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Naltrexone metabolism and sustained release following administration of an insoluble complex to rhesus monkeys and guinea-pigs

THOMAS R. MACGREGOR, MARY A. DRUM, STEPHEN E. HARRIGAN†, JAMES N. WILEY†, AND RICHARD H. REUNING*

* *College of Pharmacy, The Ohio State University, Columbus, Ohio, U.S.A. and † Warner-Lambert/Parke-Davis Co, Detroit, Michigan, U.S.A.*

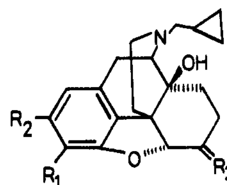
Both the release and the metabolism of naltrexone have been evaluated after intramuscular administration of a sustained release [15,16-$^3H_2$]naltrexone aluminium tannate complex in guinea-pigs and rhesus monkeys. In both species, measurable excretion of radioactivity was obtained for >50 days and complete recovery of the dose was obtained in the guinea-pig. The radioactivity excretion rate-time profile differed in the two species with guinea-pig yielding a continuously declining rate and monkey yielding a peak at 5 days. In selected monkey urine samples (days 4, 17–20 and 49–52) subjected to t.l.c., evidence was obtained for the presence of naltrexone, β-naltrexol and 2-hydroxy-3-*O*-methyl-β-naltrexol, mostly as glucuronide and/or sulphate conjugates. The t.l.c. data also suggest that in monkey a naltrexone metabolite builds up relative to naltrexone over the 52 day release period.

Naltrexone is a potent narcotic antagonist undergoing clinical studies in man. A major drawback in its use in opium addiction is its short duration of action. Therefore, sustained release preparations capable of long-term blocking of opiates have been sought (see Willette 1978 for a review of these sustained release dosage forms and their properties). One of the earlier sustained release systems to be developed was an insoluble salt-metal complex containing naltrexone (Gray 1974; Gray & Robinson 1974).

It has previously been shown that after chronic oral dosing naltrexone is extensively metabolized in the monkey, primarily to β-naltrexol and conjugates (Dayton & Inturrisi 1976). Potential metabolic consequences of sustained release drug input are either a change in clearance by one or more individual metabolic pathways or a gradual relative buildup of a metabolite having a long elimination half-life.

Our objective was to characterize both the sustained release and the metabolic profile after intramuscular administration of a naltrexone aluminum tannate complex. The release of naltrexone over a 52–58 day period was studied in the guinea-pig and rhesus monkey. In an attempt to detect any changes in metabolite elimination upon sustained release administration, quantitative thin-layer chromatography was utilized to compare relative amounts of each of the metabolites (Fig. 1) potentially present in monkey urine.

* Correspondence.



Compound	R_1	R_2	R_3
(I) Naltrexone	HO–	H–	=O
(II) α-Naltrexol	HO–	H–	H, OH
(III) β-Naltrexol	HO–	H–	OH, H
(IV) 2-Hydroxy-3-*O*-methyl-β-naltrexol	CH_3O–	HO–	OH, H
(V) 3-*O*-Methyl-β-naltrexol	CH_3O–	H–	OH, H
(VI) 2-Hydroxy-3-*O*-methylnaltrexone	CH_3O–	HO–	=O

FIG. 1. Chemical structures of naltrexone and its metabolites identified in animals and/or man.

MATERIALS AND METHODS

Compounds

The [15,16-$^3H_2$]naltrexone aluminum tannate (23·8% naltrexone, 1·8% aluminum) complex was supplied by Dr Allen P. Gray (Illinois Institute of Technology Research Institute, Chicago) and had a specific activity of 210·6 μCi mg^{-1} naltrexone base for the monkey studies and 0·62 μCi mg^{-1} for the guinea-pig studies. Synthesis of this complex has been described previously (Gray & Robinson 1974). Naltrexone (I, obtained from Endo Laboratories)

and 2-hydroxy-3-*O*-methylnaltrexone (VI), 2-hydroxy-3-*O*-methyl-β-naltrexol (IV), and 3-methoxy-β-naltrexol (V, all synthesized at Research Triangle Institute, North Carolina) were obtained as standards and used without further purification. Both α- (II) and β-naltrexol (III) were synthetically prepared (Malspeis et al 1975) and purity was verified by spectral and chromatographic techniques.

