENT OF POLYLACTIC/GIYCOLIC ACID DELIVERY SYSTEMS FOR USE IN TREATMENT OF NARCOTIC ADDICTION

Arthur D. Schwope
Donald L. Wise, Ph. D.
Dynatech R/D Company
John F. Howes, Ph. D.
Sharps Associates

INTRODUCTION

A problem universal to chemotherapeutic programs is the administration and maintenance of drug(s) at safe and effective levels for extended periods. Much technical activity has been devoted to devising improved and different means for regulating drug delivery.

One important category of dosage forms under investigation is implantable, tissue absorbable preparations which release chemical (e.g., medicaments, fertilizers, and pesticides) at a constant rate to biological systems. There are two fundamentally different approaches to achieve that end. One relies on chemical alteration of the agent so that adsorption by the system is delayed. In the second method, the active agent remains chemically unchanged, but its availability to the body is restricted by physical means. This is achieved by injecting the agent in a carrier solution (e.g., oil and saline) and by encapsulating or surrounding the chemical with an absorbable, release-regulating material.

In this study, polymers of lactide and glycolide were evaluated as the release-regulating materials. Polylacticl glycolic acids are tissue compatible and hydrolyze to the metabolites, lactic and glycolic acids. The rate of hydrolysis is controllable and dependent on the, lactic/glycolic ratio. Physical mixtures, matrices, of narcotic antagonist - naltrexone - and polymer were prepared, formed and evaluated in vitro and in vivo as long term, constant delivery, dosage systems.

This paper describes the procedures which have been used to prepare and

evaluate drug/polymer matrix systems which will provide the sustained release of naltrexone for one month and and for six months. Results of the evaluations of selected systems are presented.

METHODS AND MATERIALS

Sample preparation

L(+)-lactide and glycolide, synthesized from the respective acids, were polymerized into polylactic acid and polylactic/glycolic acid copolymers using triethylaluminum and suitable catalysts. Polymers ranging from 75 wt % L(+)lactide/25% G to 100% L(+) have been prepared. Molecular weights of the polymers determined by gel permeation chromatography, membrane osmometry and light scattering ranged from 40,000 - 200,000. Intrinsic viscosities measured at 37°C in tetrahydrofuran ranged from 0.4 to 1.0.

Naltrexones base and pamoate (supplied by NIDA) were used. The base was tritium labeled at the 15 and 16 positions.

Chemical and polymer were blended into an intimate, uniform mixture using the solvents tetrahydrofuran and methylene chloride. The solution was cast and the solvent removed by evacuation (<1 mm Hg) at 50°C for at least 24 hours. After drying, the chemical/polymer matrix was formed into implantable shapes. Beads 1/16" diameter were prepared by transfer molding and 1/16" diameter rods by extrusion. A thin, polymer coating was added to selected samples by dipping the beads or rods into a 10 wt % polymer solution. Upon removal from the dip-coat bath, the sample was dried under vacuum at 50°C for 48 hours. The resultant coating was estimated to be 0.001 to 0.003 of an inch thick.

In vitro analysis

The in vitro release rate of chemical into 50 ml of 37°C, pH 7 phosphate buffer was determined by measuring the concentration of naltrexone in the buffer daily. The sample was suspended in the solution inside a Whatman extraction thimble. The 50 ml buffer solution was changed weekly in order to maintain the agent concentration below 20% of saturation. The naltrexone base concentration was determined by means of a Beckman LS100 liquid scintillator. Riafluor® (New England Nuclear) was used as the cocktail. Naltrexone pamoate concentration was measured with a Hilger-Watts spectrophotometer.

In vivo analysis

Male albino Charles River mice (18-22 g) were used throughout the study. The mice were anesthetized with Penthrane (Abbott) and a small slit was made in the scapular region. A single rod or three beads were inserted below the slit and the wound was closed by a silk suture. The animals were allowed to recover. Groups of ten animals were then placed in cages and allowed to develop normally until the test date. Ten individual mice were placed in separate metabolism cages for collection of urine.

The urine was collected daily for the duration of the experiment. The cages were rinsed with distilled water and the washings were added to the urine. The combine and washings were urine made up to 10 ml with water. A 1.0 ml sample was taken and added to 10.0 ml of Aquasol liquid scintillation cocktail (New England Nuclear) in a vial. The radioactivity was measured using a Beckman LS-230 liquid scintillation counter. A quench curve was constructed using a series of ^{3}H quench standards and this curve was used to correct all counts.

The mouse tail flick procedure was used throughout this study. A ten second cut-off time was employed, and a control reaction time of two to four seconds was used. A rheostat, incorporated into the instrument was used to adjust the intensity of the light falling on the tail of the mouse such that each reaction time fell within the stated range. Animals with a control reaction time outside the stated range were rejected. The rheostat adjustment was only made if a significant proportion (more than two out of every ten mice) of the reaction times were outside the range of two to four sec. Groups of ten mice were medicated with an ED_{80} dose (14.0 mg/kg ip) of morphine sulphate intraperitoneally and re-exposed to the noxious stimulus twenty minutes later. The analgesic response was calculated as the percentage of the maximum possible response time (Harris and Pierson, 1964). The following formula was used for these calculations: (test-control/

10-control) x 100 = % maximum possible effect.

Each group of animals was tested by the narcotic antagonist procedure only once. Groups were planned so that testing was performed every 7 or 10 days.

At the termination of each experiment, the animals were sacrificed and the implant site was examined. The condition of the implanted material was noted and signs of encapsulation, injection or irritation.

RESULTS AND DISCUSSION

The objectives of this ongoing study are to develop implantable, biodegradable naltrexone - polylacticlqlycolic acid matrix systems which will sustain the delivery of chemical to a biological system for both one and six months. Primary to achieving these objectives are the development of reproducible polymerization procedures using pharmacologicallv suitable catalysts. the fabrication of matrices from-polymers which hydrolyze within specified times, and the selection of a practical sterilization process. Also, as the study proceeded, it was expected that a relationship between the in vitro and in vivo performances of the implants would be developed.

Experiments were performed to ascertain the effects on chemical release rate and duration of the

- degree of chemical loading,
- solubility of the chemical in both the surrounding environment and the polymer,
- surface area/weight ratio and porosity of the matrix, and
- hydrolysis rate of the polymer (i.e., composition of the polymer).

Quantitative evaluations of most of these parameters have been completed.

The degree of chemical loading and the solubilities have been identified as most influential on the performance of the implant. The duration of chemical delivery is inversely proportional to the drug loading level and the solubility of the chemical in the surrounding environment. Thus, matrices designed to meet the six-month goal are prepared with naltrexone pamoate which is less soluble in aqueous solutions than naltrexone base. The duration is directly proportional to the solubility of the drug in the polymer. Chemical/polymer solubility effects are especially important in this study - the base form of naltrexone forms a solid solution with polylactic/glycolic acids. This behavior permits the use of the very water soluble naltrexone base for long duration implants.

In other experiments it has been determined that dip-coating the implants with a thin layer of polylacticlglycolic acid reduced the initial surge of chemical from the surface of the implant and extended the duration of delivery. The releases of chemical from rods, spheres, and micron-size particle matrices have been measured. As expected the rate is directly proportional to the surface area/unit weight of implant.

Based on the above findings, matrices have been fabricated specifically to meet the forementioned goals. These systems have been tested in vitro and in vivo. One matrix system, dip-coated rods of 33% by weight naltrexone pamoate in 90 L(+)/10 polylactic/glycolic acid, has proved antagonistic to morphine challenge for 180 days in mice (8 mg naltrexone pamoate/mouse). Using the one-to-one in vitro-to-in vivo relationships which has been-observed throughout the study, it is anticipated that this system will continue to release chemical at an antagonistic level for 20 more days. Inspection of the implant site after 180 days found no signs of foreign body reaction (i.e., no inflamation or walling-off of the implant) and a rod which crumbled when probed'with forceps. It is concluded that the formulation of these rods is ideal for a 6-month naltrexone delivery system.

With another system, uncoated beads of 33% by weight ^{3}H-naltrexone base in 90 L(+)/10 polylactic/glycolic acid, chemical releases were measured both in vivo (urine of mouse) and in vitro and significant levels of antagonism to morphine challenge was realize; for 55-60 days. Plots of the cumulative results are presented in Figure 1. The daily levels of ^{3}H in the urine reported as percentage of total ^{3}H implanted are presented graphically in Figure 2. In this test, the release rate approximated zero-order throughout a major portion of the delivery period; the one-to-one in vitro-to-in vivo relationship was clearly demonstrated: and similar to other tests in this study, roughly 50 percent of the implanted ^{3}H

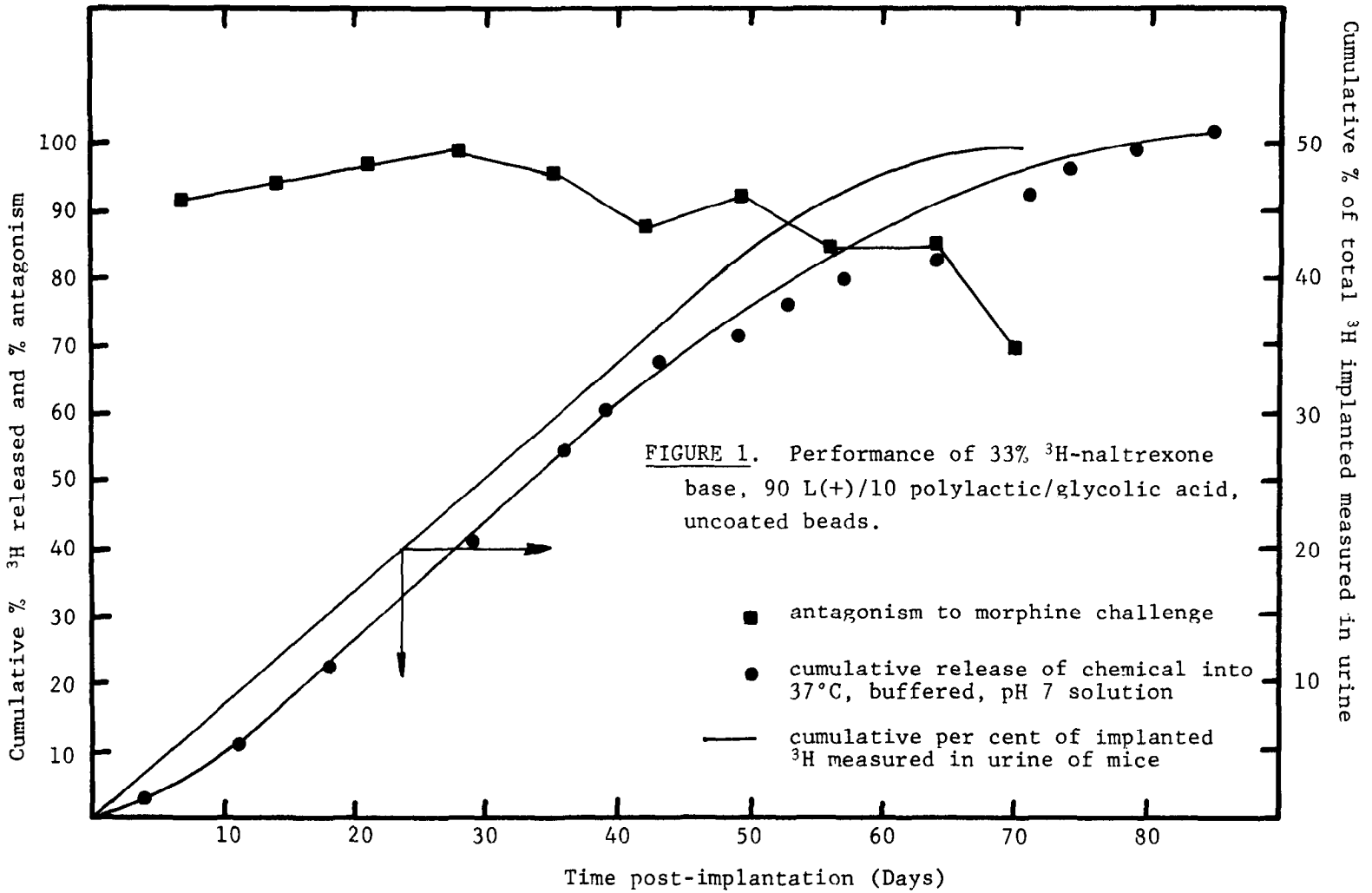


FIGURE 1. Performance of 33% ^{3}H-naltrexone base, 90 L(+)/10 polylactic/glycolic acid, uncoated beads.

