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THE PRECLINICAL DEVELOPMENT OF MEDISORB® NALTREXONE, A ONCE A MONTH LONG-ACTING INJECTION, FOR THE TREATMENT OF ALCOHOL DEPENDENCE

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1. ABSTRACT

Oral naltrexone, a nonselective opioid antagonist, is approved for the treatment of alcohol and opioid dependence. However, the efficacy of oral naltrexone is limited by poor patient compliance. To overcome this limitation, attempts have been made to develop an injectable extended-release formulation of naltrexone, including encapsulation into biodegradable polymer microspheres (e.g. Medisorb® Naltrexone, Vivitrex® (naltrexone long-acting injection)). In 1980, NIDA established development goals that they considered optimal for an extended-release formulation. At Alkermes, different formulations were tested with *in vitro* assays and *in vivo* models to select a lead formulation. Pharmacokinetic studies in rats confirmed that the principle formulation produced stable, pharmacologically relevant plasma levels of naltrexone for approximately one month following a single injection. The pharmacodynamic effects (antagonism of morphine analgesia) of extended-release naltrexone corresponded well with the pharmacokinetic profile from the same animals. While brain mu-opioid receptor density was found to increase over time in these rats, it did not appear to affect the ability of naltrexone to suppress morphine analgesia. Finally the pharmacokinetic profile of extended-release naltrexone in monkeys confirmed long duration of elevated plasma concentrations of naltrexone. Both naltrexone and the PLG polymer matrix in which it is encapsulated are well tolerated. Clinical trials of Vivitrex are currently ongoing in alcohol dependent patients.

2. INTRODUCTION

Alcohol dependence is a disease which is characterized by the following symptoms: 1) a strong need or compulsion to drink (craving); 2) an inability to limit drinking (loss of control); 3) the development of withdrawal symptoms (which may include nausea, shakiness, sweating, anxiety, and, in severe cases, hallucinations and seizures) following the discontinuation of alcohol use after a period of long time abuse (physical dependence); and 4) the need to drink more alcohol in order to feel its euphoric effects (tolerance) (1). While the majority of people drink alcohol sensibly, there are a substantial number of drinkers who are alcohol dependent, which leads to social, economic and medical problems. Alcohol dependent individuals affect not only themselves, but family, friends and communities. Alcohol dependency is a serious chronic problem seen worldwide. In the United States alone, it is estimated that alcohol related costs including lost work productivity and health problems are about $185 billion annually (2).

Based on retrospective analyses, the age at onset of alcohol dependence is in the 20s to mid 30s (1, 3), with men outnumbering women 2 to 1 (4). While the progression of symptoms is similar from person to person, the time course may vary and include periods of remission, controlled or non-problematic drinking and relapse (return to heavy drinking). Prognosis for recovery varies depending on a number of factors and the treatment approaches employed (5, 6). However, recovery from alcohol dependence is a life-long process with the perpetual possibility of relapse.

Marketed now for over 55 years in the United States, disulfiram (Antabuse®) was the first medication approved by the Food and Drug Administration (FDA) for

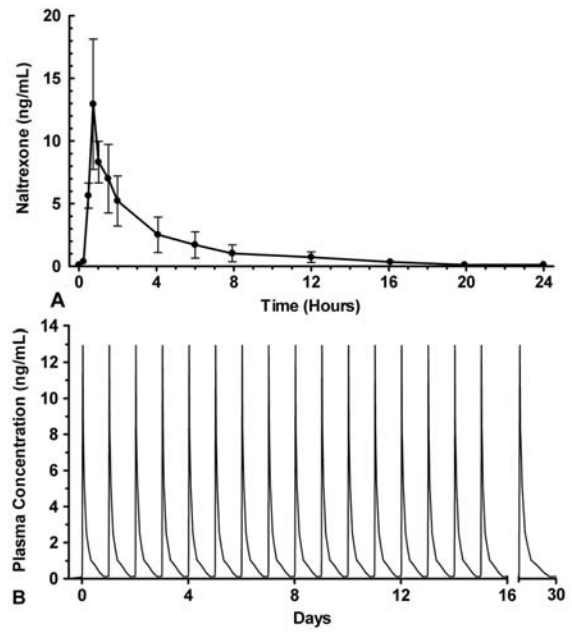

Figure 1. (A) Typical profile of plasma naltrexone levels over 24 hours following a 50 mg oral dose in humans. Oral naltrexone was given daily to normal healthy volunteers (N=6) for 5 consecutive days. This graph shows the plasma naltrexone concentrations following the 5th dose. Note the high concentration peak of naltrexone within the first hour of oral dosing followed by a fairly rapid decline in plasma levels to below the minimum therapeutic levels (2 ng/mL) within 8 hours of dosing. (B) Simulation of the daily fluctuations in plasma levels of naltrexone over the course of a month, assuming the patient adheres to the daily dosing of oral naltrexone (at about the same time every day) required for treatment of alcohol dependence.

the pharmacological treatment of alcohol dependence. The rationale behind the use of this drug is that it keeps the individual abstinent from alcohol by producing an aversive reaction when alcohol is consumed (e.g., flushing, nausea, vomiting, headaches, hypotension and rapid heart rate) (7-9). The numerous side effects of this drug (10, 11) together with the serious alcohol-induced reactions while taking disulfiram have been a significant hindrance to patients using this drug on a regular basis (12). Additionally, there is little clinical evidence that disulfiram has a significant effect on the treatment of alcohol dependence in double-blind, placebo controlled studies (13, 14).

Naltrexone (ReVia®), a non-selective, high affinity opiate antagonist, has been explored to treat both alcohol and opioid dependencies. In the mid 1960's, oral naltrexone was first investigated for the treatment of narcotic addiction and subsequently approved by the FDA in 1985 for the treatment of opioid dependence. Interest in using opioid antagonists for treating alcohol dependence arose from theories that the endogenous opioid system mediates many of the reinforcing attributes of alcohol (animal and human studies in support of this involvement are reviewed in (15-19)). In 1994, oral naltrexone was approved by the FDA for the treatment of alcohol dependence (20).