Dose administration

The injection suspension for monkeys consisted of 589 mg [15,16-$^3H_2$]naltrexone aluminum tannate in 2·5 ml 2% aluminum monostearate (Mallinckrodt) in U.S.P. peanut oil (Ruger). The drug was administered to three rhesus (*Macaca mulatta*) monkeys (2 females and 1 male, 3·4–4·5 kg) intramuscularly via the right *rectus femoris* muscles at a dose of approximately 9 mg kg^{-1}.

After the injections in monkeys all syringes, mortars, and other items used to prepare the dose were rinsed with n-amyl alcohol and assayed for radioactivity to determine the administered dose. The alcohol rinse was filtered through weighed filter paper, which was dried and reweighed. Five weighed aliquots of the dried residue were combusted in the sample oxidizer, each combusted sample was diluted to 50 ml with Monophase 40 (Packard) and five 500 μl aliquots from each dilution were assayed by counting. The filtrate was diluted to 100 ml with additional n-amyl alcohol. Five 100 μl aliquots were dried, combusted and assayed. The activity in the alcohol rinse was found to be 6·66 mCi, leaving 22·8 mCi received by the three monkeys. The dose per monkey was arrived at by determining the total radioactivity received by all three animals, then calculating the percentage of this activity each monkey received, based on the volume injected per monkey.

The injection suspension for guinea-pig consisted of 10 mg [15,16-$^3H_2$]naltrexone aluminum tannate in 1·0 ml of 2% aluminum monostearate in peanut oil. Just before injection, the suspension was agitated to assure a uniform suspension. The suspension was given intramuscularly at a dose of 20 mg kg^{-1} to four male guinea-pigs (400–540 g, Camm) via an 18 gauge needle into the thigh muscles, the dose being equally divided between both rear legs. In addition, the injection site upon autopsy was examined for gross evidence of necrosis before assay. There was no evidence of muscle injury from the preparation.

Sampling and assay for total radioactivity

In the monkey blood samples were taken at 2, 4, 8, 16 and 24 h, daily through day 8, and on days 12, 16, 33 and 52 from the saphenous vein. Plasma was separated by centrifugation. Urine (total excreted) was collected at 8 and 24 h, daily through day 8, and pooled samples were obtained for 4-day periods through day 52. All faeces were collected daily through day 8 and at 4-day intervals thereafter, as for the urine. After collection, faeces were homogenized with 9 parts water to obtain uniformity. Monkeys were housed in such a manner as to collect total urine and faeces separately as much as possible although some urine collections appear to be incomplete due to inadequate design of the cages.

Weighed aliquots of urine, plasma and faeces homogenate from each collection period for each monkey were combusted and assayed by liquid scintillation counting. After 52 days two of the monkeys were overdosed with pentobarbitone and the rectus femoris muscle excised, cut into small pieces, combusted and assayed for radioactivity. The excised tissue had no evidence of necrosis and contained negligible amounts of radioactivity.

For the guinea-pig, urine and faeces were collected separately in pooled 3 day collection periods. Weighed aliquots of urine and faeces homogenate from each collection for each guinea-pig were combusted and assayed by liquid scintillation counting.

Thin layer chromatography (t.l.c.)

Thin layer chromatographic conditions and retention indices for naltrexone and metabolites are given in Table 1. The presence of filter paper lining the development tank (20 × 20 × 5 cm) had an influence on the separation and tailing of the compounds. As is apparent from Table 1, the quantitative estimation of each metabolite in monkey urine required utilization of both solvent systems A and C.

Urine preparation for t.l.c.

An aliquot of rhesus monkey urine (0·5–1·0 ml) was concentrated by vacuum distillation (Lester 1964) and the residue reconstituted in 100 μl of distilled water or methanol. Negligible (<0·2% of sample radioactivity) amounts of radioactivity were found in the distillate. Quantitative t.l.c. was then performed using 10 μl of reconstituted urine.

Enzyme hydrolysis

Urine (50 μl) was incubated at 37 °C with Glusulase (Endo Laboratories) diluted 1:10 with acetate buffer

Table 1. Thin layer chromatography of naltrexone and its metabolites.