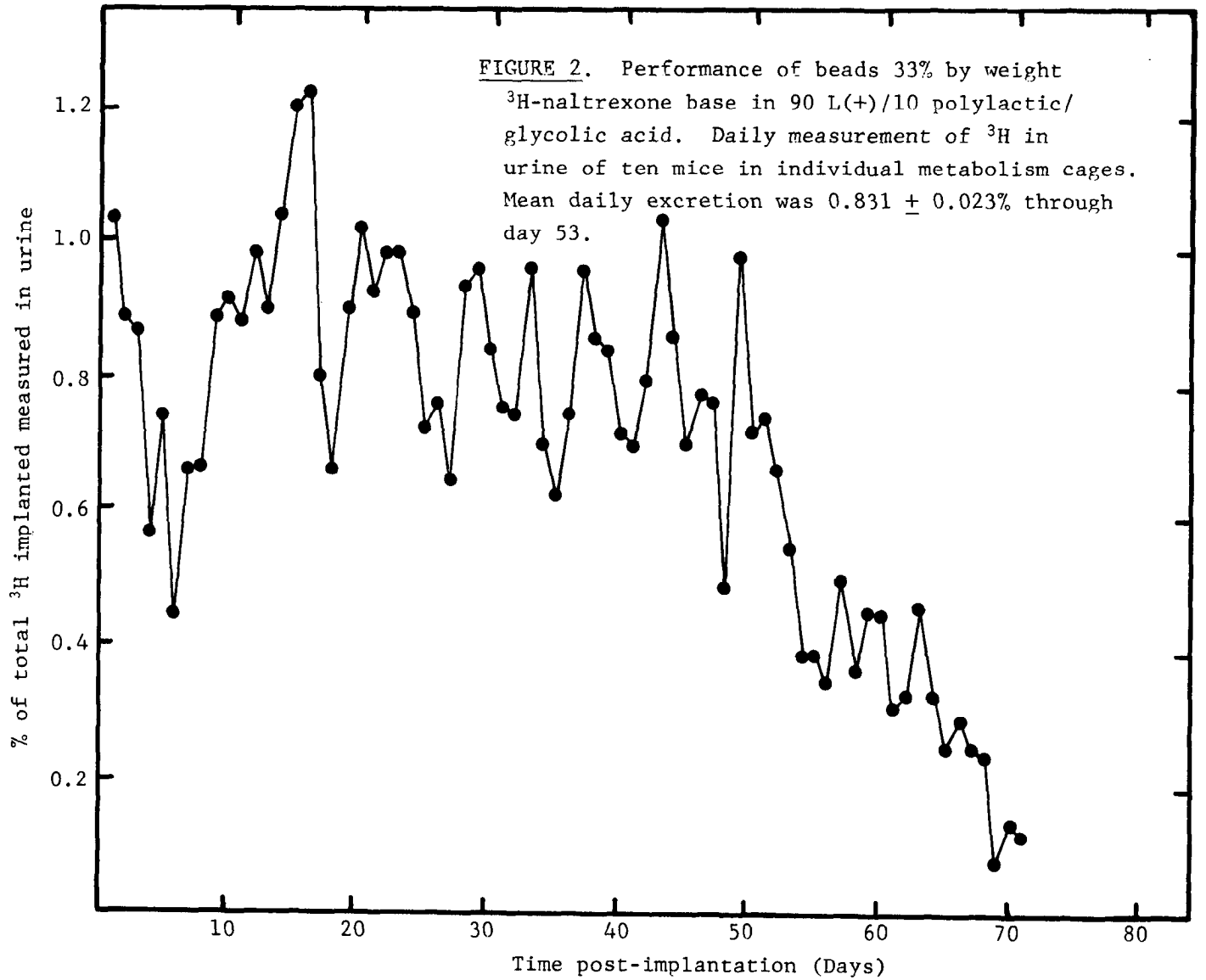


FIGURE 2. Performance of beads 33% by weight ^{3}H-naltrexone base in 90 L(+)/10 polylactic/glycolic acid. Daily measurement of ^{3}H in urine of ten mice in individual metabolism cages. Mean daily excretion was 0.831 $\pm$ 0.023% through day 53.

was found in the urine. Since the release duration is inversely proportional to loading, it is hypothesized that an uncoated bead matrix loaded to 50% by weight with naltrexone base will meet the 30-day goal.

The above two systems exemplify typical sample evaluation procedures and results. The samples were prepared with polymers synthesized from lactic and glycolic acids. Extensive study of the polymerization has identified reactant purity and ambient atmosphere as the most influential factors affecting the molecular weight of the polymer. Reactants with sharp melting points and an oxygen and moisture-free atmosphere (e.g., <1 mm Hg vacuum) are required. Using infrared analysis, the compositions of the copolymers were determined to be similar to the reactant ratio charged to the reaction vessel. This point is important to note since some catalysts react preferentially with one reactant over another. It has also been concluded that the product is a random, straight-chained polymer.

In anticipation of more intensive animal tests, and ultimately, clinical trials, sterilization procedures have been screened to select a method applicable to the polylactic/glycolic acid matrix drug delivery system. Normal methods such as autoclaving and high temperature, dry heat cannot be used since the temperatures are above the melting point of the polymer. And because the implant is porous, ethylene oxide should not be used because complete degassing cannot be ensured. Therefore, radiation sterilization was selected for evaluation. Several types of irradiation (e.g., high energy electrons and gamma ray) are under evaluation.

SUMMARY

The utility of the implantable polylactic/glycolic acid matrix system as a means of providing the sustained delivery of naltrexone for periods up to 200 days has been demonstrated. Concurrent with drug delivery, the polymer hydrolyzes to non-toxic metabolites which are naturally eliminated. No foreign body reaction to the implants has been observed in mice. A one-to-one correlation between in vitro and in vivo performance (i.e., release of chemical) has been established.

Pharmacologically suitable catalysts have been used to reproducibly prepare polymers under controlled reaction conditions. Polymers have been characterized using standard techniques and a relationship between intrinsic viscosity and weight average molecular weight established. Irradiation has been selected as the most practical method of sterilization for polylacticl glycolic acid implants.

DEVELOPMENT OF INJECTABLE MICROCAPSULES FOR USE IN THE TREATMENT OF NARCOTIC ADDICTION

Curt Thios, Ph.D.

Department of Chemical Engineering,
Washington University,
St. Louis, Missouri 63130

Long-acting injectable narcotic antagonist formulations represent a potentially useful means of treating heroin addicts. Conceptually, the necessary slow release drug formulations can be prepared by microencapsulating the antagonist and then simply injecting the capsules. The capsule wall should cause the capsules to release their payload slowly, thereby providing prolonged antagonistic action. This project is concerned with reducing the above concept to practice. The overall goal is to synthesize injectable microcapsules that will last one to six months in vivo.

The concept of an injectable microcapsule is relatively simple, but the capsules used must meet a number of stringent requirements. Naturally, a prime requirement is that they release their drug payload slowly and perform for four weeks or more in vivo. This requires deposition of relatively defect-free capsule walls around individual drug particles. The coating used to form the microcapsule wall must be biocompatible and bioabsorbable. The rate of bioabsorption preferably must be such that the capsule wall will be completely absorbed within six to twelve months after injection.

The capsules must be small enough to freely pass through a 21 gauge needle. This requires capsules smaller than 150 to 200μ. The capsules preferably will be injected as a saline dispersion and the maximum volume of capsule dispersion administered as a single injection should be one to two cc.

In an effort to prepare capsules that meet the above requirements, several new encapsulation procedures have been developed. D1-poly(lactic acid) (d1-PLA) and lactide/glycolide copolymers are used to form the capsules because they are established biocompatible and bioabsorbable polymers. Narcotic antagonists encapsulated successfully include cyclazocine free base, naltrexone free base and naltrexone pamoate. Active contents of most capsules isolated range from 50 to 75 wt. percent. At least 40 to 50 percent of the capsules isolated from a given capsule batch are less than 300μ diameter; in several cases, up to 85% of the capsules isolated from a given batch are below 180μ diameter. Although capsules with lactide/glycolide coating have been made, thus far capsules which release their antagonist payload the slowest have a d1-PLA wall.

Capsule quality in vitro is established by extracting the capsules in a rotating bottle extractor (37°C; pH 7.4 phosphate buffer) and spectroscopically assaying the extracts for the amount of antagonist released. Release curves obtained in this manner are not zero order. The capsules consistently show

a relatively high initial rate of antagonist release which declines steadily as extraction continues. This behavior is attributed to a distribution in quality of the capsules being evaluated. Scanning electron micrographs of capsule surfaces reveal that the walls of some capsules contain numerous macroscopic defects like craters and pit holes while other capsules are relatively free of such defects. It is hypothesized that the capsules with numerous defects release their payload rapidly upon immersion in the extraction medium thereby yielding the observed burst of antagonist release. Once the defective capsules are empty, antagonist release continues as a steadily declining rate from capsules with progressively fewer coating defects.

Support for the above hypothesis is provided by a recent advance in encapsulation technology. This advance enables one to isolate high yields of <180μ spherical capsules. When viewed under a scanning electron microscope, the walls of such capsules appear to have substantially fewer defects than previous capsules. The latest capsules release naltrexone free base over more than a two-week period in vitro and appear to be the best capsules made to date. In vivo evaluations are now in progress, so their in vivo lifetimes are unknown. Nevertheless, it is relevant to note that the best previous naltrexone pamoate capsules had an in vitro lifetime of about two weeks. They provided 60 to 90% antagonism in vivo for 28 days after injection when injected into mice through an 18 gauge needle as a peanut oil/aluminum monostearate suspension at a 40 mg antagonist/kg mouse dose level. Percent antagonism was established by the mouse tail-flick test procedure with 10 mg morphine/kg mouse as the challenge dose.

Significantly, the above in vivo experiment was not terminated because the capsules ceased to function. Termination occurred because only four groups of test animals were innoculated for the experiment and data points were obtained 7, 14, 21 and 28 days after injection. The capsules provided 80% antagonism on day 28 after injection, so it is clear that they still were releasing naltrexone free base at that time. How much longer the capsules would have been effective is unknown. Current in vivo experiments are being carried out for longer periods and are designed to answer this question. The <180μ naltrexone pamoate capsules isolated recently are expected to last at least 30 - 35 days and perhaps longer when injected as a peanut oil suspension. The latest capsules also are being injected as a suspension in saline containing 0.1 wt.% methylcellulose. The ultimate goal is to eliminate the methylcellulose entirely and simply use saline.

Naltrexone free base is far more water-soluble than naltrexone pamoate. Thus, it has been more difficult to synthesize naltrexone free base capsules capable of releasing their payload over a prolonged period. The best naltrexone free base capsules made to date provide 75 to 95% antagonism for 14 days after injection. The injection medium was peanut oil/alminum monostearate; the dose administered was 40 mg antagonist/kg mouse. Recent encapsulation work has focused exclusively on naltrexone pamoate, so it is not known yet if the latest encapsulation technology will yield longer-lasting naltrexone free base capsules.

LONG-ACTING NARCOTIC ANTAGONIST COMPLEXES

Allan P. Gray, Ph.D.
William J. Guordina, Ph.D.
IIT Research Institute

INTRODUCTION

The overall goal of this research is the developmnt of improved drug therapy for the treatment of narcotic dependence.

Narcotic antagonist drugs are of potentiai value for this purpose but suffer fran the drawback of being too short-acting. The current consensus is that durations of the order of atleast l-3months are desirable. Although such durations would not be acceptable for narcotic substitutes such as methadone, renewed interest has recently been expressed in methadone preparations effective for periods of several days.[1]

Our approach to achieving the goal has involved the preparation, in vitro and in vivo study of sparingly soluble salts and ionic-complexes of potentially useful drugs since there are mple precedents in other therapeutic areas showing that intramuscular injection suspensions of such preparations can provide slow release of drug and a useful prolongation of action (Gray, Robinson, 1972; 1974; Gray, 1974). Our earlier work, which has been reviewed (Gray et al., loc. cit.), cocentrated on complexes of narcotic antaganist drugs (cycclazocine, naloxone, naltrexone and, more recently, diprenorphine). We are, however, currently turning some of our attention back to the study of related complexes of the narcotic replacement drug, methadone.

Two of our preparations, naltrexone zinc tannate (Gray, Robinson, 1974) and naltrexone aluminum tannate (Gray, 1974) appear most promising. Administered in an alunium monostearate gel suspension by intramuscular injection to mice, antagonist activity remains at a constant high level and then drops off rapidly at the end of the period (Gray et al. loc. cit.; Reuing[2]). Durations of activity in miceof 3-4 weeks have been observed. Toxicological studies have thus far revealed no contra-indicative effects.[3] The pharmacokinetics of these preparations are being evaluated,[2] as well as their effects in addicted monkeys[4].

REVIEW OF EARLIER WORK

We have evaluated the ability of close to 100 mono- and polybasic organic acids to form water-insoluble salts with methadone and one or the other of the narcotic antagonist drugs, cyclazocine,nalcmone,naltrexoneand,more recently, diprenorphine. About half the acids yielded insoluble salts. Polybasic acids affording insoluble salts were evaluated for their ability to form drug: metal; acid complexes with the polyvalent metal ions, Zn^{++}, Al^{++},$Mg^{++}$and Ca^{++}. Optimum conditions for forming complexes have been developed and the consistencyoftheir composition has been established.