When administered orally, naltrexone has been shown to reduce relapse to heavy drinking in alcohol dependent patients, decrease the number of drinks consumed when relapse does occur, and promote abstinence (reviewed in (8, 13, 21)). Oral naltrexone is also reported to reduce both the craving for and the reinforcing euphoric qualities of alcohol (22-26). Despite the evidence that naltrexone is effective in the treatment of alcohol dependence, it is not widely used in the clinic (27, 28). Oral naltrexone is associated with a number of limitations which may subsequently diminish its efficacy in treating alcohol dependency and could increase the risk of relapse. First, daily dosing of oral naltrexone results in fluctuating plasma concentrations of the drug during the day (Figure 1A). This pattern repeats itself daily over the course of multiple months of treatment (Figure 1B). Secondly, compliance is a fundamental issue (29-31). This is compounded by the requirement of daily self-medication (at the same time each day). Typically, alcohol dependent individuals want the euphoria associated with taking a drink (24) and thus are less willing to take the oral medication every day over months of treatment. Often reasons for non-compliance include: indifference in getting treatment for dependency; poor social environment; forgetting to take the medication; heavy drinking; and the adverse effects associated with oral naltrexone (27, 32, 33). Finally, there is the issue of marginal efficacy as a result of too low a dose of naltrexone. The approved dose of oral naltrexone for treating alcohol dependence is 50 mg daily (34). This dose was chosen because it was found to be effective in the treatment of opiate dependence by blocking abused opiates at the receptor level (35). However, it is not known whether this is also the optimum oral dose for treating alcohol dependency. In fact, animal and human studies suggest that the effects of naltrexone on alcohol drinking are dose-dependent, with greater efficacy at higher doses (i.e., 100 mg) (36).

3. EXTENDED-RELEASE FORMULATION OF NALTREXONE

Extended-release delivery of drugs has several advantages to optimize the maintenance of treatment (37). First and foremost, extended-release of drugs improves compliance/adherence without the restrictions of daily medication. Next, the delivery of medication is assured. It takes the option and responsibility of daily treatment from the patient to the delivery system. This is an advantage in treating such chronic diseases as schizophrenia and drug dependency. Also, there is an improvement in bioavailability by avoiding first pass metabolism. Lastly, there is a reduction in drug dose. With a microsphere preparation, the drug is released slowly and steadily, avoiding the peaks and troughs associated with daily drug administration. This "smoothing out" of drug levels in the blood may decrease the incidence of adverse events associated with peaks, while improving efficacy by avoiding drug concentration troughs.

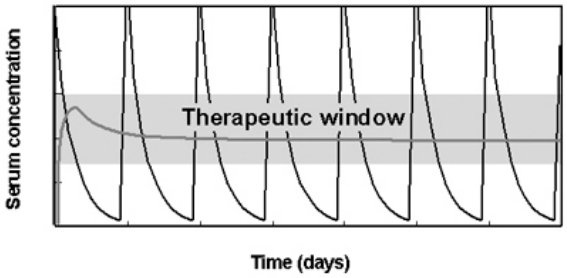

Figure 2. A simulated plasma profile following an injectable, extended-release formulation of naltrexone. An extended-release formulation would reduce repetitive peaks in plasma levels (as illustrated in Figure 1B), extend the duration of the therapeutic plasma levels, eliminate first pass metabolism in the liver, and eliminate the need for daily dosing by the patient.

An injectable extended-release formulation of naltrexone could directly address the limitations associated with daily oral naltrexone administration by:

1. Improving adherence. The drug is injected once a month for extended delivery of naltrexone and because it is injected intramuscularly it is impervious to patient manipulation.
2. Stabilizing plasma levels of naltrexone. Extended-release naltrexone would reduce the frequency and magnitude of peak plasma levels (associated with oral naltrexone) and maintain continuous therapeutic plasma levels for a month (Figure 2). Further, while oral naltrexone is readily absorbed through the gut, it suffers significant first pass hepatic metabolism (with an oral bioavailability of only 5-40%). An injectable extended-release naltrexone would eliminate this first pass metabolism.
3. Improving convenience to the patient. An injectable extended-release naltrexone formulation would eliminate the need for a conscious daily decision by the patient to take their medication.

3.1. Historical attempts to develop extended-release naltrexone

3.1.1. NIDA recommended goals for an optimal extended-release naltrexone formulation

To develop an effective extended-release formulation of naltrexone, a number of goals were established by the National Institute of Drug Abuse (NIDA) to assist in the development (38). These included:

1. The final formulation must rapidly achieve consistent plasma concentrations of naltrexone above the putitive therapeutic level (2 ng/mL; (35)) during the extended-release phase, with minimal initial drug release.
2. The formulation must release drug for at least one month.
3. The ratio of peak plasma level (C_{max}) to the minimum plasma level (C_{min}) of naltrexone should be minimized (compared to that observed following oral naltrexone) to achieve a more desirable therapeutic index.
4. The microsphere formulation must be injected in a minimal volume through a 20G or smaller hypodermic needle.
5. There must be no tissue reaction caused by allergic reactions or abscesses and minimal pain/discomfort at the injection site.
6. The formulation must be impervious to patient manipulation.
7. The last remnants of the residual microsphere polymer will be gone within a reasonable period of time after the drug has been eliminated.
8. Finally, the preparation meets pharmaceutical standards. These include a final drug product which: is a sterile preparation essentially free of foreign matter, has reproducible release characteristics, is easily prepared for injection, and can be manufactured on a large scale.

3.1.2. Early approaches to extended-release naltrexone

A number of approaches have been attempted (either alone or using a combination of approaches) to develop a prolonged release preparation of naltrexone (reviewed in (38-40)). One approach was to reduce the solubility of naltrexone by formulating insoluble salt or metal complexed (e.g., aluminum or zinc) forms of the drug, thereby prolonging its absorption in the blood (41, 42). Another line of development involved embedding or encapsulating naltrexone in a hydrophilic material. This material formed a hydrated gel around the drug that created not only a diffusion barrier but protected the drug from immediate metabolism as well. Similarly, using hydrophobic polymers, a water-resistant matrix around the drug particle was formed until eventual surface erosion occurred and slowly released naltrexone. Lastly, there was the development of a prodrug by chemically binding naltrexone to a polymer support. The drug was insoluble until the bonds were hydrolyzed or enzymatically cleaved (43, 44).

Previous efforts to develop a prolonged release preparation of naltrexone have utilized non-biodegradable polymers, polylactic (45) and lactic-glycolic acid polymers (39, 46-54), synthetic glutamic acid-leucine copolymers (46), cholesterol and polyglycerides (40). Unfortunately, these early attempts to develop a preparation of naltrexone that would release the drug over several weeks suffered several problems:

1. The formulations were not “patient-friendly”, requiring either surgical implantation under the skin or a large bore needle (>18G) to administer the suspension. Additionally, some formulations resulted in significant local site reactions.
2. The formulation did not provide a suitable pharmacokinetic profile because the duration of drug release was insufficient and/or the plasma levels were too low. Similarly, the ratio of C_{max} to C_{min} during the extended-release was too large, resulting in a pharmacokinetic profile with too steep a slope.

