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DISPOSITION OF NALOXONE-7,8-^{3}H IN NORMAL AND NARCOTIC-DEPENDENT MEN[1]

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ABSTRACT

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Naloxone-7,8-^{3}H was administered intravenously and orally on separate occasions to the same normal male subject and its disposition was examined. The fate of intravenous naloxone-7,8-^{3}H was also studied in an opiate-dependent subject both while on heroin maintenance and after withdrawal. In all cases the urinary excretion was rapid but incomplete, never exceeding 70% of the dose over 72 hours. Initial plasma concentrations of naloxone were low with a rapid rate of disappearance. Oral naloxone entered plasma quickly but in a metabolized form. The volume of distribution, plasma half-life and metabolic clearance rate of naloxone as calculated from the intravenous studies were about 200 liters, 90 minutes and 2500 liters/day, respectively.

Naloxone is a potent narcotic antagonist (Blumberg *et al.*, 1961; Foldes *et al.*, 1963) which is virtually free of agonist activity (Jasinski *et al.*, 1967). It is presently in clinical use as an antidote to narcotic overdosage (Narcan, Endo Laboratories, Garden City, N.Y.) and is also under active investigation as a therapeutic agent in postaddict chemotherapy (Zaks *et al.*, 1971). For the latter purpose, it suffers from the disadvantages of a short duration of action when given parenterally and from a low effectiveness when taken orally. With the exception of the identity of some of its metabolites in human urine (Fujimoto, 1970; Weinstein *et al.*, 1971), little is known about the fate of naloxone in man. Information about the disposition of naloxone in man would be of particular value since it may reveal the reasons for its short duration of action and oral ineffectiveness and permit the design of methods to circumvent these undesirable features of its pharmacology.

The synthesis of naloxone-7,8-^{3}H of high specific activity and biological isotope stability (Fishman *et al.*, 1973) provided the necessary substrate for the investigation of the fate of this material in man. We have administered naloxone-7,8-^{3}H to a volunteer normal subject both intravenously and orally and followed its plasma distribution and urinary excretion. To examine the effect of narcotic agonists on the disposition of intravenous naloxone, we have administered naloxone-7,8-^{3}H to a volunteer opiate-dependent subject while on heroin maintenance and then again when he was opiate-free. The results of these studies with particular emphasis on the pharmacokinetics of naloxone are reported in this communication.

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Methods

Naloxone (N-allylnoroxymorphone) was obtained as the hydrochloride from Endo Laboratories. The free base was obtained from an aqueous solution of the hydrochloride by precipitation with ammonia.

Naloxone-7,8-^{3}H was prepared in these laboratories (Fishman *et al.*, 1973) and was diluted with inert material to a specific activity of 0.5 c/mmol and 25 mc/mmol.

Subject JA was a normal 42-year-old man weighing 81 kg who was free from all medication during and immediately prior to the study. Subject FR was a 23-year-old man weighing 64 kg with a history of opiate dependence. At the time of the first study, he was being maintained on a total of 35 mg of heroin a day at the Montefiore Hospital Clinical Center. Heroin maintenance was then terminated and methadone in conjunction with small doses of tranquilizers was substituted. This regimen with decreasing amounts of methadone was continued for about five weeks at which time the methadone was stopped completely. The second study with naloxone-7,8-^{3}H was carried out one week later while the subject was receiving only chlorpromazine and diazepam.

The naloxone-7,8-^{3}H was dissolved in freshly distilled propylene glycol. A weighed aliquot of the dose was retained for counting and the remainder (1–2 ml) was injected as a bolus into the antecubital vein. The weight of the material injected was determined by the weight difference of the syringe empty and full and thus permitted calculation of the dose administered. For oral administration a weighed amount of the propylene glycol solution was diluted with orange juice and administered in the morning after overnight fasting. Subject JA received naloxone-7,8-^{3}H, specific activity 25 mc/mmol, which represented a dose of 125 μg i.v. and 100 μg p.o. To prevent withdrawal symptoms, subject FR received naloxone-7,8-^{3}H with a specific activity of 0.5 c/mmol and the doses contained 4 and 4.5 μg. Blood samples of 10 ml each were collected into heparin-treated containers at specific intervals after administration. The plasma and red cells were separated by centrifugation and their radioactive content was determined. The red blood cells were first digested in NCS (supplied by Amersham/Searle, Chicago, Ill.), bleached with benzoyl peroxide, and then counted in toluene-phosphor to show no counts above background. The plasma was counted directly in a Packard liquid scintillation counter in diotol with suitable quenching corrections by means of an internal standard. Aliquots of the plasma samples in each study was diluted with ~10 mg of naloxone and extracted with ethyl acetate and the extracts were counted after removal of solvent. The ethyl acetate extracts were then purified on preparative thin-layer chromatography in the system chloroform–methanol–acetic acid (100:60:2). The zone corresponding to free naloxone visualized by ultraviolet absorption was eluted with ethanol and the specific activity of the naloxone was determined.