Salts were analyzed spectrophotometrically for drug content, and complexes were analyzed for drug and metal content. The in vitro degree of dissociation at equilibrium was determined for the preparations-suspended in a simulated physiological buffer, pH 7.3, at 37°.

Preparations showing relatively low degrees of dissociation in vitro, since it early appeared that a high of dissociation contraindicated a prolonged duration of pharmacological action, were evaluated in mice after intramucular administration at several dose levels by the mouse tail-flick test for the potency and duration of their morphine antagonist activity.

Cur most promising preparations to date, showing the most prolonged durations of action without evidence of gross toxicity, have been zinc and aluminum tannate comlexes. In particular, the zinc and aluminum tannate complexes of naltrexone are undergoing detailed evaluation as potential clinical candidates. Thus far, the most useful of several injectable dosage forms studied is a suspension in an aluminum monostearate gel in peanut oil. The particle size of these complexes in suspension has been shown to be distributed predominantly in a range below 16μ.

Because of the interest in the naltrexone complexes, zinc and aluminum tannates of radiolabeled (15,16-^{3}H) naltrexone[5] have been prepared and are being evaluated for Maintenance of blood and urine levels and for their pharmocokinetic behavior here and at Ohio State University.[2]

RECENT IN VITRO STUDIES

The work reported here has been carried out in the period January l-April 15, 1975.

Naltrexone Complexes

Additional 15,16-^{3}H-naltrexone aluminum tannate (6) was prepared. 15,16-^{3}H-naltrexone base, specific activity 47.7 mCi/mg,[5] was diluted in methanol solution with cold naltrexone hydrochloride to give 15,16-^{3}H-naltrexone hydrochloride, specific activity 0.63 μCi/mg.

To a stirred aqueous solution of 360 mg (10.6 meq) of tannic acid neutralized with 10.6 meq of 0.5 N NaOH was added 10.6 mq of a freshly prepared 3 N aluminum nitrate solution followed by an aqueous solution of 200 mg (5.3 mmole) of the 15,16-^{3}H-naltrexone hydrochloride. The mixture was stirred for one hour, allowed to stand for 16 hours and filtered. The water-washed precipitate was dried to constant weight in vacuum, first at room temperature and then at 60°. A yield of 3.14 g of complex was obtained having the following analysis:

% Naltrexone 19.0% (spectrophotometric assay)

% Aluminum 2.4% (Eriochrome cyanine R dye method)

% Dissociation 10.1% (at equilibrium in physiological buffer, pH 7.3, at 37°)

Specific activity 0.7 μCi/mg drug base (by scintillation counting).

Naltrexone preparations supplied for outside evaluation during this period are listed in Table 1.

Additional supplies of naltrexone zinc and aluminum tannate complexes have been prepared and analyzed.

Methadone Complexes

Methadone zinc tannate and methadone aluminum tannate were prepared by the same method (method 6) described for naltrexone complexes by use of 2 equivalents of neutralized tannic acid and 2 equivalents of metal salt (zinc sulfate or aluninum nitrate) per mole of methadone hydrochloride.

Table 1

Date	Naltrexone Complex	Lot No	Wt, g	Recipient	Study
3/4-3/18/75	Zinc tannate (6)	C21885 E-8-20	750 mg	D. A. McCarthy Parke-Davis	Addicted monkeys
3/4-3/24/75	Aluminum tannate (6)	C21950 E-10-21	750 mg	D. A. McCarthy Parke-Davis	Addicted monkeys
3/4/75	Zinc tannate (6)	C22089 E-14-13	2.5 g	C. W. Mastri Industrial Biotest	Toxicology
3/4/75	Aluminum tannate (6)	C22040 E-12-10	6.6 g	C. W. Mastri Industrial Biotest	Toxicology
4/16/75	15,16-^{3}H-labeled Aluminum tannate (6)	C22142 E-16-17	1.25 g	R. H. Reuning OSU	Pbarmaco-kinetics

Analytical data are given in Table 2.

Table 2

	Zinc Tannate	Aluminum Tannate
% Methadone	31.9	27.6
% Metal	4.7[a]	2.0
% Dissociation	4.1	4.3

[a]By atomic absorption.

To assist in the in vivo evaluation of methadone complexes,their radiolabeled counterparts have been prepared. o,o'-^{3}H-Methadone base, specific activity 53.9 mCi/mg,[5] was diluted in ethanol solution with cold methadone hydrochloride to give o,o'-^{3}H-methadone hydrochloride, specific activity 1.2 μCi/mg. o,o'-^{3}H-Methadone zinc tannate and aluminum tannate complexes have been prepared fran the tritium-labeled hydrochloride by method 6; analysis is not yet complete.

Thin-Layer Chranatography of Radiolabeled Hydrochloride Salts

The purity and stability of 15,16-^{3}H-naltrexone hydrochloride and o,o'-^{3}H-methadone hydrochloride are being studied by thin-layer chromatography. After experimentation with the cold salts, identifying spots by UV-fluorescence and by various spot reagents, we have elected to work primarily with the following two solvent systems:

a) chloroform:methanol:ammonia (90:10:1)

b) n-butanol:acetic acid:water (60:15:30)

With the tritim-labeled bases and hydrochloride salts, chromatograms were developed on Polygram Sil G sheets. The sheets were cut into l-cm-wide strips and the radioactivity of each strip was determined by scintillation counting. Significant amounts of impurities were noted in the radiolabeled hydrochloride samples. Further investigation is in progress.

Dissociation Under Simulated Sink Conditions

It had previously been noted that when an amount of naltrexone zinc or alluminum tannate complex calculated to contain 15 mg of drug base was magnetically stirred in 10 ml of isotonic phosphate buffer, pH 7.3, at 37°, equilibrium dissociation of the complex was attained in less than 15 minutes. Therefore, in order to simulate sink conditions, the following procedure was adopted.

An amount of complex calculated to contain 15 mg of naltrexone base was stirred in 10 ml of buffer, pH 7.3, for 15 minutes at 37+0.l°. The mixture was centrifuged, an 8-ml aliquot of the supernatant removed and replaced by 8 ml of fresh butter. The process was repeated. The concent ration of naltrexone in each removed aliquot was determined spectrophotometrically in 0.1 N hydrochloric acid in the usual way.

Results for naltrexone zinc tannate are shown in Figure 1. A total of 10.7 mg, approximately 70%, of the naltrexone had been removed by 26 portions of buffer. Although, as can be seen from Figure 1, the amount of drug released with each portion of buffer was not constant, it does follow a smooth curve which appears to be approaching zero release asymptotically.

The amount of drug remaining in the residual complex was determined spectrophotometrically to be 2.3 mg.

For comparison, a parallel experiment is being carried out with naltrexone aluminum tannate.

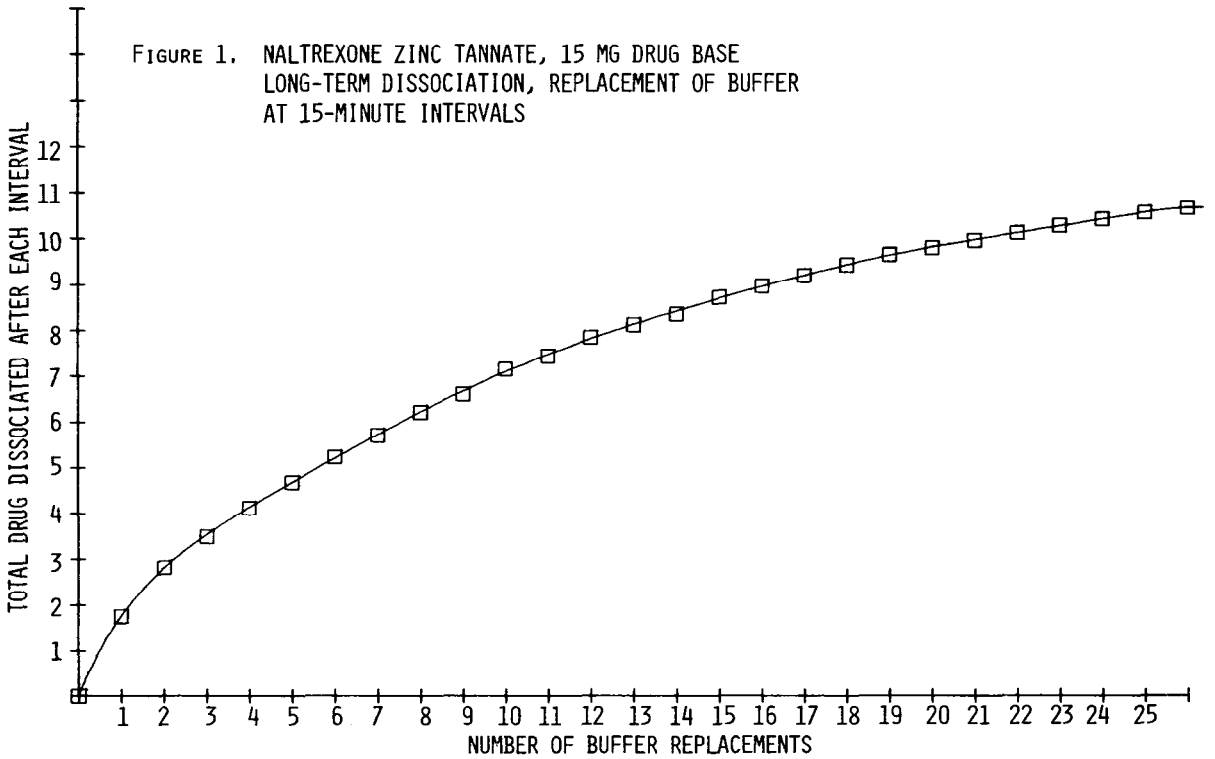


FIGURE 1. NALTREXONE ZINC TANNATE, 15 MG DRUG BASE LONG-TERM DISSOCIATION, REPLACEMENT OF BUFFER AT 15-MINUTE INTERVALS

RECENT IN VIVO STUDIES

Diprenorphine Complexes

ED_{80} Determinations

The ED_{80} dosages for diprenorphine HC1, diprenorphine zinc tannate, and diprenorphine aluminum tannate in peanut oil are 0.03 mg/kg, 0.048 mg/kg, and 0.092 mg/kg, respectively. The antagonistic effect of diprenorphine to the analgesic effect of morphine (4Omg/kg/ip) on the tail flick test was measured 40 minutes after the intramuscular injection of diprenorphine and 30 minutes after the intraperitoneal injection of morphine. The ED_{8O} dosages for the diprenorphine preparations are somewhat greater than those for the long acting preparations of naltrexone possibly because diprenorphine is released at a slower rate fromthe site of injection. The duration of antagonism of analgesia by the ED_{80} dose of diprenorphine hydrochloride is shown in Table 3.

Antagonism of Analgesia

Table 4 shows the duration of the antagonism to morphine of the long acting preparations of diprenorphine (4 mg/kg in peanut oil) compared to that of the hydrohloride.

The diprenorphine complexes exhibited durations of action comparable to those of the corresponding naltrexone complexes at

Table 3

ANTAGONISM OF ANALGESIA BY THE ED_{80} DOSE OF DIPRENORPHINE

Drug (in peanut oil)	%Antagonism Produced by a Dose Containing 0.03 Mg/kg. Drug at: Hours		
	1	3	5
Diprenorphine HCl	82	64	24

Table 4

ANTAGONISM OF ANALGESIA[a]

Drug (in peanut oil)	%Antagdsm Produced by a Dose Containing 4.0 mg/kg. Drug at: Hours						
	2	4	5	24	48	72	96
Diprenorphine HCl	94	95	83	16	-	-	-
Diprenorphine Zinc Tannate	-	-	98	63	14	19	-
Diprenorphine Aluminum Tannate	-	-	97	78	64	42	16

[a]Morphine 40 mg/kg/ip injected 30 min before the tail flick test.

this dose level in peanut oil. Diprenorphine appears to offer no advantage in duration of action and, moreover, exhibits definite agonist properties.

Methadone Complexes

Measurement of the duration of action of long acting preparations of methadone is being attempted by radiolabeling as well as pharmcologicaltechniques. In one series of experiments which will begin shortly, radiolabeled methadone HC1, methadone zinc tannate, or methadone aluminum tannate will be injected intramuscularly in mice. The plasma and brain tissue levels of radioactivity from these injections will then be measured over several days.

In another series of experiments, methadone HCl, methadone zinc tannate, ormethadone aluminum tannate (4O mg/kg methadone base) is substituted for morphine in morphine dependent mice. Mice are addicted to morphine by a series of intraperitoneal injections of morphine given at one hour intervals as described (Iorio et al., 1975). The following five doses of morphine are administered in ascending order: 12.5, 25, 50, 50, and 50 mg/kg/ip. An intramuscular injection methadone preparation is substituted for morphine at the sixth injection. The duration of the addiction in these mice is tested by the presence of jumping precipitated by naloxone (25 mg/kg/ip).