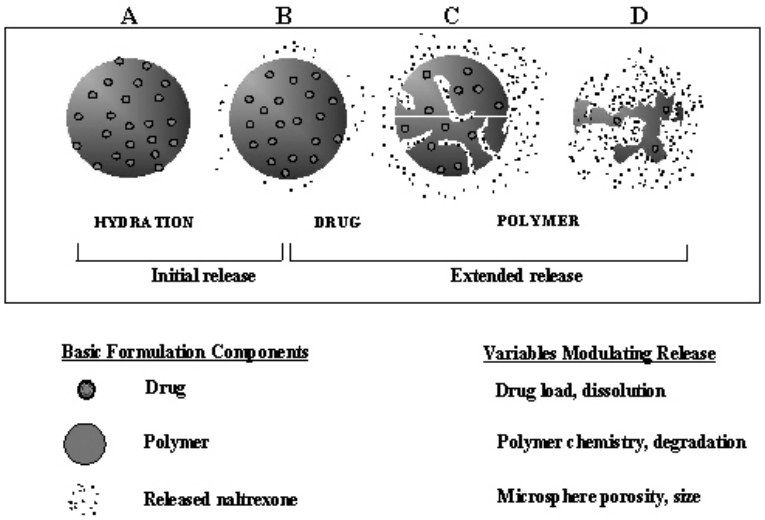

Figure 3. Components of extended-release naltrexone and the variables affecting drug release. Upon injection, the microspheres (A) begin to absorb water almost immediately, leading to a swelling of the microspheres (B). This process begins an initial release phase where a small amount of drug at or near the surface of the microspheres is slowly released. As water absorption continues, hydrolysis begins to breakdown the polymer resulting in the gradual collapse and erosion of the microspheres'internal structure (C). This polymer erosion process results in the extended-release of the drug from the microspheres. The polymer matrix eventually breaks down into lactic acid and glycolic acid, which are completely metabolized by the body and eliminated as carbon dioxide and water (D) (from (57)).

4. MEDISORB FORMULATION OF EXTENDED-RELEASE NALTREXONE

One proven technology for extended-release drug delivery is the encapsulation of drug in polymeric microspheres made of poly(d,l-lactide-co-glycolide) (PLG) (55). PLG is a common biodegradable copolymer with a history of safe human usage as sutures, orthopedics, bone plates and extended-release pharmaceuticals (e.g., Nutropin Depot®, Riperdal Consta®, Lupron Depot®, Zoladex®, Decapeptyl® SR and Sandostatin LAR® Depot). Such polymers can be fabricated into small diameter, injectable microspheres (<100 microns) and formulated to provide improved *in vivo* release of drugs, such as naltrexone, for predefined periods of time ranging from days to months. Drug release from PLG microspheres is governed by water uptake, diffusion of the bioactive molecule through the polymer matrix and the biodegradation of the polymer (55, 56). Biological degradation of PLG occurs primarily through hydrolysis, with the degradation products being the monomers, lactic acid and glycolic acid (55, 56). These monomers are further metabolized and eliminated from the body as carbon dioxide and water.

4.1. Process for Formulating Medisorb Naltrexone Microspheres

A proprietary water-based solvent extraction process is used to make Medisorb naltrexone microspheres (57). In this process, both drug and polymer are dissolved in a common organic co-solvent system. This "oil" phase is then emulsified (oil/water) with an aqueous solution. The emulsion undergoes a partial solvent extraction, via exposure to limited water volume, prior to transfer to a large aqueous "quench" solution. At this point, the oil droplets are precipitated into drug encapsulated microspheres. The microspheres are recovered, dried and undergo a secondary extraction step to further reduce the residual solvents. They are again recovered and dried a final time. The dried microspheres are aseptically filled into glass vials.

4.2. Microsphere Release Mechanism

At the time of administration, the microspheres are suspended in a sterile aqueous microsphere diluent, to aid in the suspension and injection of the extended-release Medisorb naltrexone. Following injection, initial release of drug from the microspheres is diffusion controlled. Subsequently, release is controlled by diffusion and polymer degradation (see Figure 3 for a more detailed explanation).

4.3. Selection of a Lead Formulation

Drug release is controlled by a number of factors. Among the strongest are drug loading, PLG monomer ratio (lactide: glycolide ratio) and the nature of the polymer chain end-group. These factors control not only the drug release kinetics but they also affect the dose volume and polymer degradation rate (55). Different PLG formulations were made in which the lactide: glycolide ratio and the naltrexone loading varied (57).

The formulations were initially characterized with numerous *in vitro* tests which measured microsphere size, uniformity, release rate and stability. Several different formulations were tested in rats to provide *in vivo* pharmacokinetic (PK) data. Of these, selected formulations

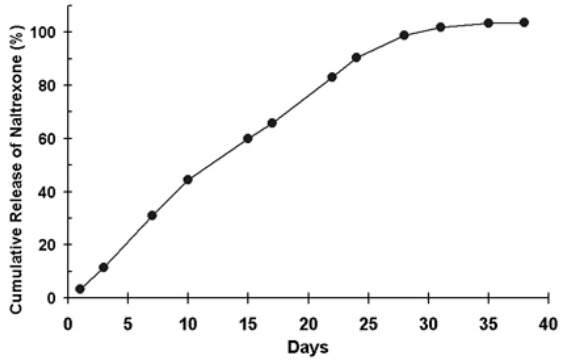

Figure 4. Representative *in vitro* drug release profile of the lead formulation of extended-release Medisorb naltrexone as measured in an aqueous buffer media at physiological pH and temperature (from (57)).