	R_F value[1]		
A[2,3]	B[2,4]	C[2,4]	D[2,4]
Naltrexone			
0·76(0·05)	0·24(0·02)	0·43(0·02)	0·16(0·03)
α-Naltrexol			
0·39(0·09)	0·29(0·02)	0·17(0·02)	0·06(0·01)
β-Naltrexol			
0·35(0·07)	0·34(0·03)	0·23(0·01)	0·06(0·01)
3-*O*-Methyl-β-naltrexol			
0·83	—	0·61	—
2-Hydroxy-3-*O*-methyl-β-naltrexol			
0·57	—	0·59	—
2-Hydroxy-3-*O*-methylnaltrexone			
0·89	—	0·45	—

[1] Mean (standard deviation) upon development to 150 mm.
[2] The t.l.c. systems consisted of glass backed silica gel plates with fluorescent indicator and the following developing solvents and conditions: (A) chloroform–methanol–ammonia (90:10:0·5 ml, no paper), (B) ethyl acetate–acetic acid (9:1, paper lined), (C) chloroform–acetone–diethylamine (2:16:3, paper lined), and (D) cyclohexane–ethyl acetate–ammonia (30:70:0·75 ml, no paper).
[3] For naltrexone and α- and β-naltrexol n = 24 and for the others n = 1.
[4] For naltrexone and α- and β-naltrexol n = 17 and for the others n = 1.

(0·2 M, pH 5·0) for 24 h according to Ludden et al (1978). The enzymatically-treated urine was then vacuum distilled and chromatographed as before.

Scintillation counting

Biological samples and solutions were all combusted on a Packard Tri-Carb 306 Oxidizer and supplemented with the universal cocktail Monophase 40. To determine the distribution of radioactivity on the thin layer sheets, the silica gel was removed in small, measured increments (2–10 mm) by scraping, the scrapings placed in scintillation vials, distilled water (4 ml) added to each vial, the mixture suspended with 10 ml Instagel (Packard) and the suspension assayed by liquid scintillation counting. All samples were 'counted' using a Beckmann LS-345 refrigerated liquid scintillation counter equipped with automatic external standardization. Reference to a quench curve was made and the efficiency did not vary significantly from vial to vial for either the combusted or t.l.c. samples.

RESULTS AND DISCUSSION

An essentially complete recovery of the dose (102% mean s.d. 6%) was obtained from urine and faeces collected for 58 days following intramuscular administration of naltrexone aluminum tannate in guinea-pigs. The plots of rate of excretion of total radioactivity as a function of time in the four guinea-pigs (Fig. 2) reveal that an initially high rate was followed by a slowly declining excretion rate. Plots of amount remaining in the animal vs time were close to being monoexponential but exhibited systematic curvature, either concave or convex. The time required, mean (s.d.), for 50% elimination of the dose was 9·5 (1·6 days) and 90% elimination was 35·7 (6·6) days. The faeces collected over the 58 days contained 21·5 (4·2) of the radioactivity administered which was comparable to the 12·4 (3·8)% reported for a single intramuscular naltrexone dose in the guinea-pig (Ludden et al 1978).

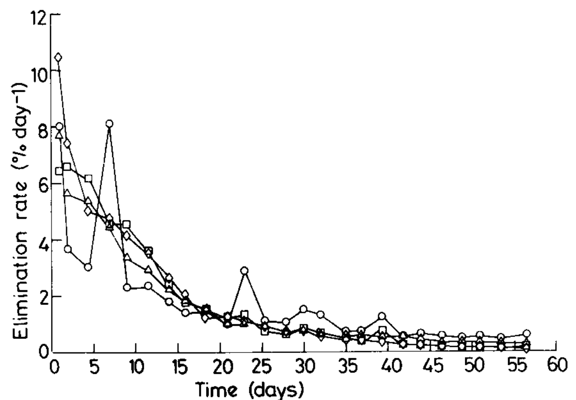


FIG. 2. Rate of elimination of radioactivity (sum of urine and faeces), expressed as percent of the recovered dose per day, as a function of time after intramuscular administration of [15,16-$^3H_2$]naltrexone aluminum tannate (20 mg kg^{-1} naltrexone base equivalent) in 2% aluminum monostearate-peanut oil to four guinea-pigs (○ □ △ ◇).