Groups of mice were tested at 1, 24, 48, and 72 hours after the intramuscular injection. Mice receiving the vehicle (peanut oil) or methadone (40 mg/kg) jumped at nearly the same rate following an injection of naloxone (25 mg/kg) at each of the test periods. These studies are being continued.

In another group of experiments, mice were addicted to morphine with injections given twice daily for two weeks; they received morphine (150 mg/kg/ip) on the last two days. These studies showed that methadone (4O mg/kg) zinc tannate in peanut oil vehicle prolonged naloxone (10 mg/kg/ip) induced jumping by two days as compared to vehicle and methadcne HC1 treated mice.

Radiolabeled Naltrexone

^{3}H Naltrexone HC1 (4mg/kg) in the aluminum monostearate vehicle was injected intra-umscularly in mice. Mice were sacrificed by decapitation 1, 2, 4, and 24 hr after the injection. The brains of the mice were removed and immediately digested in NCS tissue solubilizer. The plasma fraction of the blood was separated via centrifugation. The levelofradioactivity in each sample as measured 24 hrs later (see Table 5).

The plasma levels of radioactivity following an intramuscular injection of naltrexone HCl in an aluminum monostearate gel vehicle decreased steadily over the 24 hr sampling period. In contrast, the brain levels of radioactivity did not decrease during the same period of time. These results suggest that the plasma levels of naltrexone do not directly relate to the brain levels of naltrexone. The work is being continued.

Table 5

PLASMA AND BRAIN LEVELS AFTER INTRAMUSCULAR INJECTION IN MICE OF RADIOLABELED NALTREX0NE HC1 IN ALUMINUM MONOSTEARATE GEL VEHICLE

Time (Hours)	Level of Radioactivity in Plasma and Brain as ng/ml and ng/g, Respectively, 0f Naltrexone	
	Plasma	Brain
1	408	86
2	380	77
4	200	79
24	128	a
48	a	a

[a]Experiments in progress.

FOOTNOTES

1. R. E. Willette, personal communication.
2. R. H. Reuning, NIDA Progress Reports, persona1 communications.
3. C. W.Mastri, personal communication.
4. D. A. McCarthy, Parke-Davis.
5. Obtained from J.A. Kepler, Research Triangle Institute.

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SUSTAINED RELEASE OF NALTREXONE FROM GLYCERIDE IMPLANTS

M.F. Sullivan Ph.D.
D. R. Kalkwarf, Ph. D.
Battelle, Pacific Northwest Laboratories

INTRODUCTION

This research is concerned with developing injectable and biodegradable systems that release the narcotic antagonist, naltrexone, in vivo for at least 30 days at pharmacologically effective rates. Such preparations should improve the efficiency of treatment of heroin addicts with naltrexone by compensating for the rapid metabolism of this antagonist. Natural glycerides that are solids at body temperature yet can be melted and molded at relatively low temperatures appear to be appropriate vehicles for delivering the drug at effective and constant rates. These substances provide a reservoir from which the drug can diffuse gradually during treatment and that is itself eventually absorbed by the body with negligible toxic effects. This report describes their utilization in naltrexone-delivery systems.

DEVELOPMENT AND TESTING PROCEDURES

Implant preparation:

Small pellets and particles were prepared containing naltrexone dispersed homogeneously in various glycerides. Crystals of naltrexone (diameter <125μ) were added with vigorous stirring to molten combination of a-monopalmitin, 1,2- and 1,3- dipalmitin and tripalmitin at 80 C. To form pellets, the liquid suspensions were drawn -into cylindrical molds, 1.7-mm in diameter, and cooled rapidly until they solidified. The cylinders were extruded, cut into 3-mm lengths for use in mice and into 10-mm lengths for use in rats, rabbits, monkeys and swine. Particles were prepared in two size ranges, 125-250 μ and < 125 μ by grinding and sieving the solid dispersions, They were then suspended in an aqueous solution containing 1.5% methyl cellulose at a concentration that would allow injection of the desired amount of naltrexone in a 0.25 ml volume.

In Vitro Evaluation

The release rates of naltrexone from the preparations were measured in vitro to determine if the release characteristics justified further testing in vivo. The in vitro evaluation was done by placing a sample in a continuous flow of iso-

tonic saline solution at 37 °C using the apparatus shown in Figure 1. The flow rate was increased until naltrexone was removed from the surface as fast as it diffused there from the interior, The rate of drug release into this "perfect sink" was determined from spectrophotometric measurements on the effluent solution at 281 nm,

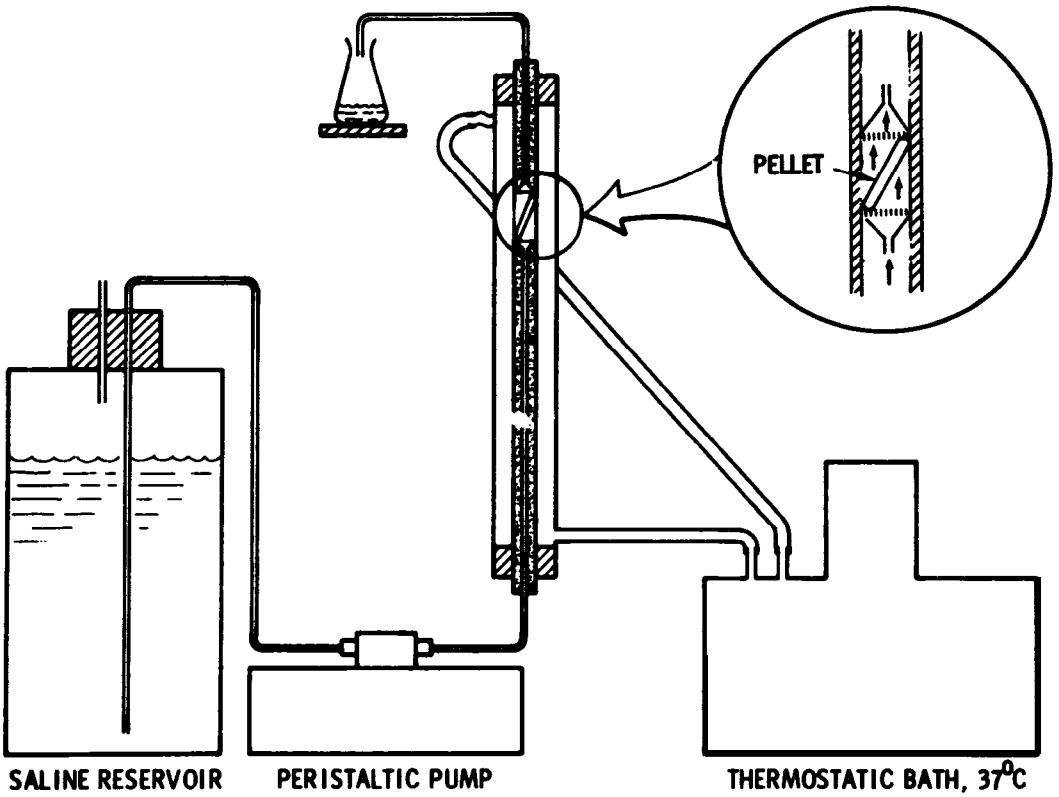

FIGURE 1. CONTINUOUS FLOW SYSTEM FOR DETERMINING DRUG RELEASE RATES IN VITRO

In Vivo Evaluation

The mice used in these studies were of the Swiss-Webster strain weighing between 20-30 gm, Experimental and control animals were maintained in groups of 15 and fed Purina mouse pellets ad libitum. Their weights were determined weekly, The in vivo measurement of the pharmacologic effectiveness of the various preparations was done by either implanting pellets subcutaneously into the nape of the neck with a 12-gauge trocar or by injecting a suspension of naltrexone loaded particles in 1.5% methylcellulose as the suspending agent. Both vehicle-implanted and normal mice were used as morphine-injected controls, A dose of 15 mg/kg of morphine sulphate was injected subcutaneously and the tail-flick response was measured 30 minutes later according to the procedure of D'Amour and Smith, 1941 as modified for mice by Harris et al., 1969, At least six antagonist-implanted mice, two vehicle controls and two normal controls were used for each test, unless otherwise specified,

At the conclusion of the pharmacologic measurement, the mice were anesthetized, the pellets were removed, and the implantation sites were inspected for any gross signs of tissue incompatibility. Residual naltrexone in each pellet was determined spectrophotometrically after dissolving it in chloroform. Residual glyceride was determined by esterifying a sample of the chloroform solution with methyl alcohol in the presence of boron trifluride and determining the amount of ester with a gas chromatograph.

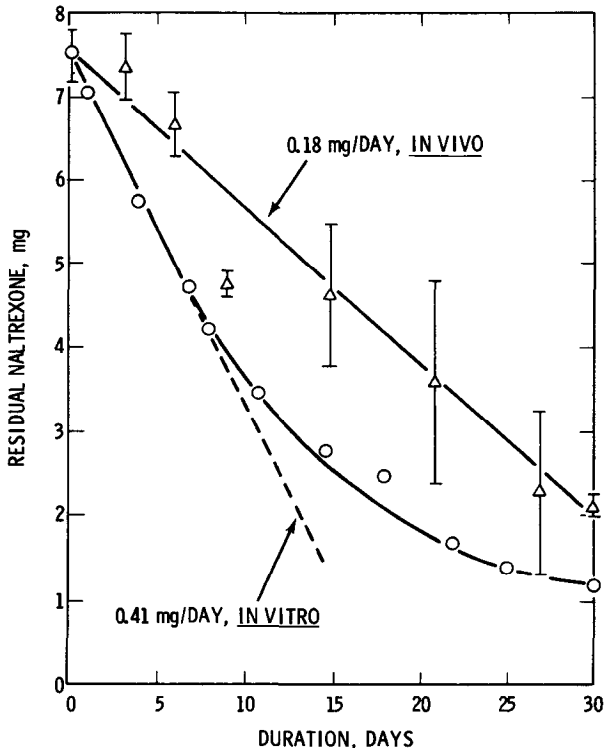

FIGURE 2. RELEASE OF NALTREXONE FROM 1.7 x 10.0 mm PELLETS CONTAINING 30% NALTREXONE IN 25:75 DIPALMITIN: TRIPALMITIN.

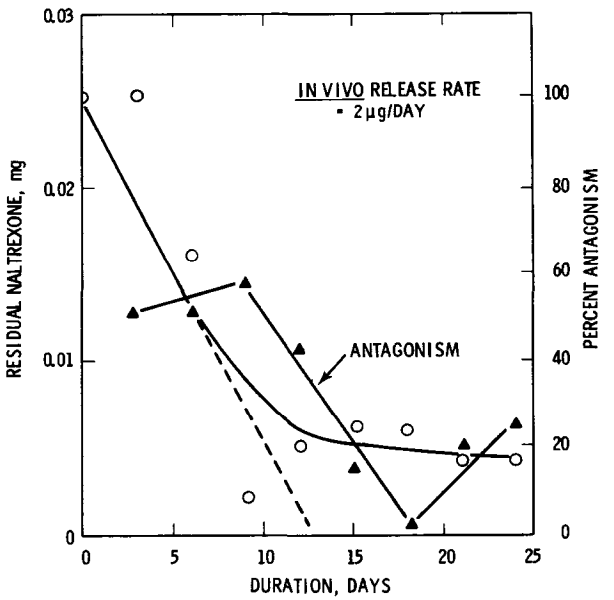

FIGURE 3. RELEASE OF NALTREXONE IN MICE FROM A 1.7 x 3.0 mm PELLET CONTAINING 0.3% FREE BASE IN 25:75 DIPALMITIN: TRIPALMITIN AND COMPARISON WITH THE REACTION TIME OF THE MICE IN THE TAIL-FLICK TEST.

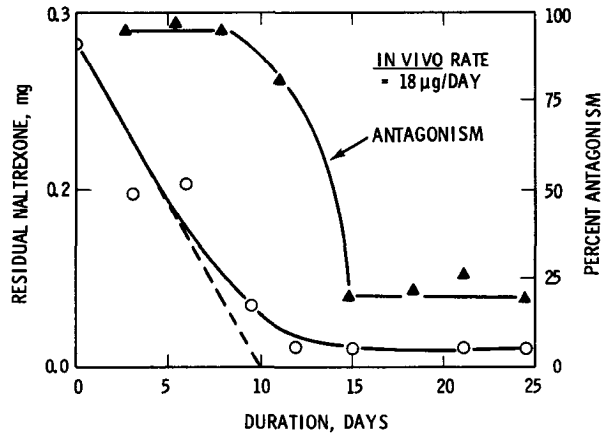

FIGURE 4. RELEASE OF NALTREXONE IN MICE FROM A 1.7 x 3.0 mm PELLET CONTAINING 30% FREE BASE IN 25:75 DIPALMITIN: TRIPALMITIN AND COMPARISON WITH THE REACTION TIME OF THE MICE IN THE TAIL-FLICK TEST.