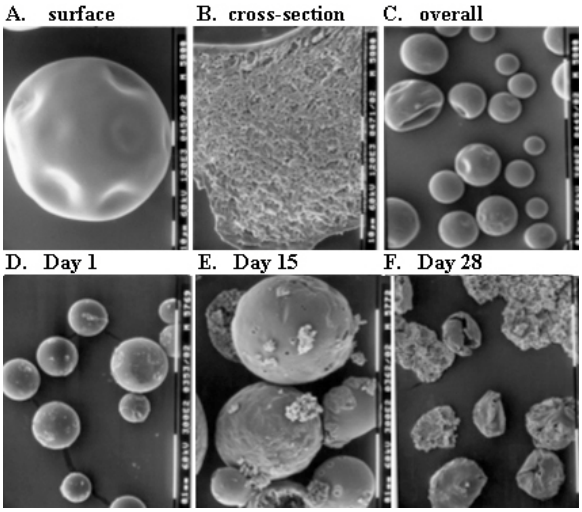

Figure 5. Scanning electron photomicrographs showing microsphere morphology and degradation of Medisorb naltrexone microspheres during *in vitro* release at 37°C in a physiological buffer. (A) Dry microsphere with dimpled surface (x1200); (B) Cross-section of dry microsphere showing dense structure (1200x); (C) Multiple dry Medisorb naltrexone microsphere (300x); (D) Day 1. Note loss of dimpled character commonly associated with hydration and swelling (300x); (E) Day 15. Swelling of microspheres continues (300x); (F) Day 28. Significant erosion of the interior of the microspheres has occurred with the residual polymer shell continuing to degrade (300x) (from (57)).

were tested in non-human primates to assess tolerability and confirm the PK profile observed in rats. One formulation was selected as the lead. This formulation entered into a formal development program involving GLP safety/toxicokinetics in rats and rabbits to establish a relationship between PK and pharmacodynamics (PD) in rats before progressing to a Phase I safety/PK study in humans.

4.3.1. *In vitro* Release of Naltrexone from Microspheres

The analytical results indicated high encapsulation efficiency for all the formulations (57). Increasing drug content (% loading) typically resulted in increased drug release within the initial 24 hours and increased the overall rate of release. Increasing the glycolide content also increased the overall rate of release, but to a lesser extent. A representative *in vitro* release profile of the lead formulation (microspheres of approximately 100 microns in diameter) at 37°C in a physiological buffer is shown in Figure 4. A near linear *in vitro* release profile was seen which provided an extended-release of naltrexone for over one month with minimal initial drug release. Additionally, there was a simultaneous degradation of the microsphere polymer with the drug release (Figure 5).

4.3.2. *In vivo* Pharmacokinetic and Pharmacodynamic Evidence of Efficacy in Rats

Pharmacological blockade of opiate receptors following an acute injection of naltrexone attenuates the analgesic effects of morphine in rats (58). With extended-release Medisorb naltrexone it is necessary to evaluate the extended pharmacological effects of this drug by repeatedly testing the same animal on a behavioral measure of analgesia over the course of several months. The behavioral rat model employed for this study (59), defined as the 'morphine-induced analgesia test', is a standard means for assessing analgesia by measuring the response of individual animals to a thermal stimulus (60, 61). In this test, rats are placed on a moderately heated (48°C) platform (a 'hot plate') and after a short period of time, the animals respond to the heat by licking their hind paw. The rats are quickly removed from the hot plate as soon as a response is noted. However, if an opioid analgesic, such as morphine, is first given to the rat, the reaction to the heat is abolished or greatly attenuated (up to a maximum of 60 seconds). Pharmacological blockade of opiate receptors in the CNS by extended-release naltrexone should block the effects of morphine and return the performance of morphine-treated animals to control levels. This test is ideally suited for evaluating the extended-release effects of naltrexone because it permits each rat to be tested multiple times over a period of several weeks to months.

Naltrexone-containing (loading density of 35% (w/w) naltrexone base) or non-loaded (placebo) microspheres were suspended in 1 mL of the aqueous diluent (0.9% saline, 0.1% Tween-20 and 3% carboxymethylcellulose) and injected intramuscularly (i.m.) with a 22G needle to provide a total of 50 mg/kg of naltrexone or a comparable mass of placebo microspheres (approximately 100 microns in diameter). Animals received an intraperitoneal (i.p.) injection of morphine (1 mg/kg) or saline on the hot plate test days. Half the animals receiving extended-release naltrexone were sacrificed 36 days after the injection, a time when the behavioral effects of naltrexone were diminished. The placebo and the remaining naltrexone treated rats received a second, identical microsphere injection 34 days after the first injection. These animals were retested on the hot plate and sacrificed on day 37 (following the second injection), a time when the