Urines were collected at intervals for 72 hours. Aliquots of each urine collection were diluted with water and counted. In every case all efforts were made to obtain complete collections, and creatinine determinations were used to ensure that this was achieved. Aliquots of the combined urine collections were frozen and lyophilized. The water so obtained was counted and the highest specific activity obtained was 1 cpm/ml above background, indicating essentially complete biological stability of the tritium at C-7 and C-8. Other aliquots of the urine were adjusted to pH 7.8 and were subjected to continuous ether extraction for 24 hours and were also extracted with chloroform–ethanol (7:3). The organic extracts were taken to dryness and the residues were counted. All counting was carried out in a Packard Tri-Carb liquid scintillation counter with an efficiency of 48.7%.

TABLE 1

Urinary excretion of radioactivity after naloxone-^{3}H administration (as % of dose)

	JA		FR	
	i.v.	Oral	Heroin (i.v.)	Narcotic-free (i.u.)
Dose (cpm × 10^6)	10.35	8.14	6.87	7.24
Urine collections				
0–6 hr	37.7	23.5	24.7	37.4
6–12 hr	6.2	11.1	9.4	4.1
12–24 hr	9.5	12.1	18.3	10.1
24–48 hr	9.0	9.1	11.1	5.1
48–72 hr	1.4	3.3	4.3	3.6
Total	64.8	59.1	67.8	60.3

Results

The doses administered and the urinary excretion of radioactivity in all four studies are given in table 1. The excretion in all cases is very rapid with 24 to 37% of the dose appearing in the first 6 hours with very little radioactivity appearing in the final 48 to 72 hours. The ex-

creted material was all in the conjugated form since none of it was extractable with organic solvents. In the normal subject, the oral dose was excreted more slowly when compared to the intravenous dose. In the case of the opiate-dependent subject, FR, the excretion within the first 6 hours was 25% when he was on heroin compared to 37% after withdrawal. Thereafter, however, the excretion while on heroin was faster with the eventual total excreted being somewhat higher, 67 to 61% when opiate-free. The total urinary excretion in all four studies was quite similar ranging from 59 to 67% of the dose. The fate of the remainder of the dose is unknown at this time, since fecal excretion was not monitored.

The change in plasma specific activity following both intravenous and oral administration of naloxone-7,8-^{3}H to the normal subject, JA, is recorded graphically in figure 1. To permit direct comparison, the specific activity values from each experiment have been adjusted to a common dose of 1×10^7 cpm. The intravenous disappearance curve reveals an initial rapid component presumably reflecting distribution, followed by a slower rate of decrease for the next 5 hours. At that time the plasma radioactivity content was already very small and it had completely disappeared in the next plasma sample measured (20 hours). Oral naloxone-7,8-^{3}H results in a surprisingly rapid appearance of radioactivity in the plasma with the initial 15-minute sample already containing 75% of the radioactivity in the corresponding sample from the intravenous

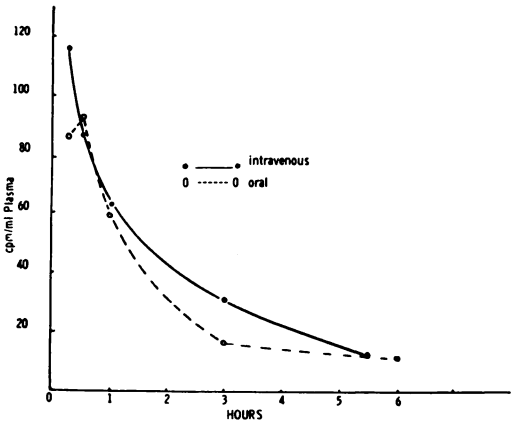

FIG. 1. Plasma radioactivity after intravenous and oral naloxone-7,8-^{3}H administration to normal subject.

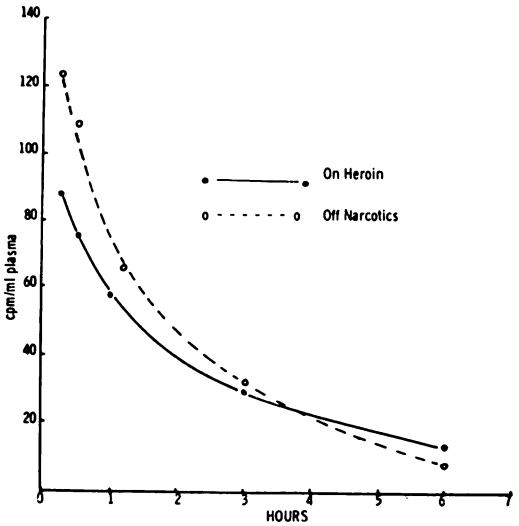

FIG. 2. Plasma radioactivity after intravenous naloxone-7,8-^{3}H administration to opiate-dependent subject while on heroin and after withdrawal.

dose. The plasma radioactivity from the oral dose reaches a maximum at 30 minutes and proceeds to decay faster than in the intravenous study for the next 3 hours. After that, a time plateau is apparent for the next 3 hours with only minimal counts left in the 20-hour sample.