A plot of rate of excretion of radioactivity as a function of time after intramuscular injection of the sustained release preparation in the three rhesus monkeys (Fig. 3) shows the radioactivity excretion rate increased initially with a peak at 5 days followed by a gradually diminishing excretion rate until the end of the sampling at 52 days. Thus, the naltrexone aluminum tannate suspension provided measurable release of naltrexone for more than 50 days in both species. However, much drug was wasted in the initial rapid release phase.

Research in mice (Gray & Robinson 1974) has shown that naltrexone zinc tannate in peanut oil has a short duration of action but that the addition of 2% aluminum monostearate to the vehicle slows down release as well as acting as a suspending agent. Presumably, the vehicle protects the naltrexone complex from aqueous attack by biological fluids. Therefore, it is probable that the excretion rates for

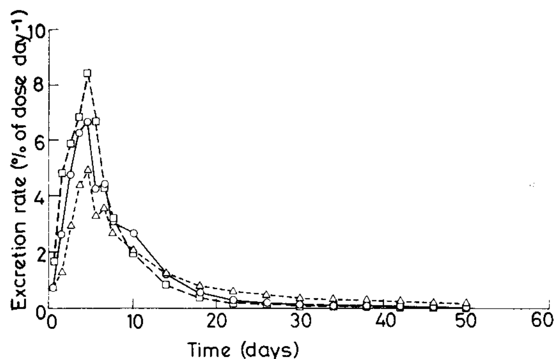


FIG. 3. Rate of excretion of radioactivity (sum of urine and faeces), expressed as percent of the administered dose per day, as a function of time after intramuscular administration of [15,16-$^3H_2$]naltrexone aluminum tannate (9 mg kg^{-1} naltrexone base equivalent) in 2% aluminum monostearate-peanut oil to three rhesus monkeys (○ □ △).

the monkeys (Fig. 3) are representative of radioactivity release from the delivery system suspension and that both the aluminum tannate complex and the vehicle are essential components of the sustained release system. Comparison of the plasma radioactivity levels with the excretion rates in urine and faeces (Fig. 4) indicates that there is no significant delay between changes in plasma level and changes in excretion rates. This result further confirms that the rate limiting step for excretion is not elimination of radioactivity from the animal but rather release of radioactivity from the sustained release system. A comparison of the amount of radioactivity excreted in urine with that excreted in faeces for one monkey is also shown in Fig. 4. Overall in the three monkeys the faeces contained approximately 20% of the intramuscular dose. However, a total of only 60% of the dose was recovered after 52 days due to occasional urine sample loss from the metabolism cage.

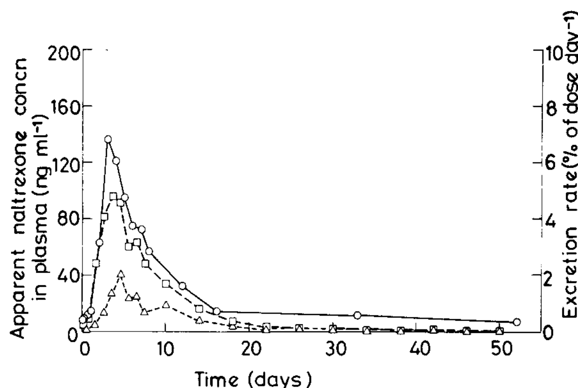


FIG. 4. Time profile of plasma radioactivity, expressed as apparent naltrexone concentration, and of urinary and faecal excretion rates in a rhesus monkey that received [15,16-$^3H_2$]naltrexone aluminum tannate in 2% aluminum monostearate-peanut oil intramuscularly. ○ Plasma □ Urine △ Faeces.

The reason for the difference between guinea-pig and rhesus monkey with respect to the radioactivity excretion rate-time profile is not apparent from the data. Possible explanations include (1) a difference between the different lots of the naltrexone aluminum tannate, or (2) the difference between species in the injection site with perhaps the smaller muscle mass of the guinea-pig permitting part of the injection to leak into subcutaneous areas and release its naltrexone more rapidly. A third possible explanation would be a difference between the two species in physiology and/or biochemistry that somehow influences the stability of the sustained release system and thus the release of naltrexone from that system. The fact that the main difference between species occurred during the first 5 days after administration suggests that differences in the intramuscular injection site may be responsible.

