Naltrexone Zinc Tannate: A Prolonged-Action Narcotic Antagonist Complex

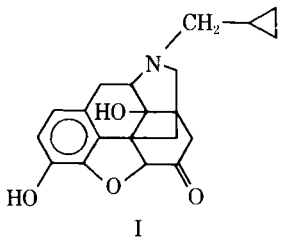
Keyphrases □ Naltrexone zinc tannate—prepared as prolonged-action narcotic antagonist □ Narcotic antagonists—preparation and evaluation of naltrexone zinc tannate □ Drug dependence—preparation and evaluation of naltrexone zinc tannate as prolonged-action narcotic antagonist □ Zinc tannate complex with naltrexone—prepared as prolonged-action narcotic antagonist

Sir:

No fully satisfactory therapeutic regimen is yet at hand for the treatment of narcotic dependence, a major sociomedical problem. Psychotherapy alone may be useful for some drug users but demands strong motivation on the part of the subject and, in any event, would hardly be practicable on a scale adequate to treat the numerous addicts [an estimated 150,000 in New York City alone (1)] currently at large in the United States. Methadone is useful but introduces its own complications since it is basically a substitute for heroin and is itself an addicting drug subject to abuse (1, 2).

A potentially more promising therapeutic approach involves the use of narcotic antagonist drugs. A pure narcotic antagonist has no effects of its own in the dosages used but blocks the effects, including the euphoric effect, of heroin and other narcotics. A drug-dependent subject, first relieved of his or her physical addiction by a brief period of methadone detoxification and then treated with a narcotic antagonist, would not experience a euphoric reaction to heroin. The psychological dependence would not be reinforced and, ideally, time would be available for other treatment modes and social changes to reduce and eventually eliminate dependence on drugs. The difficulty with existing narcotic antagonist drugs is that they are too short acting since, unlike methadone, they provide no incentive to the subject to return for frequent maintenance therapy (1, 3, 4). A need has thus been indicated for injectable narcotic antagonist preparations with a prolonged duration of action (1, 3).

Our approach to this problem has involved development of sparingly soluble salts and salt complexes of existing narcotic antagonists inasmuch as precedents in other therapeutic areas show that intramuscular injection of such form can provide slow release of drug and a useful prolongation of action (5–7). We prepared numerous complexes of the antagonists naloxone and cyclazocine (8), evaluated their duration of action in mice, and found zinc tannate complexes to provide the most significant prolongation of effect (8). Naloxone zinc tannate showed a prolongation of action much greater than that shown by naloxone pamoate (8, 9)[1,2].



I

Since naltrexone (I), a close analog of naloxone, has been reported to be essentially as pure an antagonist as is naloxone and to be two to three times as potent with about twice the duration (9), we then prepared complexes of this drug. We found naltrexone zinc tannate to provide a highly promising increase in duration of action in mice. Duration was further enhanced when the complex was incorporated in an aluminum monostearate gel.

Our methods were essentially as described earlier (8). Naltrexone tannate was prepared by stirring an aqueous solution of naltrexone hydrochloride[3] with an aqueous solution of an equivalent amount of gallotannic acid [assuming 5 equivalents/mole and an approximate molecular weight of 1700 (10)] just neutralized with 0.2 *N* NaOH. The resultant precipitate of naltrexone tannate was washed with water and dried *in vacuo* over phosphorus pentoxide.

Naltrexone zinc tannate was prepared by stirring an aqueous suspension of the tannate with 1 equivalent of 1 *M* solution of zinc sulfate and washing and drying as before.

To analyze for naltrexone, an aqueous suspension of a weighed amount of complex was treated with a pH 10.4 phosphate buffer, sodium chloride was added, and the pH was adjusted to 9.5–10 with 20% NaOH. The aqueous mixture was extracted with chloroform containing 1% 2-propanol, and the organic extract was shaken with 0.1 *N* HCl. The acid solution was diluted to standard volume, the absorbance at 281 nm was determined with a UV spectrophotometer[4], and naltrexone content was read off a standard curve.

Zinc content was determined by shaking a suspension of a weighed amount of complex in 1 *N* HCl, centrifuging, serially diluting an aliquot of the supernatant solution with distilled water and 0.1 *N* HCl, and measuring the responses of an atomic absorption flame-emission spectrophotometer[5] in comparison with the dose–response of standard zinc solutions (Fisher standard reference solution of zinc oxide in dilute nitric acid, serially diluted with 0.1 *N* HCl).

[1] Naloxone pamoate showed a significant increase in duration of action only when incorporated in an aluminum monostearate gel. We thank Dr. H. Blumberg and Dr. A. Rubin for information on the composition of this gel.