The plasma radioactivity data obtained in the opiate-dependent subject, FR, after intravenous naloxone-7,8-^{3}H administration both when on heroin maintenance and after narcotic withdrawal are recorded in figure 2. These results have also been adjusted to a common dose of 1×10^7 cpm, to allow for comparison. The main difference between these two studies is in the initial (15-minute) plasma concentration which is about 30% higher in the narcotic-free state. The rate of decay in that experiment is faster, however, so that it intercepts the disappearance curve from the heroin study at 3 hours. To obtain an approximation of the percentage of the dose radioactivity contained in the total body plasma volume we have used a value of 38 ml of plasma per kg b.wt. to give calculated plasma volumes of 3.0 liters for subject JA and 2.35 liters for subject FR. With these values, the percentage contained in the blood at various times in all experiments can be calculated. Fifteen minutes after administration to subject JA, 3.5 and 2.5% of the intravenous and oral dose radioactivity, respectively, is contained in the total blood volume. The percentages for subject FR are 2.0%

while on heroin and 2.9% when drug-free. It is noteworthy that the latter figure is much closer to that found in the normal subject after parenteral administration.

Analysis of the plasma radioactivity was accomplished by ethyl acetate extraction and reverse isotope dilution with carrier naloxone. In all studies in which the antagonist was given intravenously, the results were closely comparable. A representative set of values obtained with subject JA is recorded in table 2 and shows that the initial sample consists mostly of naloxone but that thereafter progressively contains a greater percentage of nonextractable and presumably conjugated material. These results have been plotted graphically in figure 3 and are in contrast to those in figure 1 which record undifferentiated plasma radioactivity. The plasma analysis after the oral dose is also recorded in table 2. In this case the solvent-extractable radioactivity is comparable to the intravenous studies, but it is associated with naloxone to a much lesser extent. The nature of the non-naloxone radioactivity is at present unknown. It should be stressed that the rapid decay of plasma radioactivity limits the accuracy of the above analyses particularly in the later samples. The results in these samples are therefore subject to considerable error and the values recorded should be considered in this context.

TABLE 2

Analysis of plasma radioactivity in subject JA

Plasma Sample	Ethyl Acetate Extract		Thin-Layer Chromatography and Reverse Isotope Dilution	
	i.v.	Oral	i.v.	Oral
15 min	86[a]	83[a]	98[b]	17[b]
30 min	65	68	89	14
60 min	42	37	84	11
180 min	27	26	79	
360 min	19		70	

[a] Percentage of total plasma radioactivity.
[b] Percentage of ethyl acetate extract.

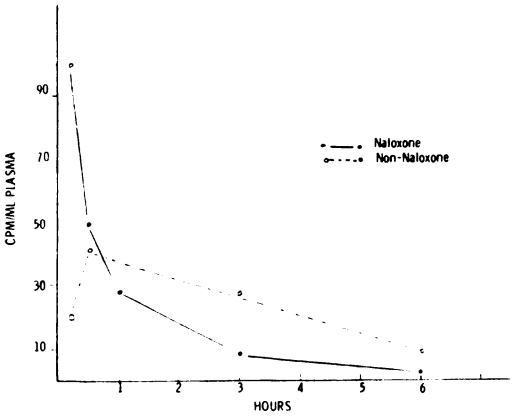

FIG. 3. Disappearance of naloxone and non-naloxone plasma radioactivity after naloxone-^{3}H intravenous administration (subject JA).

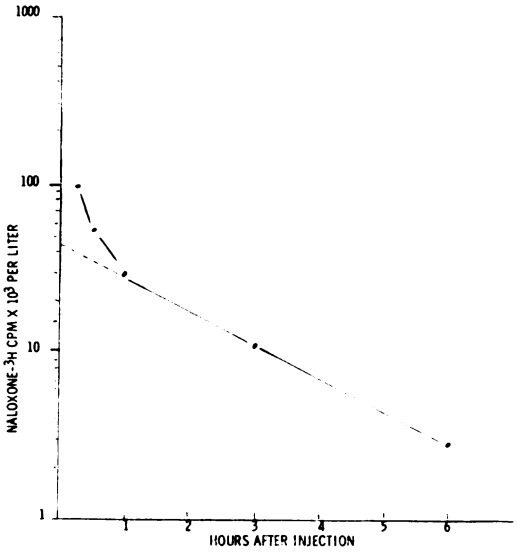

FIG. 4. Decay of naloxone-^{3}H in plasma after intravenous administration (subject JA).