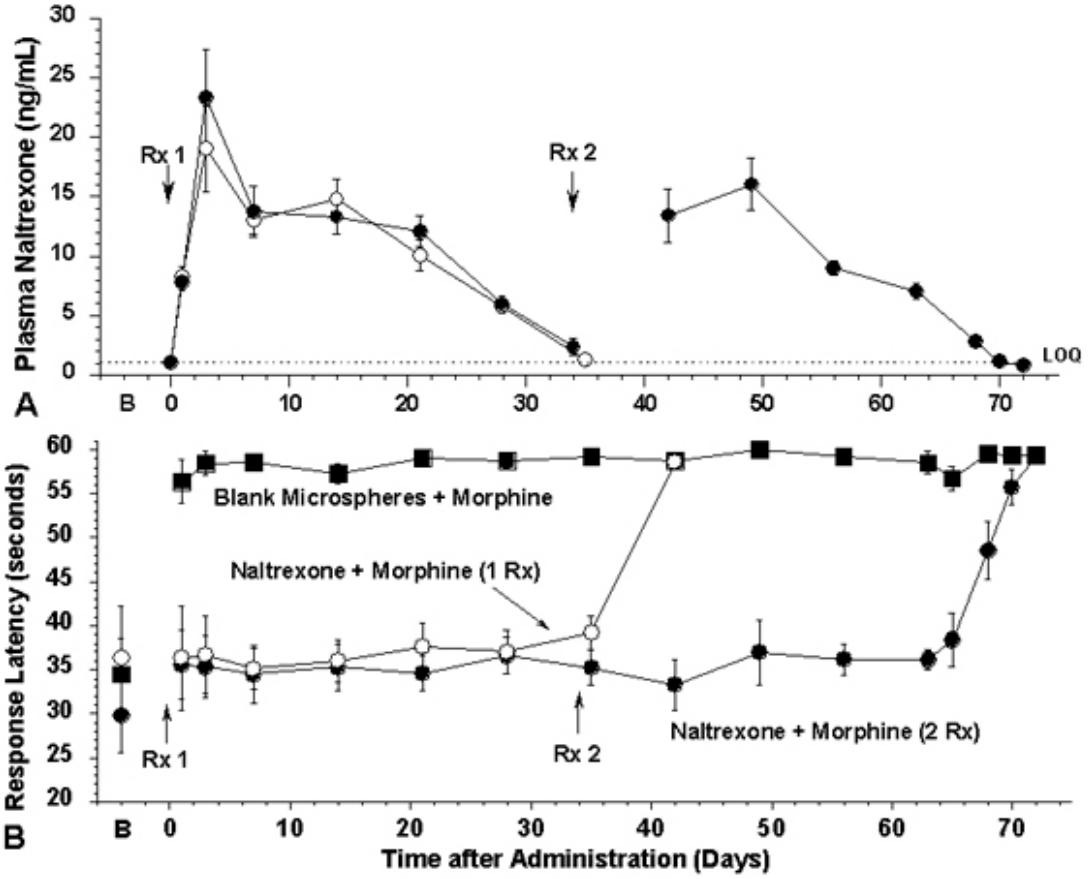

Figure 6. (A) Plasma levels of naltrexone in rats following either a single i.m. injection of extended-release Medisorb naltrexone (open circles) or a second, identical injection 34 days following the first treatment (closed circles). Plasma levels of naltrexone were maintained for approximately 21 days following a single injection. This effect was repeated with a second injection after an additional 34 days. Each point represents the mean ± SEM naltrexone plasma concentration (ng/mL) from nine rats. LOQ: lower limit of quantitation (<1 ng/mL). RX 1, 2: time of first and second Medisorb naltrexone injection. (B) Morphine (1 mg/kg) analgesia following either a single or second injection of extended-release Medisorb naltrexone microspheres in the same rats (as above) as measured on the hot plate test. Naltrexone antagonized morphine-induced analgesia, with responses equivalent to those observed under baseline conditions. By 41 days after a single injection of Medisorb naltrexone microspheres, the analgesic actions increased to levels observed in the placebo + morphine treated group. In contrast, two naltrexone treatments consistently suppressed morphine-induced analgesia for a total of 68 days. Data represent the mean ± SEM of latency to lick a hind paw by nine rats. B: baseline condition (from (59) with copyright permission from Nature Publishing Group, http://www.nature.com).

pharmacodynamic effects of naltrexone had completely disappeared.

Injections of extended-release Medisorb naltrexone or placebo were well tolerated by the rats independent of the number of injections. This was evidenced by the absence of local site reactions, such as redness, swelling, exudation, or scratching, upon *in vivo* and *ex vivo* examination. While there was a statistically significant difference in body weight between the extended-release naltrexone and placebo treated rats ($p<0.01$), the percent weight gain at the end of each drug delivery period were similar (7.6% vs 6.5% at Day 35 and 14.8% vs 15.7% at Day 70 for the placebo and extended-release naltrexone, respectively).

For quantitation of plasma levels of naltrexone, blood samples were collected via tail vein from all animals immediately after each behavioral test. Plasma levels of naltrexone were determined by LC-MS-MS (62) and were below the level of quantitation (LOQ) in all rats prior to treatment. Maximum plasma levels were observed by 3 days. Plasma concentrations of naltrexone did not significantly differ from each other between 3 and 14 days, with detectable levels of naltrexone maintained to 35 days (Figure 6A). A similar pattern was observed in animals receiving a second injection of extended-release naltrexone microspheres. Further, naltrexone was no longer quantifiable in plasma (<1 ng/mL) 35 days after the second injection.

Table 1. Mu-Opioid Receptor Binding in Rat Brain Following Extended-Release Naltrexone

Brain Region	Treatment				
	Control	**Naltrexone 1 month**	**% Increase**	**Naltrexone 2 month**	**% Increase**
Central Gray	7.48 (0.27)	17.74 (0.74)*	140%	19.90 (0.62)*	160%
Dentate Gyrus	7.10 (0.39)	15.68 (0.82)*	125%	17.34 (0.89)*	140%
Dorsal Raphe Nucleus	7.22 (0.86)	18.84 (2.31)*	160%	22.92 (1.06)*	220%
Habenular Nucleus	19.41 (1.41)	36.40 (2.50)*	90%	40.92 (2.42)*	120%
Hippocampus CA1	4.77 (0.29)	12.03 (0.79)*	150%	13.48 (0.75)*	190%
Inferior Colliculus	10.35 (1.72)	23.15 (1.87)*	130%	27.39 (1.54)*	170%
Lateral Orbital Cortex	8.84 (0.72)	20.16 (0.81)*	120%	23.68 (1.31)*	170%
Nucleus Accumbens	12.45 (1.11)	25.05 (2.16)*	90%	21.94 (1.31)*	70%
Perirhinal Cortex	6.41 (0.24)	12.52 (0.51)*	100%	14.54 (0.57)*	130%
Striatum	8.04 (0.67)	15.23 (0.79)*	80%	13.73 (0.38)*	75%
Subiculum	20.04 (1.56)	33.77 (2.26)*	70%	40.40 (1.75)**	100%
Substantia Nigra	8.69 (0.77)	17.95 (0.68)*	100%	20.54 (1.48)*	140%
Superior Colliculus	8.90 (1.15)	20.40 (1.57)*	120%	20.06 (1.42)*	120%
Tenia Tecta	9.18 (1.07)	22.67 (1.33)*	150%	28.73 (1.71)**	215%
Thalamus	11.27 (1.51)	21.46 (1.41)*	100%	21.18 (1.07)*	90%

Brains from naltrexone-treated animals were processed for quantitative autoradiography of mu opioid receptor binding using [^{3}H] DAMGO (D-ala^2, N-methyl-phe^4, glycol^5] enkephalin. One group of animals was sacrificed when the behavioral effects of naltrexone were still maximal (i.e., 29 days following treatment). The remaining animals received a second microsphere treatment 34 days following the first injection and were sacrificed after an additional 29 days to gain insight into the relationship between the duration of extended-release naltrexone (see Figure 6) and the magnitude of opiate receptor changes. Significant increases in mu-opioid receptor binding were observed in all brain regions examined. Note that these increases were seen within 1 month with only the subiculum and tenia tecta showing further changes with two months of naltrexone. Data are presented as mean ± SEM microCi/gram. * naltrexone vs control ($p<0.05$), ** one vs two months naltrexone ($p<0.05$) (from (59) with copyright permission from Nature Publishing Group, http://www.nature.com).

The PD effects of extended-release Medisorb naltrexone corresponded well with the PK profile derived from the same animals (Figure 6B). Animals were tested on the hot plate 30 minutes following an injection of morphine. Animals receiving placebo demonstrated a pronounced analgesic response to morphine (as determined by their latency to lick one hind paw approaching the maximum allowable duration of 60 seconds). In contrast, animals receiving extended-release naltrexone (containing 50 mg/kg naltrexone) showed a complete antagonism of the effects of morphine, responding at or very near the level of those that received no morphine (i.e., approximately 35 seconds response time). This response continued for approximately 1 month following a single injection of naltrexone microspheres. After one month, the rats' response rapidly returned to that of the rats receiving placebo. In the groups receiving a second dose of extended-release naltrexone at day 34, morphine continued to be blocked and ineffective through the second month (Figure 6B). Once again, at the end of that month, the rats' responses rapidly reverted to the non-naltrexone controls. These data demonstrate that following i.m. injections of extended-release formulation, naltrexone is gradually released and successfully blocks the opiate receptors in the brain for a full month antagonizing the analgesic properties of morphine.