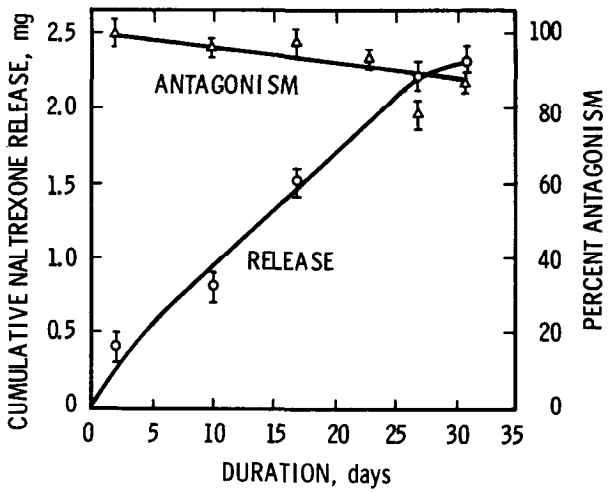

FIGURE 5. RELEASE OF NALTREXONEAND RESULTING ANTAGONISTIC EFFECT FROM A 1.7 x 3.0 mm PELLET CONTAINING 30% NALTREXONE (FREE BASE IN DIPALMITIN AND IMPLANTED S.C. IN MICE

RESULTS

An early preparation that we evaluated was a solid implant, 1.7 x 10 mm, that was loaded with 30 percent naltrexone. The pellet matrix was dipalmitin:tripalmitin in the ratio of 25:75. The release rate into isotonic saline at 37 C under "perfect sink" conditions is shown in Figure 2 along with the in vivo release that occurred from subcutaneous implants that were removed in groups of 3 at intervals after treatment. The release rate of naltrexone in vitro gradually decreased with time as its concentration near the surface diminished; however, the release rate in vivo remained constant, indicating that biodegradation had continually exposed fresh surface, Rats that were implanted with a single pellet were unaffected by morphine for 33 days after treatment as judged by their tail-flick response.

Small pellets (1.7 x 3.0 mm) were implanted in mice to determine the effect of the naltrexone dose on the pharmacologic response to morphine analgesia. The naltrexone-release rate and the duration of action of the implants is partially dependent on the concentration of the antagonist in the glyceride matrix. This can be seen in Figure 3 and 4 where a 0.3% naltrexone loading resulted in the release of 2.0 μg/day. However, both preparations were ineffective for antagonizing morphine beyond 2 weeks after they were implanted.

The duration of naltrexone effectiveness by a single injection is extended when it is administered as naltrexone pamoate. Pellets containing various combinations of dipalmitin and tripalmitin along with naltrexone pamoate were tested for their release characteristics in vitro. Naltrexone pamoate was released from a dipalmitin-tripalmitin matrix more rapidly than when it was present as the free base, This formulation appeared to be unsuitable for providing 30-days of effective antagonism.

Pellets loaded with 30% naltrexone in an equal mixture of 1,2- and 1,3- dipalmitin were tested both in vitro and in vivo and were found to release the antagonist at pharmacologically effective rates for 30 days, This is shown in Figure 5. Three of the ten mice tested at each time interval were implanted with pellets containing ^{3}H-naltrexone. Urine collected daily from these animals during the 2 days prior to the tail-flick test showed that urinary excretion of tritium paralleled the in vitro release of naltrexone as shown in Figure 6. Both types of data show that the release rate of naltrexone was high initially but decreased rapidly to a constant value, Our earlier studies with ^{3}H-naltrexone showed that approximately 50% of the dose could be recovered from the urine of mice after administration by single injection or by continuous infusion. It was on this basis that the quantity of naltrexone metabolized daily was calculated. The dis appearance of drug and dipalmitin from 1.7 x 3.0 mm pellets is shown in Figure 7. Earlier tests showed that 23 of 29 1.7 x 10 mm dipalmitin implants disappeared completely from intramuscular sites in the buttocks of rabbits within 3 months. The data points in Figure 7 are not sufficient to predict the disappearance rate of the 1.7 x 3.0 mm pellets in mice accurately, but a line is drawn through them to present a "best estimate" of the biodegradation profile.

In an effort to obtain pellets that biodegraded more rapidly, glyceride mixtures containing either 10% α-monopalmitin and 90% dipalmitin or 20% a-monopalmitin and 80%-dipalmitin were loaded with 30% naltrexone and tested, The biodegradation rates of the preparations did appear to be more rapid, but the release rates of naltrexone were also greater, as shown in Figure 8. Consequently, the drug contents of the pellets were depleted within a few days.

In a further effort to obtain effective preparations that biodegraded more rapidly, particles containing 30% naltrexone dispersed in either dipalmitin or tripalmitin were prepared and tested. Suspensions of these particles in aqueous methyl cellulose solution were injected subcutaneously in mice and tested at intervals thereafter for their antagonist potency by the tail-

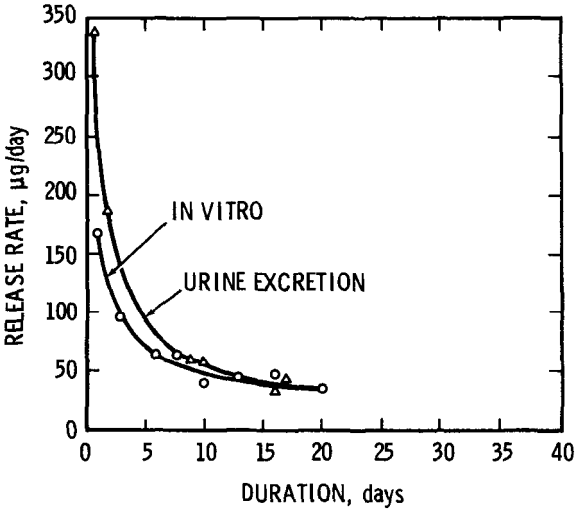

FIGURE 6. COMPARISON OF NALTREXONE RELEASE RATES FROM A PELLET IN VITRO AND AS DETERMINED BY URINARY EXCRETION OF ^{3}H-NALTREXONE FROM MICE. PELLETS WERE 1.7 x 3.0 mm IN SIZE AND CONTAINED 30% NALTREXONE IN DIPALMITIN

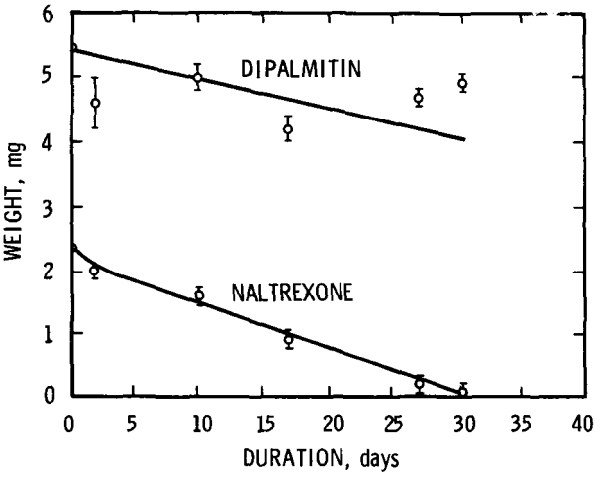

FIGURE 7. LOSS OF NALTREXONE AND GLYCERIDE FROM A 17 x 3.0 mm, 7.8 mg PELLET IMPLANTED SUBCUTANEOUSLY AND CONTAINING 30% NALTREXONE (FREE BASE) IN DIPALMITIN

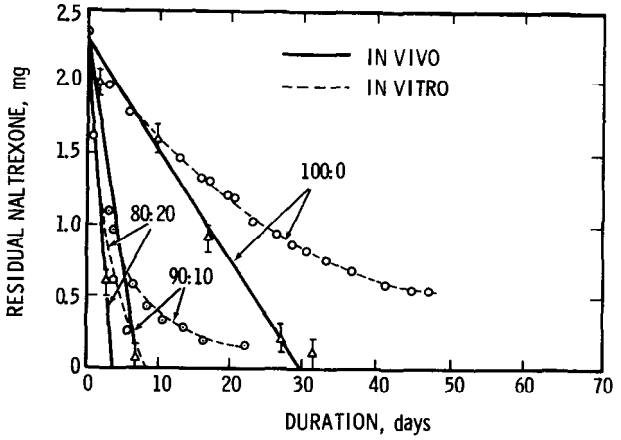

FIGURE 8. COMPARISON OF IN VIVO AND IN VITRO RELEASE OF NALTREXONE FROM 1.7 x 3.0 mm PELLETS CONTAINING 30% NALTREXONE (FREE BASE) IN DIPALMITIN: MONOPALMITIN MIXTURES, 100.0, 90:10, OR 80:20

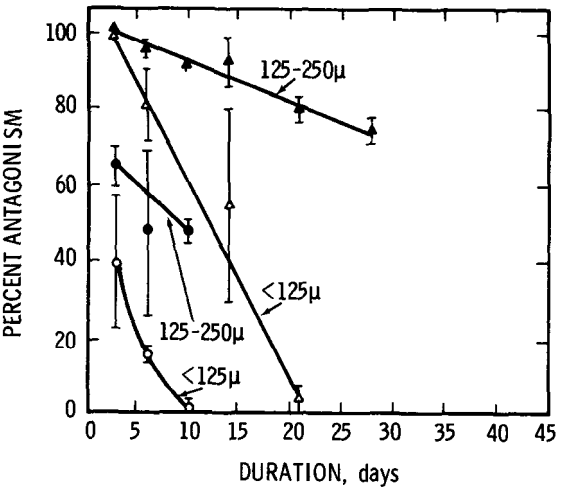

FIGURE 9. EFFECTIVENESS OF 8.0 mg OF TRIPALMITIN (△, ▲)OR DIPALMITIN (○, ●)) PARTICLES OF DESIGNATED SIZE CONTAINING 30% NALTREXONE FOR ANTAGONIZING MORPHINE (15 mg/kg) USING THE MOUSE TAIL-FLICK TEST

flick response. The results are shown in Figure 9. This preliminary evaluation in only a few mice showed that particles less than 125 μ and dipalmitin particles were relatively ineffective for any prolonged duration of action. On the other hand evaluation in at least 6 mice per interval showed that tripalmitin particles in the 125-250 μ range provided at least one month of antagonist activity. Further refinement of these particles is now in progress to provide a more uniform glyceride coating that may extend the life of this preparation.

No appreciable tissue response was found after implantation of either dipalmitin or tripalmitin in rats, rabbits, or swine at intramuscular, subcutaneous, or intraperitoneal sites. No significant amount of fibrosis was seen as long as 1 year after treatment with the slower biodegrading glycerides. There has been no gross indication that any of our more recent preparations are toxic.

CONCLUSIONS

These studies have shown that glycerides and naltrexone can be combined to regulate the release of that antagonist in-laboratory animals for periods of at least one month. The dose released is dependent on the drug concentration within the matrix of the release vehicle and on the glyceride composition of the matrix itself. The rate of biodegradability of a solid implant can be increased by increasing the proportion of the lower molecular weight glyceride in the matrix. However, this change also speeds up the drug release rate thereby limiting the usefulness of this manipulation. Hopefully, the biodegradation rate may also be increased by administering slow-release vehicles in particle form due to the increased surface area.

This form of the vehicle would be less retrievable by an addict that wished to remove the implant and may be preferred by the therapist. Our current efforts are directed toward improving both the particles and the pellet implants in order to offer a range of slow release devices that can be chosen to fit the needs of the addict being treated with narcotic antagonists.

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USE OF SYNTHETIC POLYPEPTIDES IN THE PREPARATION OF BIODEGRADABLE DELIVERY VEHICLES FOR NARCOTIC ANTAGONISTS

Kenneth R. Sidman
Douglas L. Arnold, Ph.D.
William D. Steber
Lita Nelsen
F.E. Granchelli, Ph.D.

Suresh G. Sheth

ARTHUR D. LITTLE, INC.

INTRODUCTION

Under contract No. ADM-45-74-218 we have been working on the development of practical, clinically acceptable narcotic antagonist delivery vehicles prepared from synthetic polypeptides such as glutamic acid/leucine copolymers. The synthetic polypeptides were chosen because they potentially exhibit diffusional release rates and biodegradation rates in the regions of interest, and have been shown to be biocompatible. Two types of systems are being sought: One capable of administering naltrexone for one month and another for approximately six months. It is desired that these systems biodegrade at a sufficiently rapid rate that they are essentially "absorbed" as soon after they are exhausted of antagonist as possible.

In addition, in view of the constantly evolving concepts advanced in the areas of antagonist development, psychopharmacology, and protocols for patient treatment, it is important that the delivery system developed in this program be amenable to other antagonists, and be capable of being fabricated into a variety of dosage forms, including integral shapes such as rods and fibers, as well as discontinuous forms such as microcapsules and microbeads.

Although the antagonist may be released from the matrix by diffusion, by the degradation of the matrix, or by a combination of both mechanisms, we are pursuing the development of a system which releases naltrexone essentially by diffusion alone because, at this stage, such a system can most easily be fabricated. Biodegradation subsequently removes the implant matrix.