When the logarithm of plasma naloxone concentration is plotted *vs.* time in all studies where naloxone was given intravenously, the disappearance rate could be fitted by a straight line after the first hour, consistent with a one-compartment model. A representative curve is presented in figure 4, with the extrapolated y intercept designated by the dashed line. Analysis of these data permitted calculation of the apparent volume of distribution, plasma half-time and metabolic clearance rates of naloxone in all the intravenous studies. These are listed in table 3 and it must be cautioned that since these values depend largely on the later plasma samples they may be subject to considerable error. No calculations are recorded for the oral study since these would have little significance in view of the unknown nature of the plasma radioactivity.

The apparent volume of distribution of intravenous naloxone was 230 liters in subject JA,

while in subject FR it was 250 liters when on heroin compared to 165 liters after opiate withdrawal. The plasma half-life of naloxone in subject JA was 90 minutes, while in the opiate dependent patient it was 100 minutes when on heroin and only 70 minutes when opiate-free. The metabolic clearance rates were uniformly high at 2340 to 2550 liters/day, with only small differences between the two subjects when corrected for body area.

TABLE 3

Pharmacokinetics of intravenous naloxone

Abbreviations used are: V_D, apparent volume of distribution of naloxone; $T_{1/2}$, time in which any plasma concentration of naloxone is reduced by 50%; MCR, volume of blood from which naloxone is completely and irreversibly removed in unit time; MCR/m², metabolic clearance rate per square meter of skin area.

	JA	FR on Heroin	FR Narcotic-Free
V_D (liters)	230	250	165
$T_{1/2}$ (min)	90	100	70
MCR (liters/day)	2550	2540	2340
MCR/m² (liters/day)	1270	1450	1330

Discussion

It is evident from these studies that naloxone is rapidly cleared from the body irrespective of its route of administration. The administered material is rapidly excreted in urine and its plasma life is of short duration. While plasma concentration and pharmacological potency need not necessarily be connected, particularly when a blood-brain transfer is involved, the results obtained are sufficient to explain the short 3 to 4 hour duration of action of naloxone. Indeed, the plasma life of naloxone calculated in these studies corresponds well with its observed pharmacological lifetime of 3 to 4 hours. The recently reported plasma half-life of morphine in man (Spector and Vesell, 1972) showed it to be substantially longer than that of naloxone and corresponds to the greater duration of action of this narcotic agonist. The large volume of distribution of naloxone and the small percentage of the dose present in plasma when considered in conjunction with the high metabolic clearance rate and urinary excretion point to a rapid and extensive metabolism of the administered material as the principal reason for its short duration of action.

The oral dose is obviously rapidly absorbed into the blood and reaches comparable plasma levels to that attained by the intravenous dose. The material in plasma is, however, not free naloxone, but a metabolite of it which presumably does not have the same biological potency and hence serves to diminish the effectiveness of the orally administered material. The oral result further illustrates the rapid metabolism of naloxone since apparently nearly all of it is metabolized in the first hepatic transit. It is noteworthy that the material entering the blood after oral naloxone is largely extractable with organic solvent and is therefore not conjugated but is a structurally altered naloxone molecule. Due to the lack of sufficient counts, the nature of this transformation product or products could not be determined in this study. The present experiments suggest that protection of the naloxone molecule from transformation during its first transport through the liver would lead to more effective oral activity since absorption from the intestinal tract does not appear to constitute the problem.

The differences in the pharmacokinetics of naloxone when administered coincidentally with heroin with those obtained after opiate withdrawal are of some interest. The dose of naloxone given was not pharmacological (<5 μg) and hence withdrawal effects were not involved. This is an isolated study and any generalization should be considered with great caution. It may be significant, however, that the calculated values obtained in the opiate-free study, when corrected for body weight differences, are uniformly closer to those obtained in the normal subject than those obtained while the subject was on heroin. Thus the volume of distribution of 165 liters in the 63-kg addict compares better with a 230-liter volume of the 81-kg normal patient than a value of 250 liters. Similarly the $T_{1/2}$ value of 70 minutes is closer to the normal 90 minutes than the 100-minute value obtained on heroin when these are adjusted for body weight differences.

The similar results obtained from the JA intravenous study in which a near pharmacological dose of 125 μg was given and the narcotic-free FR experiment in which only 4 μg were administered suggests that comparable values

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