4.3.2.1. Brain Mu-Opioid Receptor Changes

Increases in mu-opioid receptor density (receptor up-regulation) are commonly observed in the brains of rodents in response to multiple daily or continuous administrations of opioid antagonists (63-66). Therefore, the effect of mu-opioid receptor density following the administration of the extended-release naltrexone formulation was investigated as a biochemical measure of the pharmacodynamic efficacy (59). The time course of the changes in these receptors following the administration of extended-release microspheres was explored using receptor binding and immunohistochemical assays. At the conclusion of the behavioral testing (hot plate) following one or two injections of naltrexone microspheres (spaced 34 days apart), animals were sacrificed and the brains removed.

Frozen brains were cut into 20 micron thick sections and were used for quantitative autoradiography of mu-opioid receptor binding using [^{3}H] DAMGO (67). Brain sections adjacent to those used for receptor binding were processed for mu-opioid receptor immunoreactivity (68) using autoradiography with Ab-1 (Oncogene Research Products) as the primary antibody to mu-opioid receptors and [I^{125}] anti-rabbit IgG as the secondary antibody. Autoradiography revealed that radioligand binding to mu-opioid receptors was significantly increased above control brains in all regions examined, ranging from 90% in the habenular nucleus to 160% in the dorsal raphe nucleus after 1 month (Table 1). In most brain regions, these densities continued to increase at 2 months.

Immunohistochemistry using brain sections adjacent to those used in the radioligand autoradiography revealed significant increases in mu-opioid receptor immunoreactivity in only two of 15 brain regions examined after one month of treatment with extended-release naltrexone (Table 2). Following two months of treatment, increased immunoreactivity compared to controls was observed in 14 of 15 regions. However the magnitude of

Table 2. Mu-Opioid Receptor Immunoreactivity in Rat Brain Following Extended-Release Naltrexone

Brain Region	Treatment				
	Control	**Naltrexone 1 month**	**% Increase**	**Naltrexone 2 months**	**% Increase**
Central Gray	2.89 (0.19)	3.22 (0.33)	11%	3.98 (0.25)**	38%
Dentate Gyrus	2.60 (0.18)	2.87 (0.17)	10%	3.19 (0.17)*	23%
Dorsal Raphe Nucleus	2.44 (0.13)	2.90 (0.17)	19%	3.09 (0.21)*	27%
Habenular Nucleus	2.90 (0.17)	3.29 (0.18)	13%	3.86 (0.24)*	33%
Hippocampus CA1	2.57 (0.18)	2.61 (0.13)	0%	2.96 (0.18)	15%
Inferior Colliculus	2.96 (0.16)	3.34 (0.17)	13%	3.73 (0.13)*	26%
Lateral Orbital Cortex	2.77 (0.18)	3.05 (0.24)	10%	3.37 (0.13)*	22%
Nucleus Accumbens	2.90 (0.20)	3.54 (0.24)*	22%	3.79 (0.11)*	31%
Perirhinal Cortex	2.49 (0.13)	2.53 (0.18)	0%	2.72 (0.07)	9%
Striatum	2.82 (0.15)	3.25 (0.20)	15%	3.61 (0.20)*	28%
Subiculum	3.26 (0.13)	3.82 (0.25)	17%	4.26 (0.21)*	31%
Substantia Nigra	2.50 (0.15)	3.08 (0.16)*	23%	3.52 (0.17)*	41%
Superior Colliculus	2.64 (0.10)	2.72 (0.14)	0%	3.17 (0.12)	20%
Tenia Tecta	2.75 (0.12)	3.03 (0.14)	10%	3.31 (0.08)	20%
Thalamus	2.79 (0.16)	3.33 (0.16)	19%	3.80 (0.16)*	36%

Brain sections adjacent to those used in mu opioid receptor binding (Table 1) were processed for immunohistochemical visualization of mu-opioid receptors using a radiolabeled secondary antibody for autoradiographic quantitation. The increases in immunoreactivity following extended-release naltrexone were seen in only select brain regions and were less robust compared to the changes in receptor binding (Table 1). Note that, in contrast to the increases in binding that were relatively constant between one and two months, the majority of brain regions examined showed increases in immunoreactivity only after two months of extended-release naltrexone. Data are presented as mean ± SEM nCi/gram. * naltrexone vs control ($p<0.05$), ** one vs two months naltrexone ($p<0.05$) (from (59) with copyright permission from Nature Publishing Group, http://www.nature.com).

these increases was lower than those observed with receptor binding, ranging from 9% in the perirhinal cortex to 41% in the substania nigra.

To further examine the increase in mu-opioid receptor density, an independent group of rats was treated with extended-release naltrexone and sacrificed at various time points following the injection. The brains were rapidly removed and the cortex, midbrain and striatum were dissected free and used in a saturation radioligand ([^{3}H]DAMGO) binding assay (69) to examine the time course of the changes in mu-opioid receptor density. Evidence of increased mu-opioid receptor density (B_{max}) was observed as early as 5 days after administration (Figure 7). By 1 week after the naltrexone microsphere injection, the B_{max} for DAMGO binding to the midbrain and striatum was significantly increased (110% compared to placebo treatment). These increases in receptor density were sustained throughout the subsequent 33 days, at least 1 week after significant decline in pharmacodynamic efficacy was observed. The density of cortical mu-opioid receptors, however, did not begin to increase until 30 days after the extended-release naltrexone administration and reached significance (120% increase over placebo treated) at 40 days. In contrast to receptor density, no significant changes in mu-opioid receptor affinity (K_d) was observed in any brain region, at any time point.