Quantitative thin-layer chromatography of the monkey urines at three time intervals revealed evidence for the presence of β-naltrexol and 2-hydroxy-3-*O*-methyl-β-naltrexol following intramuscular injection of naltrexone aluminum tannate. Comparison of Table 2 with Table 3 shows that these

Table 2. Thin layer chromatography of radioactivity from untreated rhesus monkey urine.

Compound	Radioactivity found at the R_F value corresponding to each compound[1]		
	day 4[2]	days 17–20[2]	days 49–52[2]
Origin-R_F 0·07	92(2)	90(3)	88(5)
Naltrexone	1·5(0·2)	2·3(0·4)	5·9(4·8)
α-Naltrexol[3]	0·2(0·1)	0·4(0·2)	0·2(0·3)
β-Naltrexol	0·9(0·2)	1·2(0·2)	1·0(0·6)
2-Hydroxy-3-*O*-methyl-β-naltrexol	0·3(0·1)	0·6(0·2)	0·7(0·2)
3-*O*-Methyl-β-naltrexol[3]	0·1(0·1)	0·1(0·1)	0·1(0·1)
2-Hydroxy-3-*O*-methylnaltrexone[3]	0·1(0·1)	0·1(0·1)	0·2(0·2)

[1] Mean (standard deviations) where n = 6 determinations (3 monkeys × 2 solvent systems) except for 3-*O*-methyl-β-naltrexol and 2-hydroxy-3-*O*-methylnaltrexone where n = 3 determinations (3 monkeys × solvent system of resolution). Values are expressed as a percent of total radioactivity found on the plate.

[2] Interval for urine collection expressed as the number of days after administration of the naltrexone salt-metal complex.

[3] Considered to be trace amounts, if present.

Table 3. Thin layer chromatography of radioactivity from Glusulase-treated rhesus monkey urine.

Radioactivity found at the R_F value corresponding to each compound[1]		
day 4[2]	days 17–20[2]	days 49–52[2]
Origin-R_F 0·07		
46(10)	55(15)	63(10)
Naltrexone		
30(6)	18(6)	12(3)
α-Naltrexol[3]		
1·3(0·6)	1·8(1·1)	0·7(0·5)
β-Naltrexol		
14(3)	14(6)	5·3(3·0)
2-Hydroxy-3-*O*-methyl-β-naltrexol		
2·3(1·1)	4·2(0·9)	2·4(1·4)
3-*O*-Methyl-β-naltrexol[3]		
1·3(1·3)	0·3(0·3)	0·4(0·2)
2-Hydroxy-3-*O*-methylnaltrexone[3]		
0·3(0·3)	0·2(0·2)	0·9(0·7)

[1,2,3] as in Table 2.

metabolites are largely present as glucuronide and/or sulphate conjugates. The finding of 13% conjugated β-naltrexol and 28% conjugated naltrexone in the urine compares favourably with results from chronic oral dosing of naltrexone in monkeys reported previously (Dayton & Inturrisi 1976). The finding of 2-hydroxy-3-*O*-methyl-β-naltrexol in the urine of monkeys after administration of a sustained release naltrexone preparation parallels the finding of this metabolite in man following chronic oral dosing of naltrexone (Verebely et al 1975). Our data show this metabolite to be present to the extent of approximately 2–4% and largely as glucuronide and/or sulphate conjugates in monkey. The other known metabolites were found only in trace amounts as glucuronide and/or sulphate conjugates.

There appears to be a significant fraction of the radioactivity in the region near the origin (R_F = 0–0·07) after enzymatic hydrolysis. This region did not correspond to the R_F of any of the known non-conjugated metabolites of naltrexone. A check of the enzymatic conditions needed for maximal hydrolysis of any hydrolysable conjugates showed that ample time and enzyme were available. Triple extraction of Glusulase-treated urine with ethyl acetate revealed that essentially all the radioactivity was extractable. It is therefore possible that a very polar unknown metabolite of naltrexone is present and the data suggest a build up of this metabolite relative to naltrexone during the 52 days of sustained release of naltrexone in the monkey. Thus, either the metabolite has a much longer half-life than naltrexone, or the clearance of naltrexone and/or the metabolite changes during the 52 day study.

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