[2] W. R. Martin, NIMH Addiction Research Center, and L. S. Harris and W. L. Dewey, Medical College of Virginia, personal communications.

[3] Endo Laboratories.

[4] Beckman model DU-2.

[5] Jarrell-Ash model 82-270.

Table I—Analytical Data: Amount of Tannic Acid in Complexes Assumed by Difference

Naltrexone Complex	Melting Point	Drug, %		Zinc, %	Mole Ratios		Dissociation, % at 37°[a]
		Calc.	Found		Drug/ Acid	Zinc/ Acid	
Hydrochloride	>250°	90.3	—	—	—	—	100
Tannate	197–199°	50.1	49.4	—	4.9	—	38.2
Zinc tannate	>250°	—	31.3	4.19	2.4	1.7	11.5

[a] Percent dissociation is percent of drug dissociating from complex suspended in isotonic phosphate buffer, pH 7.3.

Table II—Duration of Antagonism for Dose Containing 4.0 or 40.0 mg/kg Naltrexone Base Administered Intramuscularly at Indicated Time before Tests

Dose Containing 4.0 mg/kg Naltrexone Base

Naltrexone Complex	Vehicle	Percent Antagonism[a,b] at: Hours			Days						
		4	8	16	1	2	3	4	5	6	7
Hydrochloride	Peanut oil	100	76	28	0	—	—	—	—	—	—
Tannate	Peanut oil	100	76	37	19	5	—	—	—	—	—
Zinc tannate	Peanut oil	99	94	86	67	31	43	29	7	—	—
Zinc tannate	Aluminum monostearate gel	—	—	—	100	91	72	53	23	29	0

Dose Containing 40.0 mg/kg Naltrexone Base

Naltrexone Complex	Vehicle	Percent Antagonism[a,b] at (Days):											
		1	2	3	5	7	9	12	14	15	17	19	21
Hydrochloride	Peanut oil	39	18	—	—	—	—	—	—	—	—	—	—
Tannate	Peanut oil	84	59	25	—	—	—	—	—	—	—	—	—
Zinc tannate	Peanut oil	100	—	96	39	18	9	—	—	—	—	—	—
Zinc tannate	Aluminum monostearate gel	100	—	94	83	78	86	86	74	73	68	71	39

[a] Each value represents the mean of 12 mice. Each mouse was used only once. [b] Percent antagonism to a standard dose of 20 mg/kg morphine sulfate administered intraperitoneally 30 min before test.

To determine *in vitro* dissociation, a weighed amount of complex calculated to contain 15 mg of naltrexone was suspended in 10 ml isotonic phosphate buffer, pH 7.3 (125 ml of 0.2 *M* KH_2PO_4, 85 ml of 0.2 *N* NaOH, and 2 g NaCl, diluted with distilled water and pH adjusted to 7.3 with additional 0.2 *N* NaOH; final volume 500 ml). The suspension was stirred (magnetic stirrer) for 1 hr in a 37.0 ± 0.1° bath and filtered, and an aliquot of the filtrate was bought to a pH of about 10 with 20% NaOH and treated with an additional 2 g NaCl. The mixture was extracted with chloroform containing 1% 2-propanol, and the naltrexone content was determined spectrophotometrically as before. Analytical and dissociation data are given in Table I.

Antagonism of analgesia was determined by the tail-flick procedure of Harris and Pierson (12) with the mouse substituted for the rat as the test animal (11). Dose–response curves and ED_{80} (antagonism) values were obtained based on measurement of reaction time 40 min after intramuscular administration of naltrexone preparation and 30 min after intraperitoneal administration of 20 mg/kg of morphine sulfate, which was the ED_{80} (analgesia) dose under normal conditions. Surprisingly, naltrexone hydrochloride, tannate, and zinc tannate, administered by intramuscular injection in peanut oil suspension, all showed the same ED_{80} (antagonism) value, *i.e.*, the dose producing 80% antagonism at 40 min contained 0.02 mg/kg naltrexone base.

Duration of antagonist activity was evaluated by dosing mice intramuscularly with naltrexone preparations, challenging groups of these mice at the intervals indicated in Table II with a 20-mg/kg ip dose of morphine sulfate, and testing their reaction time in the tail-flick test 30 min thereafter. Naltrexone preparations were injected in peanut oil suspension and also incorporated in an aluminum monostearate gel. The gel was prepared by slowly heating, with stirring, a mixture of 2 g aluminum monostearate[6] in 100 ml of peanut oil to 125° and then allowing the gel to cool to room temperature[1]. Duration of activity was determined at dose levels calculated to contain 4.0 and 40.0 mg/kg of naltrexone base (Table II). At least 12 mice were used for each determination (at each dose level and time interval), and no mice were used for more than one determination. Thus, mice were not repeatedly exposed to the test procedure; each mouse was exposed only to two control tail flicks and one test tail flick.