4.3.2.2. Suppression of Morphine Analgesia by Extended-Release Naltrexone: Extent of Opioid Receptor Blockade

Concurrent with these neurochemical changes in mu-opioid receptor density, extended-release naltrexone was found to still be effective in blocking morphine-induced analgesia for 28 days as measured using the hot plate (see above). However, this study was done with only a single, low dose of morphine (1 mg/kg). To better understand the depth of opioid receptor blockade mediated by extended-release naltrexone microspheres, the analgesic response of naltrexone treated rats to a range of higher doses of morphine (0.5-50 mg/kg) was studied to determine if there is a shift in the analgesic potency of morphine (i.e., hypersensitivity). Therefore, rats were injected with extended-release Medisorb naltrexone (containing 50 mg/kg naltrexone) or placebo microspheres and tested weekly using the hotplate model of analgesia. The latency to lick the hind paw while on the hot plate was measured and dose-response curves for morphine-induced analgesia were generated. These curves provided estimates of the morphine dose required to induce analgesia in 50% of the treated rats (ED_{50}). This pharmacological index provides a point of comparison between extended-release naltrexone and placebo treated rats, quantifying the degree of suppression of morphine-induced analgesia. One week after administration, naltrexone reduced the analgesic potency of morphine by 15-fold relative to placebo microspheres (ED_{50}= 2.7, 1.3/6 mg/kg vs 42, 21/82 mg/kg, placebo vs naltrexone treated, mean, 95% CI, $p<0.05$; Figure 8). Similar reductions in morphine potency were maintained for the duration of the study (Figure 9). Only by 4 weeks after the initiation of treatment was the 50 mg/kg dose of morphine capable of inducing full analgesia in the extended-release naltrexone treated group. Despite the previous observation of naltrexone-induced increase in brain mu-opioid receptor density, no evidence of hypersensitivity to the analgesic properties of morphine

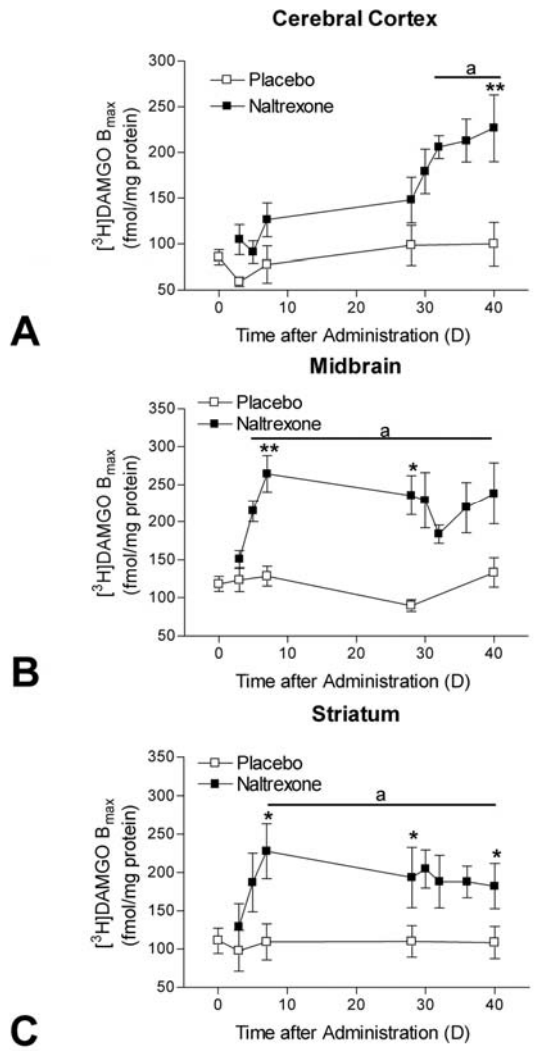

Figure 7. Regional changes in mu-opioid receptor density (B_{max}) in the brain following a single i.m. dose of extended-release Medisorb naltrexone. Data represents the mean ± SEM of data from eight rats (A, B) or eight sets of pooled striata from 16 rats (C). Significant increases in the density of mu receptors in the cerebral cortex were not observed until 32-40 days after the Medisorb naltrexone microspheres injection (A). In contrast, receptor density in the midbrain (B) and striatum (C) was significantly increased by seven days (relative to placebo treated). (a) significantly different from t_0 control ($p<0.05$) *, ** significantly different from contemporaneous control ($P<0.05$ and 0.001, respectively) (from (59) with copyright permission from Nature Publishing Group, http://www.nature.com).

was observed at any time after the extended-release naltrexone administration.

4.4.3. Pharmacokinetic Profile of Extended-Release Naltrexone in Non-Human Primates

The lead formulation of extended-release Medisorb naltrexone displayed good *in vivo* release kinetics, as indicated by plasma naltrexone concentrations in rats (see above), but it is known that there is a substantial species variation with regard to the metabolism of naltrexone (70-72). The major urinary metabolite of naltrexone (6-beta-naltrexol) in the monkey is similar to man, while in the rat no major metabolites have been reported. Thus the PK profile of extended-release naltrexone may be different in rat and non-human primates.

Four Rhesus monkeys (weighing 6.0 to 8.9 kg) received a single dose of the lead extended-release naltrexone formulation (administered subcutaneously in two injections) for a total naltrexone dose of 150 mg per animal (this is equivalent on a weight basis to 20 mg naltrexone/kg in a 7.5 kg monkey). Blood samples for PK analysis were collected at multiple time points out to 48 days following administration of the naltrexone microspheres. Plasma concentrations of naltrexone and its metabolite 6-beta-naltrexol were determined using a LC/MS/MS method.

No significant clinical observations of the injection sites were seen nor was there any appreciable weight loss in individual monkeys over the course of the study. Table 3 summarizes the PK parameters using a normalized dose of 20 mg naltrexone/kg. The plasma profile of 6-beta-naltrexol paralleled the naltrexone profile, but the plasma concentrations were generally 10 times lower then that of naltrexone (Figure 10). These monkey pharmacokinetic data confirm the rat PK profile in that the lead extended-release naltrexone microsphere formulation produced an acceptable naltrexone profile over a one month period.

4.4. Summary

The extended-release formulation of Medisorb naltrexone maintains stable, pharmacologically relevant plasma levels of naltrexone for at least 28 days. The PK profile in rats and monkeys and the PD performance of the extended-release formulation of naltrexone meet the goals set forth early in the development of this drug. Given that both naltrexone and the PLG polymer matrix in which it is encapsulated are well tolerated locally, extended-release Medisorb naltrexone should be applicable to clinical use. This formulation ought to prove safe and effective in the treatment of alcohol dependence by providing an advanced means for maintaining elevated plasma levels of naltrexone for a one month treatment cycle. Currently, Medisorb naltrexone (Vivitrex) is in ongoing multi-center clinical trials.

5. PERSPECTIVE: THE FUTURE OF ALCOHOL DEPENDENCE TREATMENT

While the specific causes of alcohol dependence are still unclear, it is known to develop as a result of a complex interaction among the pharmacological properties of ethanol, the genetic makeup, personality traits and psychological needs of the individual, and a wide variety of environmental influences. Because the causes of dependency vary between individuals, successful treatment of alcohol dependency must be tailored to the individual using a combination of treatment approaches (73).