METHODS

Preparation of Polymers

The glutamic acid/leucine (Glu/Leu) copolymers were prepared following the procedure of Fasman et.al. (Fasman, 1964). The polymers were charac-

terized by infrared spectroscopy, ultraviolet spectroscopy, intrinsic viscosity, and solubility in N, N dimethy l formamide(DMF).

Preparation of Delivery Vehicles

Film-shaped implants were prepared by casting a DMF solution of the copolymer and naltrexone (labelled with tritium and exhibiting a specific activity of 0.48 mCi/gram) into a teflon mold. By alternately casting and drying (at 40°C-60°C) thin layers of the solution, films 0.007cm to 0.07Ocm thick were built up. The films were then placed in a vacuum oven at 50°C-70°C, 30 in. Hg vacuum for twenty-four hours to remove the last traces of solvent. Individual devices exhibiting total surface areas of one to two cm were cut from the films.

Rod-shaped implants 0.04cm to 0.08cm in diameter were prepared by extrusion through a die. Typically, finely ground polymer was dry-blended in the desired proportion with the tritium-labelled naltrexone. Sufficient solvent was added to make a 50% solids paste. This was then placed in a steel compression mold that had a O.lcm diameter hole bored in its side (later the mold was modified by drilling and tapping so that it would accept a screw-in die). Rod was extruded at room temperature by exerting moderate pressure on the plunger. The rod was then dried to remove the solvent.

Tube devices with an inside diameter of 0.18cm and wall thicknesses ranging from 0.0026cm to 0.012cm were prepared by casting a solution of the polymer onto a rotating glass mandrel. Extruded naltrexone/polymer cores consisting of 80% naltrexone by weight were inserted into the tubes. Pure polymer plugs were then inserted and cemented into the ends of the tubes.

In Vitro Evaluation of Naltrexone Release Rates

The delivery vehicles were rinsed briefly with Farle's Balanced Salt Solution (IX - without phenol red, Grand Island Biological Company)and were placed in stoppered test tubes containing 10 ml of the Farle's solution. The tubes were placed in a shaker water bath at 37°C and were gently agitated. Drug concentration of the solution was periodically assayed by scintillation counting techniques to permit an evaluation of the in vitro drug-release behavior of the copolymers. (The solutions were replaced sufficiently frequently that the naltrexone concentration remained far below saturation, i.e. below ~460 mg/100 ml).

IN VITRO EVALUATION

Charles River CD #l mice were prepared for device evaluation by implanting the delivery vehicles using the following protocol:

Surgery: Under pentobarbital Na(60 mg/kg) anesthesia the drug release devices were implanted subcutaneously on the back of a mouse.

Apparatus: Immediately after surgery, the mice were fitted with coprophagy cups and placed in metabolic cages. The coprophagy cups assured complete separation of urine and feces and thus allowed for an accurate evaluation of the relative magnitudes of excretion by both routes. Urine and feces were collected daily for the first week. and two days per week thereafter.

The wire floors and draining portions of the cages were flushed to dissolve dried urine and assure a thorough removal of radioactivity from the cages. Feces samples were removed from the tail cups and held in air-tight vials until analyzed.

Analysis of Excreta: Small aliquots of diluted urine (urine and rinse) were analyzed directly by scintillation methodology. Radioactivity in the feces was determined by homogenizing the samples and then analyzing them using scintillation counting procedures.

Tail Flick Bioassay: A Dewey-Harris tail-flick apparatus constructed by Dr. William L. Dewey and his associates was used in this program.

Experimental animals were weighed and control tail-flick times were determined prior to any treatment or manipulation. Animals whose tail-flick times were outside the range of 2-4 seconds were discarded. At various times after implantation, test tail-flick times were taken thirty minutes following an AD_{80} dose of morphine - SO_4 (17.0 mg/kg).

RESULTS

Films

Initially, model delivery systems in film form were prepared using various naltrexone/polypeptide compositions to assess the compatibility of the antagonist in the polymer. Naltrexone was found to be highly compatible with Glu/Leu copolymers, showing no tendency to bloom to the surface of films containing at least 40% drug by weight. This result indicates that the

naltrexone/polypeptide compositions are capable of being stored for prolonged periods of time without changing their composition or structure.

Films prepared from 40/60, (i.e., 40 mole percent leucine/60 mole percent glutamic acid), 35/65, 20/80, and 20/80 Glu/Leu copolymers containing 10% to 40% naltrexone by weight were evaluated in vitro in mice. The naltrexone was found to be released by diffusion, exhibiting a diffusion coefficient that varied as a function of the copolymer composition and the initial naltrexone loading. For example, the diffusion coefficient increases as the glutamic acid content is raised; and, at a fixed glutamic acid content, it increases as the initial naltrexone concentration is increased. A wide range in diffusion coefficients can be achieved (see Table 1). These values are within practical ranges of interest for the development of delivery vehicles capable of meeting the program goals.

The diffusion coefficients may be used to predict release rate versus time behavior using suitable equations. One useful expression for release rate is the following. (Baker, 1974)

$$\dot{M} = \frac{\text{Release}}{\text{Rate}} = \frac{(8)(\text{Drug Content, } \mu g)}{\ell^2} \cdot \sum_{n=o}^{\infty} e^{\frac{-D(2n+1)^2 \pi^2 t}{\ell^2}} \quad \text{Eq 1.}$$

where D=diffusion coefficient, ℓ=thickness, t=time

TABLE 1

EFFECT OF COPOLYMER COMPOSITION AND ANTAGONIST LOADING ON NALTREXONE DIFFUSION COEFFICIENTS

Copolymer Composition	Naltrexone Content	Diffusion Coefficient	Time To Release One-Half of Initial Drug Loading From Films With Thicknesses Of 0.007 Cm^2	0.070 Cm^2
(Glu/Leu)	Wt. %)	(Cm^2/Hr)		
40/60	10	20×10^{-7}	1.2 Hrs	120 Hrs
	20	22×10^{-7}	1.1	110
	40	120×10^{-7}	0.2	20
35/65	10	0.31×10^{-7}	78	7800
	20	11.5×10^{-7}	2.1	210
	40	26×10^{-7}	0.9	90
20/80	20	1.0×10^{-7}	24	2400
	40	12×10^{-7}	2.0	200
10/90	40	19×10^{-7}	1.3	130

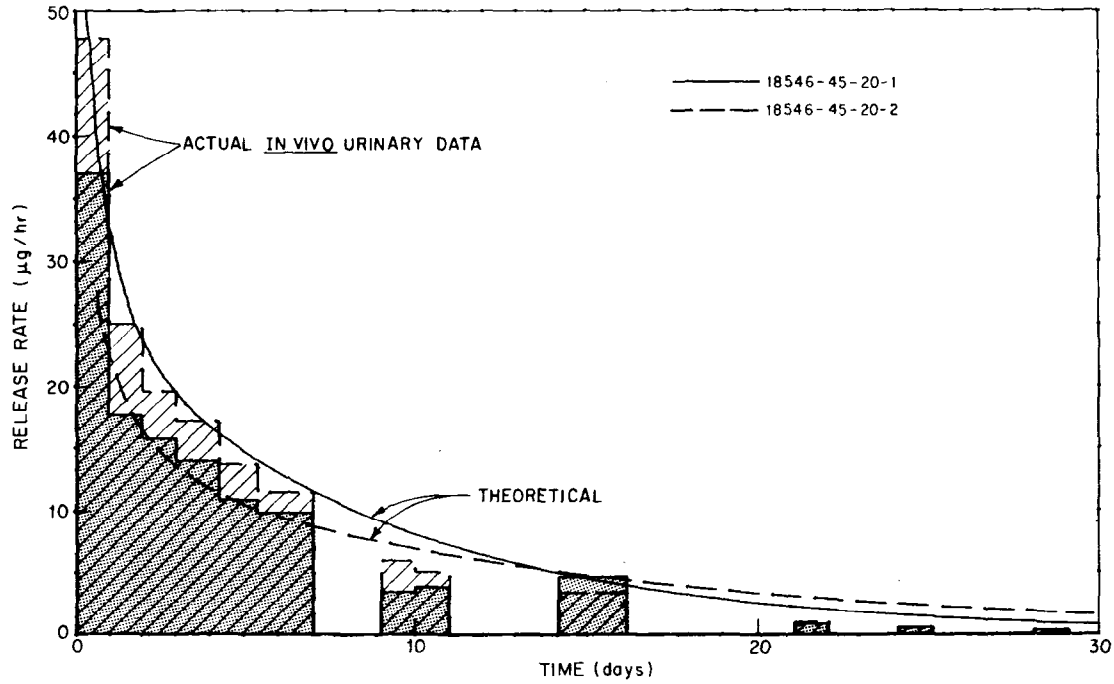

FIGURE 1 COMPARISON OF THEORETICAL AND ACTUAL IN VIVO RELEASE RATES vs. TIME
35/65 GLU/LEU COPOLYMER FILMS CONTAINING 20% NALTREXONE

TABLE 2

COMPARISON OF IN VIVO RELEASE BEHAVIOR OF 35/65
GLU/LEU FILMS CONTAINING 20% NALTREXONE

Implant Thickness cm	Initial Loading Implant of Drug µg	Mouse	Percent Antagonism* 1	4	7	9	11	14	16	18	21	24	28-Days
0.061	5800	GT	-	-	100	-	-	100	-	-	100	100	-
0.056	4900	GL	-	73	64	-	65	-	-	100	100	-	100
0.048	6600	GH	-	-	100	-	-	100	-	-	100	100	-
0.046	4050	RL	100	-	-	100	-	-	100	-	100	100	-
0.043	4650	GR	-	100	-	-	100	-	-	100	100	-	100
0.043	5500	RT	100	-	-	100	-	-	72	-	100	-	100

*Mice were challenged with 17 mg/kg morphine sulfate administered i.p., and were tested after 30 min.

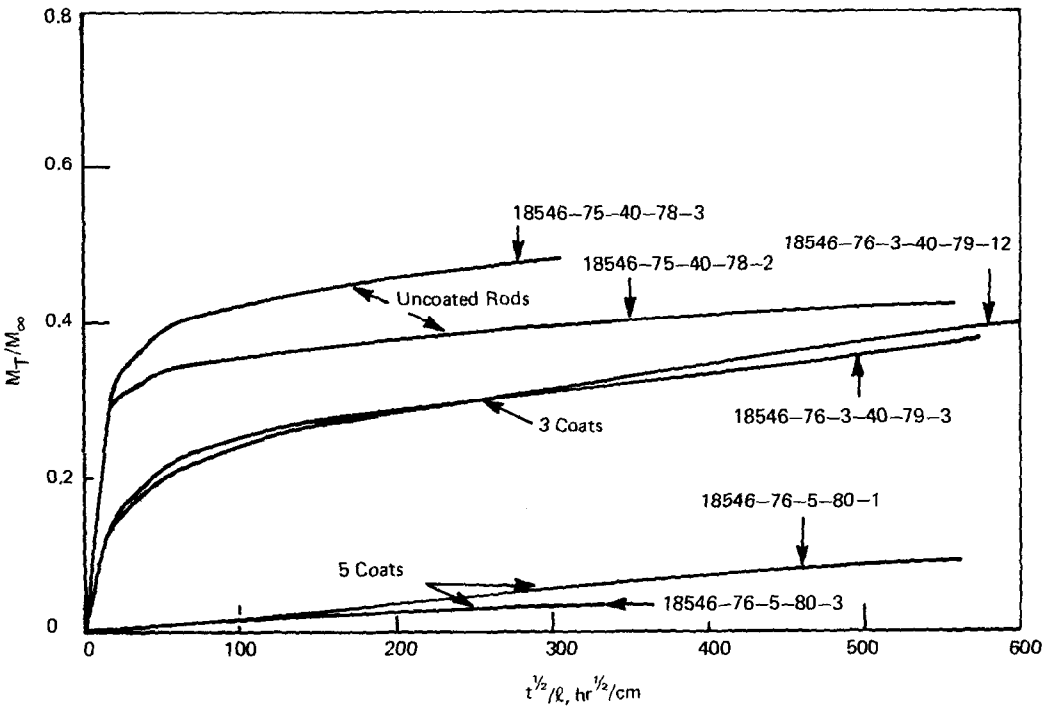

FIGURE 2 CUMULATIVE RELEASE VERSUS $t^{1/2}/\ell$ FOR 20/80 RODS CONTAINING 40% NALTREXONE RODS OVERCOATED WITH PURE POLYMER

TABLE 3

CHARACSTICS OF 20/80 GLU/LEU RODS
CONTAINING 40% NALTREXONE EVALUATED *IN VITRO*

Diameter cm	Weight mg	Sample No.	Average Diffusion Coefficient cm^2/hr
Uncoated Rods			
0.061	7.5	18546-75-40-78-3	1.3×10^{-8}
0.065	7.3	18546-75-40-78-2	
Rods with Three Coats			
0.060	6.1	18546-76-3-40-79-l	1.0×10^{-8}
0.068	6.5	18546-76-3-40-79-3	
Rods with Five Coats			
0.056	7.1	28546-76-5-40-80-3	0.38×10^{-8}
0.064	6.9	18546-76-5-40-80-l	

With this expression, a comparison of the theoretical release rate with the actual in vivo release rates may be made. Figure 1 shows such a comparison for typical 35/65 Glu/Leu films containing 20% naltrexone by weight. Here the actual urinary recovery of radioactive drug and metabolites is plotted versus time. (In these experiments the urinary radioactivity accounted for 80-90% of the total excreted, and, therefore, the urine data provided a relatively accurate indication of the rates of release from the implants). Note that the actual release rates follow the theoretical predictions quite well, confirming that naltrexone is released from films by diffusion.