It is clear that duration of activity *in vivo* followed percent dissociation *in vitro* (Table I) quite well in this limited series, although there were exceptions in more extensive series of naloxone and cyclazocine complexes (8). Naltrexone tannate showed some in-

[6] Mallinckrodt.

crease in duration relative to the hydrochloride salt, more clearly at 40.0 than at 4.0 mg/kg. The zinc tannate complex in peanut oil showed at least a threefold prolongation of the activity of the hydrochloride, and there was a further roughly threefold prolongation of activity when the zinc tannate was incorporated in the aluminum monostearate gel. Indeed, naltrexone zinc tannate in the gel medium still evidenced 70% antagonism 19 days after injection of the 40.0-mg/kg dose. The mice showed no behavioral or other adverse drug effects even at the 40-mg/kg dose.

This work is continuing. We earlier speculated as to the nature of the zinc tannate complexes (8) and indicated that the metal ion appeared to bond covalently to the same sites as were involved in ionic bonding with drug since the zinc displaced an equivalent amount of drug from the complex and the equivalents ratios remained substantially constant. We recently found[7] that by varying the method of preparation, we can increase the amount of zinc in the complex without further reducing the amount of drug. The resultant complexes show reduced percent dissociation *in vitro* and increased duration of activity *in vivo*. We also prepared analogous complexes containing aluminum in place of zinc which show somewhat enhanced duration of activity.

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Received July 2, 1973.

Accepted for publication October 15, 1973.

Supported by a contract from the City of New York, Health Services Administration, and by PHS Grant 5R01 DA00133 (formerly R01 MH 21361), National Institute of Mental Health.

We thank Mrs. Brenda A. Ley and Mrs. Marylin R. Cater for outstanding technical assistance.

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[7] Unpublished data.

Conversion of Apocodeine to Apomorphine and Norapomorphine in Rats

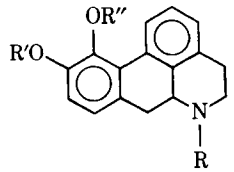
Keyphrases □ Apocodeine—metabolic conversion to apomorphine and norapomorphine, rats □ Apomorphine and norapomorphine—urinary metabolites of apocodeine, rats □ Norapomorphine and apomorphine—urinary metabolites of apocodeine, rats

Sir:

There is renewed and increased interest in the biological activities of aporphine compounds. This has resulted in part from the finding that apomorphine (I) has some usefulness in the treatment of Parkinson's disease (1–8). Apocodeine (II) is an important homolog of I. The relatively poor dopaminergic activity of II (and III) in pigeons and dogs (9, 10) led Cannon *et al.* (10) to theorize that both phenolic groups of I are required to exert central emetic effects. Recently, however, Lal *et al.* (11) reported that II induced stereotyped behavior and caused reversal of reserpine-induced catalepsy in rats similar to that of I. While its activity is described as intermittent, II is clearly one-fourth as active as I (11). Lal *et al.* (11) suggested that these latter observations might be explained by *in vivo* conversion of II to I. Our recent experiments show that I and norapomorphine (IV) are metabolites of II in rats.

Male Sprague–Dawley[1] rats (175–300 g) were injected with II-HCl (30 mg/kg ip); urine, separate from feces, was collected for 48 hr following injections and frozen. The first and second 24-hr samples (4–12 ml) were made up to 12 ml with distilled water; 3 ml of 3 *N* hydrochloric acid was added to each, and the mixtures were heated at 100° for 1 hr. The hydrolysates were adjusted to pH 7.0 with 1 *N* sodium hydroxide and extracted with three 10-ml portions of ethyl acetate. The separators were rinsed with 10 ml of ethyl acetate and the combined extracts were reduced to dryness *in vacuo;* the residues were dissolved in 1–2 ml of acetone for TLC analysis.

Sample and blank urine extracts were spotted on silica gel GF_{254} plates[2] (250 μm) and then developed in the following solvent systems: 1, chloroform–methanol (85:15); 2, acetone; and 3, acetone–methanol (1:1). Detection was *via* quenching of 254 nm-induced fluorescence and visualization of plates allowed to stand in the air for 12–24 hr. Authentic I[3]



I: R = CH_3; R′, R″ = H
II: R, R′ = CH_3; R″ = H
III: R, R′, R″ = CH_3
IV: R, R′, R″ = H

[1] Sprague–Dawley Co., Madison, Wis.
[2] Analtech, Newark, Del.
[3] S. B. Penick, New York, N.Y.