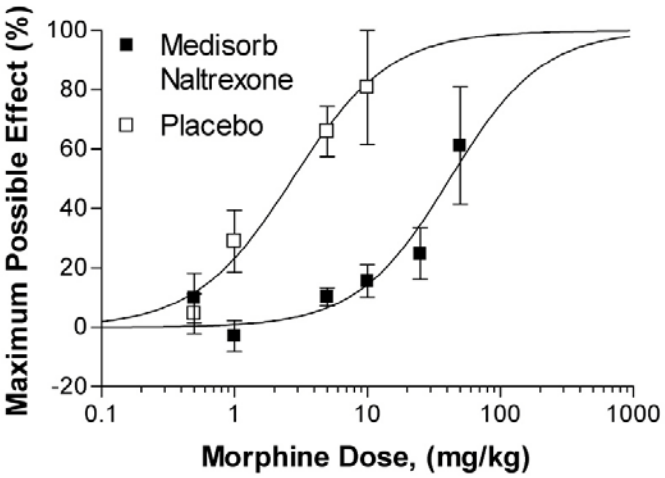

Figure 8. The ability of extended-release Medisorb naltrexone to suppress the analgesic properties of morphine was readily apparent 1 week after administration. While placebo treated rats demonstrated full analgesia (>120 seconds without licking a hind paw) after the administration of 10 mg/kg morphine, the administration of 50 mg/kg morphine to the naltrexone treated rats was still not sufficient to produce full analgesia. Data represents the mean ± SEM of data from eight rats. Percent of maximal possible effect= morphine latency – saline latency/ maximum latency (120 seconds) – saline latency

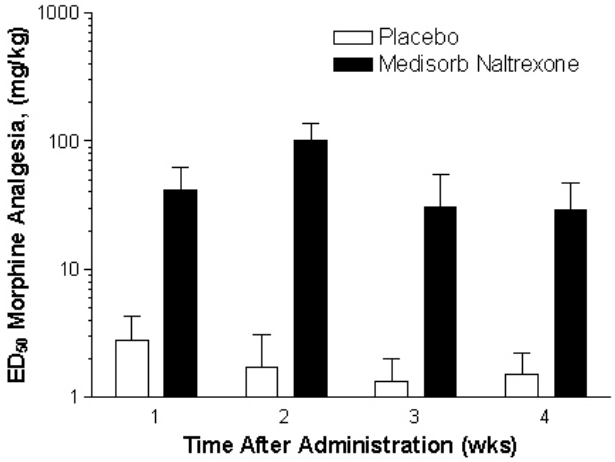

Figure 9. The mean ED_{50} values (log 10 ± SEM) between extended-release Medisorb naltrexone and placebo groups were significantly different at all time points ($p<0.05$). Within the placebo-treated or the extended-release Medisorb naltrexone treated groups, the ED_{50} values for morphine analgesia over the 4 weekly time points were not significantly different.

Table 3. Pharmacokinetic Parameters Following a Single Dose of Extended-Release Naltrexone in Rhesus Monkeys (20 mg/kg Dose)

	C_{max} (ng/mL)	T_{max} (days)	$AUC_{0\text{-}1\ day}$ (ng day/mL)	$AUC_{0\text{-}last\ day}$ (ng day/mL)
Naltrexone	38.0 ± 6.2	0.08 ± 0.0	22.0 ± 4.1	315.0 ± 62
6 beta-naltrexol	1.5 ± 0.5	1.2 ± 0.7	1.1 ± 0.2	28.0 ± 3.8

Values are mean ± SD

Naltrexone was approved by the FDA as an adjunct to psychosocial therapy in the treatment of alcohol dependence. The use of pharmacotherapy alone in the treatment of alcohol dependence is limited in that it does not address the patient's ability to avoid or cope with high risk situations that may initiate drinking (including developing strategies for enhancing patient compliance with medication treatment). While psychosocial therapy facilitates recovery from alcohol dependence by increasing the patient's coping skills with high risk cues or stresses associated with alcohol, it fails to deal with the underlying neurochemical mechanisms of alcohol craving and dependency (74). Recent research has supported the concept of using medications in conjunction with psychosocial therapy in the treatment of alcohol dependency to enhance efficacy (i.e., decrease the urge to drink, increase the number of days abstinent and diminish the risk of relapse to heavy drinking) for longer periods of time (74-76). Besides the type of psychosocial therapy employed, its intensity and duration are also important (73).

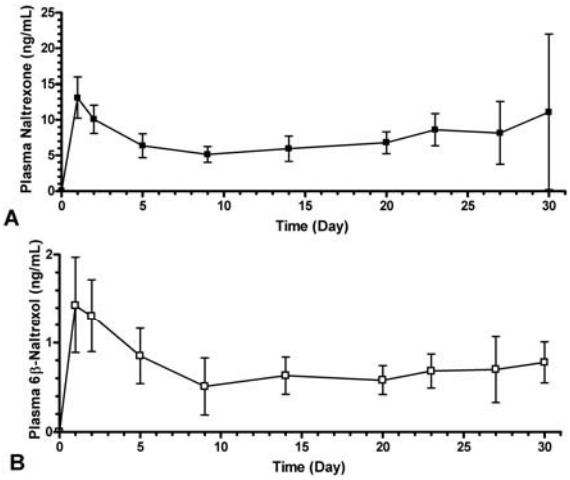

Figure 10. Plasma concentrations of naltrexone (A) and its major metabolite, 6-beta-naltrexol (B) in Rhesus monkeys following a single subcutaneous dose of extended-release Medisorb naltrexone microspheres, normalized to 20mg/kg of naltrexone. The lower limit of quantitation was 0.2 ng/mL for both. Data represents the mean values ± SD from 4 monkeys.

The optimum pharmacological and psychosocial treatment combinations may differ with various subtypes of alcoholics (76, 78).

Given the multiplicity of neurotransmitter systems which are affected by alcohol (reviewed in (79-80)) and may influence alcohol consumption, it is possible that no single drug will produce a consistent robust effect in all alcohol dependent patients (known as a "silver bullet"). Under these conditions, it may be necessary to combine naltrexone with other drugs to address the individual patient's needs and thereby enhance the clinically important treatment effects (81-82).

Naltrexone is an appealing drug in that there are a multitude of potential indications for its use (83). Similarly, extended-release naltrexone may be efficacious in treating any number of addictive disorders or diseases together with appropriate psychosocial therapy. Some of these are currently being explored by Alkermes.

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