Since a naltrexone dosage of l-2 μg/hr per mouse is all that is necessary to block an AD_{80} dose of morphine sulfate, animals receiving these film implants were blocked for more than 28 days (see Table 2).

Rods

Solid rod shaped devices released naltrexone in a manner similar to that observed with the films, indicating that a prolonged release can be achieved, albeit at a rate that continuously decreases with tine (as predicted by standard diffusion equations).

Rods prepared from 20/80 Glu/Leu, containing 40% naltrexone by weight, were found to exhibit an unexpectedly high initial rate of release, which could be substantially reduced by overcoating the rods with pure 20/80 Glu/Leu copolymer (see Table 3 and Figure 2). Rods receiving five coats of polymer exhibited diffusion coefficients that were constant with time, and corresponding release rates that were constant when plotted versus the square root of tine. After approximately 70 days, 10% of the initial drug was released from these devices.

Despite the prolonged release obtainable from monolithic devices such as films and rods, it is clear that constant rates of release by diffusion cannot be achieved with these dosage forms because the drug flux continuously decreases as the drug concentration gradient within the device decreases. Tube forms, however, behave quite differently.

Tubes

Tube devices filled with a saturated solution of the antagonist (with excess solid present) will maintain the concentration gradient across the tube wall at a fixed value. As a consequence, these devices are capable of delivering naltrexone at a constant rate over a prolonged period (until the solid phase disappears).

Preliminary experiments with tubes have begun. In these early experiments, variations in release rate were encountered because of poor transport of naltrexone from the solid drug core to the interior wall of the tube. As previously stated, a constant release rate depends on maintaining a saturated solution of naltrexone in the interior of the tubes. The tubes in these experiments were only partially filled with liquid, resulting in only partial utilization of the surface area available for diffusive release.

We are currently studying techniques for prefilling tubes with saline so that a saturated solution of naltrexone will be in contact with the entire inner surface of the tubes. This will ensure full utilization of the surface area for diffusive release, and consequently higher and more predictable release rates. Tubes with wall thicknesses ranging from 0.026mm to 0.12mm are being employed in this study so that an evaluation of the influence of wall thickness may be made.

CONCLUSIONS

Synthetic polypeptides may be fabricated into a variety of naltrexone delivery vehicles capable of administering the antagonist for prolonged periods of time. Tube dosage forms, in particular, offer advantages for this application since

- They my contain drug loadings as high as 80% to 90% by weight, allowing a minimum overall size of depot.

- They would be expected to exhibit a constant rate of antagonist release (by diffusion), and hence a high utilization of the drug payload.

- They would contain a minimum amount of polymer to be removed by biodegradation.

- They may be administered by trocar injection.

However, additional work is required to develop techniques for the preparation of vehicles that release antagonist at a reproducible and constant rate.

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DEVELOPMENT OF CHRONOMERS™ FOR NARCOTIC ANTAGONISTS

Richard C. Capozza, Ph. D.
Edward E. Schmitt, Ph. D.
Lee Richardson Sendelbeck, M.S.
ALZA Corporation

BACKGROUND

The National Institute of Drug Abuse (NIDA) is sponsoring work to develop an erodible, long-acting delivery system for the controlled administration of a narcotic antagonist to assist in the management of narcotic addiction. Based on a favorable relationship between potency and lack of undesirable side effects, naltrexone has been recommended as the drug of choice. The requirements of the system are:

(1) Ability to release naltrexone at prescribed rates for one to six months.

(2) A shape and size which will permit convenient parenteral administration.

(3) Absence of significant adverse tissue response.

(4) Bioerodibility of the drug-delivering element.

ALZA has undertaken the task of developing a system which is aimed at meeting these requirements and which is based on a dispersion of naltrexone in one of ALZA's bioerodible polymeric matrices.

OBJECTIVE

The objective of this program is to prepare a bioerodible naltrexone delivery system which can be implanted subcutaneously in humans and which can release the narcotic antagonist over one to six months at relatively constant rates sufficient to block the euphoric action of morphine-based drugs.

CHRONOMER™ MATRICES

These novel and proprietary polymeric systems, identified by the trademark CHRONOMER™, are being developed by ALZA Research, a division of ALZA Corporation, for use in the fabrication of drug delivery systems and are designed to undergo predictable erosion when placed in a biological environment. In general, the CHRONOMER™ matrix materials are hydrophobic and absorb little water. Within a given family of CHRONOMER™ matrix materials, variations of molecular structure make possible the preparation of polymers which possess a range of physical properties in-

cluding tough, glassy solids (C 101) and soft, compliant materials (C 101 ct).

The erosion process proceeds via hydrolysis at the surface of the CHRONOMER™ matrix. The drug contained within that surface layer is released into the surrounding tissues at a predetermined rate. The erosion process of the CHRONOMER™ matrices can be influenced by incorporation of additives, particularly alkaline materials, which modify the rate of hydrolysis of the polymers. If a drug *is* uniformly dispersed within a CHRONOMER™ matrix, the drug is released at a rate determined by (1) its concentration in the polymer, (2) the surface area exposed to water, and (3) the pH of the microenvironment at the interface between the matrix and its surroundings.

The drug release rate at any time is proportional to the surface area of the matrix. With some system configurations, such as thin discs, the surface area remains essentially constant throughout the system lifetime and thus results in a constant drug delivery rate. With other shapes, such as rods or spheres, the surface area decreases with time, causing a decrease in drug delivery rate.

The erosion rate of CHRONOMER" materials is inversely related to the pH of the aqueous phase in contact with the surface of the polymer. If the drug is acidic, its release can increase the acidity of the environment and hence can accelerate CHRONOMER™erosion and concomitant drug release. When the drug is basic, the local hydrogen ion concentration is reduced and the CHRONOMER™ matrix erodes at a slower rate than predicted for the pH of the bulk of the surrounding medium.

Since relatively slow release rates are required for a naltrexone delivery system, Naltrexone free base, rather than naltrexone hydrochloride, is being used in CHRONOMER™/ naltrexone composites, If necessary, release rates can be modified by incorporating bases or buffers into the matrix.

Prior to the commencement of the present NIDA sponsored contract, CHRONOMER™ drug delivery systems had been demonstrated to provide continuous, and substantially constant, release in *vitro* and *in vivo.* However, in some cases, there was a lag time of up to one week before the onset of the predicted rate of erosion.

CHRONOMER™ matrix materials are presently made in 150 g batches in a modified Atlantic Research 2CV reactor. Our ability to control and prescribe reaction conditions leading to reproducible batches of polymer has improved considerably during the last few years due to our independent effort to perfect these unique erodible matrix materials. For example, neither the molecular weight, which approaches 100,000, nor the molecular weight distribution (M_w/M_n = 3.5) of these polymers has varied by greater than 10% in the last five consecutive runs. A larger Atlantic Research 1OCV has been purchased by the Corporation and could increase our batch capacity by 200 fold. The unit has just been received, is not installed, and is currently undergoing a thorough inspection to evaluate compliance with specifications.

Toxicological studies conducted at ALZA during the past two years have not revealed limiting adverse effects of either the CHRONOMER™ materials or their hydrolysis products.

ANIMAL MODELS

The development of a long-acting, parenteral delivery system for the controlled administration of naltrexone requires a demonstration of efficacy in an experimental animal. The tail-flick test was recommended by NIDA as the means of evaluating the pharmacological activity of naltrexone. In this test, morphine delays the tail-flick response to externally applied heat and the narcotic antagonist reduces or eliminates this delay. Either the rat or the mouse can be used in this test; we selected the rat because it is the more suitable for drug release kinetic studies in *vivo.*

The ability to perform satisfactorily the tail-flick test procedure was demonstrated. The test was used to measure the effectiveness of naltrexone to antagonize the analgesic action of an 80% effective dose (ED80) of morphine (found to be 6.5 mg/kg body weight) in the rat. Single doses of naltrexone ranging between 2 and 20 µg/kg, injected subcutaneously in rats, were used to establish a single dose-response curve, A linear relationship resulted when dose (log scale) was plotted against response (probit scale). An 88% antagonist effect was achieved with a naltrexone dose of 20 µg/kg. An order-of-magnitude estimate was made to translate these data into a continuous drug delivery rate-response relationship. About 5 µg of naltrexone/kg/hr was calculated to be necessary to maintain > 80% antagonism.

Experiments to determine the parenteral dosage of continuously administered naltrexone required to antagonize the analgesic action of an 80% effective dose (ED80) of morphine in the rat were recently completed. Naltrexone was infused intravenously at doses of 0, 4 and 16 µg/kg/hr. The analgesic effect of an

expected ED80 dose of morphine was determined at the end of six hours, by means of the tail-flick procedure.

Four and 16 µg/kg/hr naltrexone resulted in 54 and 89% antagonism, respectively, against a 63.5% effective dose of morphine. A more effective morphine challenge (EDBO) is expected to lower the level of antagonism using these rates of naltrexone delivery.

DRUG RELEASE KINETICS

In order to (1) determine the naltrexone release kinetics and shape stability of CHRONOMER™/naltrexone composites *in* vitro and in *vivo* and (2) evaluate them with respect to the degree of tissue response, naltrexone was incorporated into C 101 and C 101 ct matrix materials at a level of 20 mg/g. Pure matrix materials and those filled with 10% Na_2CO_3 were used to afford a range of useful lifetimes and physical characteristics. The mixtures were fabricated into 1.3 mm thick films and cut into 1 cm diameter circles. The systems were chemically analyzed for naltrexone after fabrication, and the drug was shown to be unaffected by either the processing conditions or the CHRONOMER™ matrices.

The systems were implanted subcutaneously in rats. Naltrexone release from the systems was measured by analyzing the recovered systems for drug. Naltrexone release from samples of common origin was also measured *in vitro* (phosphate buffer), The results of those experiments are shown later in the text.

All drug release studies showed a high level of scatter. Average values of naltrexone released in vitro from each of these systems resulted in a release rate of 1.6 to 2.6 µg/hr for the first week, Systems containing Na_2CO_3 continued to release naltrexone at this rate for about the next 2½ weeks. Systems without Na_2CO_3 released naltrexone at a constant rate of 6.0 to 7.8 µg/hr after the first week of the study.

System	C 101	C 101, Na_2CO_3	C 101 ct	C 101 ct, Na_2CO_3
Shape stability	accept-able	good	poor	accept-able
Rate of drug release (µg/hr) *in vitro*				
1st week	2.0	1.6	1.5	1.7
3rd week	7.8	1.6	6.0	2.6
Tissue response*				
2nd week	6	1	0	1
4th week	0	0	1	1

*Number of moderate or severe reactions observed.

Data obtained by measuring the naltrexone in subcutaneously implanted devices, recovered two and four weeks post-implantation, showed greater scatter than that obtained *in vitro.* The C 101 ct systems without alkaline excipients exhibited poor shape stability. Both Na_2CO_3-free CHRONOMER™ matrices eroded within a few weeks time. Both CHRONOMER™ matrices containing 10% Na_2CO_3 had acceptable erosion characteristics, although they increased in size and weight after implantation, probably because of water absorption by the excipient. The four CHRONOMER systems produced some moderate tissue reaction at two weeks post-implantation. Less tissue reaction was observed at four weeks post-implantation, when the systems were significantly smaller and smoother compared with the original implant. With regard to drug release kinetics, shape stability, tissue response and longevity, the candidate based on a C 101 matrix containing 10% Na_2CO_3 was favored.

Because of the scatter observed in the drug release data, a number of experiments were initiated to better define the homogenity of the CHRONOMER™/drug mix and the drug release kinetics in *vitro.* A variety of matrix/drug formulations were prepared. Part of the source of scatter was traced to inadequate mixing. The mixing problem has since been obviated by using an inexpensive but more efficient blender, designed and developed by ALZA. The other source of scatter was found to result from instability of naltrexone in the erosion medium. Relatively small amounts

degradation products (< 3%) result in a large increase in the extinction coefficient of the ultraviolet absorption spectrum of naltrexone (as much as 40%). An improved assay procedure (liquid chromatography) is now routinely used and this technique provides measurements with less than a few percent error.

Using the improved techniques described above, two matrix materials (C 101 and C 101 ct) containing naltrexone and excipient were re-evaluated. Of the formulations investigated, CHRONOMER™ 101 ct/naltrexone systems now appear to be the most promising. After an initial lag interval of about two weeks, during which little drug is released, the C 101 ct matrix begins eroding and releases drug in a zero order fashion at a rate of 30 - 45 μg/hr/cm^2. That these C 101 ct/naltrexone systems erode and release drug concomitantly is evidenced by complete matrix erosion at the time drug has disappeared from the system. The concentration of suspended drug in the polymer does not appear to affect the rate of drug release at least to a concentration of 30%.

In contrast to the results with C 101 ct, C 101 failed to provide satisfactory functionality. The lag period preceeding drug release for CHRONOMER™ 101/naltrexone systems increased with decreasing drug content up to as long as 18 days at a loading of 2% (w/w). All C 101/naltrexone systems had substantial (50 - 80%) polymer residues remaining after complete drug release. We conclude that C 101/naltrexone systems do not erode and release drug concomitantly, and hence no further efforts will be expended on C 101 systems.

Preliminary sterilization studies showed that no adverse effects to CHRONOMER™/naltrexone systems occurred after exposure to 2.5 or 5.0 mrads of ^{60}Co irradiation.

FUTURE PLANS

The proposed program utilizes the background and experience gained in the earlier studies. The plans are directed to the development of the C 101 ct matrix system containing naltrexone and excipient. Our interpretation of (1) the long lag period between exposure to moisture and establishment of steady state drug release from these systems and (2) the large quantity of crystalline residue of C 101 matrix which remains after all the drug has been released, is based on phenomena brought about by dissolved naltrexone in the matrix, Basic materials are known to inhibit the ability of these matrices to erode and plasticizers are known to facilitate crystallization, Naltrexone is believed to play both roles. If a less soluble (in the matrix) form of the drug is to be used, the problem of delayed erosion may be significantly reduced. By shifting to C 101 ct, crystallization phenomena may be avoided completely.

TESTING OF DRUG DELIVERY SYSTEMS FOR USE IN THE TREATMENT OF NARCOTIC ADDICTION

Richard H. Reuning, Ph.D.
College of Pharmacy, Ohio State Univ.
Louis Malspeis Ph.D.
College of Pharmacy. Ohio State Univ.
Sylan Frank, Ph.D.
College of Pharmacv. Ohio State Univ.
Robert E. Notari, Ph.D.
College of Pharmacy, Ohio State Univ.

INTRODUCTION

The objective of this research is to evaluate the drug release characteristics of drug delivery systems developed for narcotic antagonists, particularly naltrexone and naloxone. The procedure which has been developed to accomplish this objective is a series of three successive testing procedures, each more quantitative than the previous in describing the release rate. The phase I evaluation consists of a test for the duration of analgesic antagonism in mice using the mouse tail-flick test. Phase II of our evaluation scheme is concerned with quantitating the excretion of tritium in urine and feces after administration of a drug delivery system containing radiolabeled narcotic antagonist to rats or guinea pigs. The third phase of this evaluation work is a pharmacokinetic description of the release rate of narcotic antagonist from the delivery systems administered to dogs and monkeys. In this phase III work the narcotic antagonist is administered in unlabeled form and samples of blood and/or urine are subsequently collected and analyzed for concentration of the narcotic antagonist by electron capture gas-liquid chromatography. Such concentration data obtained as a function of time, together with appropriate control experiments, permit a determination of the release rate of the narcotic antagonist from the drug delivery system.

In all three phases of this evaluation research, a substantial effort has been devoted to the development of the techniques and expertise necessary to carry out the evaluation in each phase. Progress in this developmental work has been such that all three phases of evaluation can now be carried out for naltrexone delivery systems.

PHARMACOLOGIC CHARACTERIZATION OF PROLONGED ANTAGONIST RELEASE

The research on pharmacologic characterization of prolonged antagonist release has been concerned with both the development of methodology and the actual evaluation of several naltrexone delivery systems. In this work, a modified version of the mouse tail-flick test for narcotic antagonist activity that was developed permits a reproducible temperature to be applied to a small portion of the mouse tail and yields data with comparatively small standard deviations. The technique has been critically tested and found to be an accurate measure of analgesia and analgesic antagonism.

Several naltrexone delivery systems have been evaluated for duration of analgesic, antagonism at a dose level of 40 mg naltrexone/kg using this modified mouse tail-flick procedure. A delivery system consisting of 35% naltrexone in 95% polylactic acid. 5% Citroflex 4 yielded a duration of analgesic antagonism of approximately 17 days, compared to 1/2 day for the same dose of naltrexone in aqueous solution. Results from additional controls indicate that the prolongation of activity was due to slow release of naltrexone from the polymer delivery system. A second delivery system consisting of naltrexone zinc tannate complex suspended in a vehicle of 2% aluminum monostearate in peanut oil yielded significant analgesic antagonism for 16 days whereas naltrexone hydrochloride suspended in the same vehicle lasted only 4 days. Results from additional controls indicate that the mechanism responsible for the prolonged activity is slow dissolution of naltrexone from the delivery system. A somewhat similar delivery system, i.e., naltrexone aluminum tannate suspended in a vehicle of 2% aluminum monostearate in peanut oil, yielded significant analgesic antagonism for 22 days. The fourth delivery system consisted of beads containing 50% naltrexone in a co-polymer of L(+)-lactic acid (75%)/glycolic acid (25%). This system yielded significant antagonism for 18 days at a 50 mg/kg dose level of naltrexone and for at least 21 days at a 100 mg/kg dosage. Again, comparison with appropriate controls indicates that the prolonged analgesic antagonism is due to slow release of naltrexone from the delivery system.

SEMI-QUANTITATIVE CHARACTERIZATION OF PROLONGED ANTAGONIST RELEASE

In order to carry out the proposed research on estimating narcotic antagonist release rates from delivery systems containing tritiated antagonist, it has been necessary to spend considerable effort in developing methodology prior to evaluating release of naltrexone from a sustained-release delivery system. Initially, both the radiochemical purity of tritiated naltrexone and the lack of exchange of label with water in biological systems was established for both 8-^{3}H-naltrexone and 15,16-^{3}H naltrexone. Stability of the tritiated naltrexone was monitored periodically by thin-layer chromatography. Essentially quantitative recovery of the administered tritiated naltrexone was obtained in urine and feces after parenteral administration of a solution of the drug to rats and guinea pigs. The quantitation achieved by combustion of urine and fecal samples followed by liquid scintillation counting indicates that, in the rat, about 60% of the administered radioactivity is excreted in feces and 40% in urine. On the other hand, in the guinea pig only 14% of the administered radioactivity was found in feces and about 84% in urine.

The naltrexone zinc tannate delivery system mentioned previously was evaluated by this radioactivity excretion technique. ^{3}H-15,16-naltrexone was administered via this dosage form intramuscularly (20 mg/kg) in guinea pigs. The overall elimination of radioactivity was found to be approximately exponential in four guinea pigs with 50% of the dose excreted in 2.7-4.9 days and 90% in 11.6-15.3 days. This compares favorably with the 16 day duration of analgesic antagonism obtained for this delivery system by the pharmacologic technique discussed in the previous section.

PHARMACOKINETIC EVALUATION OF PROLONGED ANTAGONIST RELEASE IN DOG,MONKEY AND MAN

Analytical Development

A major part of the work aimed at an eventual pharmacokinetic quantitation of narcotic antagonist release rates has been devoted to the development of sensitive gas chromatographic-electron capture (GLC/EC) assays for naltrexone and naloxone in human, dog and monkey plasma. These assays for the unchanged narcotic antagonist, which can readily be adapted to assays for drug in urine as well, permit a pharmacokinetic quantitation of the disposition of naltrexone and naloxone in the dog, monkey or man, as well as a quantitation of the release rate of these compounds from antagonist delivery systems. The assays for naltrexone and naloxone generally involve addition of an internal standard to the plasma sample, extraction of the basified plasma sample with benzene, back extraction into 0.1 N sulfuric acid, basification, re-extraction into benzene, evaporation, derivatization in benzene with pentafluoropropionic anhydride or heptafluorobutyric anhydride in benzene, washing the reaction mixture with sodium tetraborate, and injecting a portion of the derivatized material onto an OV-17 column. The assays for naloxone and naltrexone appear to be specific for unchanged drug and to clearly separate the unchanged drug from known metabolites. The sensitivity limit is 5-10 ng of antagonist in a 0.5 ml sample of plasma. We have extended the sensitivity of the assay of naltrexone in dog and monkey plasma to 1 ng/ml of plasma by the use of 2-3 ml of plasma. This appears to be the lower sensitivity limit of the GLC/EC technique. Linear calibration curves have been obtained for naloxone and naltrexone in dog and human plasma and for naltrexone in monkey plasma and for naltrexone in both unhydrolyzed and glusulase-hydrolyzed dog urine. Preliminary work on a GLC/EC assay for cyclazocine indicates that it may be possible to analyze this narcotic antagonist at concentrations less than 1 ng/ml.

Biologic Disposition of Naltrexone

In order to determine the rate of release of narcotic antagonist from a delivery system by pharmacokinetic techniques, it is necessary that the assay utilized to measure the concentration of antagonist in plasma and urine be specific for the unchanged drug. Thus, it is essential that a knowledge of the basic metabolic pathways of the narcotic antagonist be developed and that samples of authentic metabolites be synthesized or isolated and tested for interference with the assay procedure. In addition, a knowledge of the physiologic distribution of the narcotic antagonist can aid in explaining the relationship between the concentration of antagonist measured in the plasma and the amount of drug remaining in other parts of the body. Thus far, our work on biologic disposition has been limited to naltrexone.

Our research on the physiologic distribution of naltrexone has included a determination of the extent of binding of the drug in plasma from several species. It was found by equilibrium dialysis that naltrexone is bound in plasma to an extent of 20-26% in man, monkey, dog, guinea pig, rat and mouse. The extent of binding was independent of naltrexone concentration over a wide concentration range in all species. A second approach to the investigation of naltrexone distribution involved a determination of the

tissue levels of radioactivity in mice at 1, 5, and 15 minutes after intravenous administration of tritiated naltrexone. The results of this study indicate that the radioactivity is very rapidly distributed from plasma to tissues, concentrating in the liver, kidney and gastrointestinal tract, with less than 4% of the dose being present in plasma 1 minute after administration.

Considerable progress has also been made concerning the metabolism of naltrexone in several species. Two metabolites of naltrexone, α- and β-naltrexol, as well as two metabolites of naloxone, α- and β-naloxol, have been synthesized by sodium borohydride reduction of naltrexone and naloxone, respectively. Each pair of epimers was separated by preparative thin-layer chromatography and the structures were determined by spectral techniques. Use of these authentic metabolites has permitted the development of a qualitative GLC/EC method for distinguishing either α- or β-naltrexol in urine in the presence of large amounts of naltrexone . After parenteral administration of naltrexone substantial quantities of β-naltrexol and/or its conjugates were found in enzymatically hydrolyzed urine samples from man, monkey and guinea pig whereas smaller quantities were excreted by the rabbit, mouse, rat and dog. Trace amounts of the 6α-hydroxy epimer, a-naltrexol, were detected in the urine of monkeys and guinea pigs only. In vitro experiments with guinea pig liver indicate that the enzymes responsible for the reduction of naltrexone to both α- and β-naltrexol are present in the 100,000 x g supernatant of the liver homogenate.

The isolation of α- and β-naltrexol has also permitted the development of a GLC/EC analytical procedure for each of these metabolites. Linear calibration curves were obtained after extraction of these metabolites from dog plasma containing 20 to 150 ng of either α- or β-naltrexol. As mentioned previously, we have also confirmed that these metabolites do not interfere with the GLC/EC assay for naltrexone.

Pharmacokinetics of Naltrexone

The characterization of the phannacokinetics of a narcotic antagonist is a necessary prerequisite to the determination of the rate of release of that antagonist from a delivery system. Since the species most likely to be useful for testing narcotic antagonist delivery systems are the dog and monkey, the pharmacokinetics of naltrexone has been studied in these species.

Plasma concentration-time data for intravenous naltrexone in the dog, obtained by the GLC/EC analytical technique, indicate that at least two "compartments" are necessary to account for the data. The half-life of the principal exponential phase of the plasma level-time curve was 32 and 36 min in two foxhounds, and the apparent volume of distribution was 3.2 and 3.6 liters/kg. In similar i.v. bolus experiments using four monkeys, the plasma level-time half-life for naltrexone was 40-82 minutes (mean, 59 min) and the apparent volume of distribution was 4.4-10.1 liters/kg (mean, 6.4 liters/kg). These data indicate that naltrexone is distributed from blood to the rest of the body to an unusually great extent. Also, the drug appears to be rapidly eliminated in both the dog and the monkey. Since the clearance of naltrexone is greater than liver blood flow, the data indicate that some extrahepatic elimination occurs with this drug. Most importantly, however, the phannacokinetic data from these experiments will permit a determination of the release rate of naltrexone from a naltrexone delivery system administered to the dog or monkey in a subsequent experiment. Such an experiment in the monkey is currently in progress.

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