Methadone Concentrations in Plasma and Their Relationship to Drug Dosage
Kim Wolff,¹ Marlon Sanderson,¹ Alan W. M. Hay,¹ and Duncan Raistrick²

We have developed a sensitive HPLC method for measuring methadone in plasma and have used it to establish that there is a linear relationship between plasma concentration and methadone dose over the range of 3–100 mg of methadone per day in a group of 31 addicts. We found a good correlation between dose and plasma concentration (r = 0.89), with the plasma methadone concentration increasing by 0.263 mg/L for every milligram of methadone consumed per kilogram of body weight. Five patients had unexpected high or low concentrations; this finding is discussed.

Additional Keyphrases: chromatography, liquid · abused drugs

Methadone treatment has been used to treat opioid addiction for many years, but there is no scientific basis for assessing the adequacy of the dosing schedule for each individual (1).

Early methods for the measurement of methadone in plasma involved gas–liquid chromatography, but sample preparation was often lengthy and cumbersome. High-performance liquid chromatography (HPLC) has been applied recently to the analysis of methadone (2, 3); however, these methods were sensitive only to the microgram range and could not measure low plasma concentrations reliably.

Earlier studies undertaken to assign clinical significance to methadone in plasma concentrations involved few patients (4–8); most studies were usually conducted over days rather than weeks (9), and were not representative of the drug-taking population (10). These investigations also revealed great variability in the elimination half-life (t½β) of methadone and the clearance of the drug between patients, and within patients, resulting in a wide scatter of plasma concentrations obtained for fixed dosage schedules (7). Such findings supported the general beliefs, therefore, that determinations of methadone concentrations in plasma would not be helpful in adjusting drug dosages in patients experiencing withdrawal symptoms and that there was no therapeutic plasma range for methadone (11, 12).

In view of the shortcomings of earlier studies, we decided to set up a sensitive assay for methadone, using HPLC to re-examine the relationship between dose and plasma concentrations. We have therefore measured methadone concentrations in plasma under steady-state conditions in a large number of patients taking a wide range of methadone doses.

Materials and Methods

Subjects. Thirty-one patients attending the Leeds Addiction Unit over a period of 30 months participated in the study. Patients were prescribed various daily amounts of methadone linctus (1 g/L), depending on the degree of their opioid dependence. Opioid dependence was assessed by self-report interviewing and by monitoring for signs of narcotic withdrawal with the Opioid Symptom Severity Assessment (SSA) chart (13). All patients had reached steady-state conditions, defined as 5–6 half-lives after dosing had begun (t½β for methadone = 24 h (14)). Patients attended the clinic weekly, fortnightly, or monthly on the same weekday at pre-set clinic times to give a blood and urine sample for the study.

Measurements were made on successive samples obtained (weekly, fortnightly, or monthly) from patients who were maintained on methadone over a period of two to 42 weeks (mean 15.5, SD 9 weeks). Eight patients in this study were HIV seropositive, and five were infected with hepatitis B virus.

Drugs. Methadone HCl B.P. was obtained as a gift from the Wellcome Foundation (London, U.K.). Benzhexol HCl B.P. (Artane), used as an internal standard, was donated by Lederle Laboratories Division (Cyamid, Gosport, Hampshire, U.K.).

Reagents. Methanol, 1,2-dichloroethane, isopropanol, and n-butyl chloride, all HPLC grade, were obtained from Rathburn Chemicals Ltd. (Walkerburn, Peeblesshire, Scotland). Aqueous ammonium perchlorate solution (100 g/L), sodium carbonate (anhydrous), sodium hydrogen carbonate (all of AnalaR analytical grade) were supplied by BDH Chemicals Ltd. (Poole, Dorset, U.K.).

Equipment. The HPLC system consisted of a Model 510 solvent-delivery system (Millipore (U.K.) Ltd., Waters Chromatography Division, Harrow, Middlesex, HA1 2YH, U.K.) and a Model 231/401 Gilson autosampler (Anachem U.K. Ltd., Luton, Bedfordshire, U.K.), fitted with a 50-µL loop. Separation was performed on an Apex-I silica column, 25 cm × 0.46 cm (i.d.), packed with 5-µm-diameter particles (Jones Chromatography, Llambreach, Mid Glamorgan, CF8 3QQ, U.K.) protected by a 5 cm × 0.46 cm (i.d.) guard column (silica Corasil Type II). Methadone was detected at 215 nm with a Model 455LC spectrophotometer coupled to a Model 747 data-module integrator (both from Waters Chromatography Division).

Patients' samples. Blood (10 mL) was drawn by venipuncture into heparinized "Monovette" blood-collection tubes, before the consumption of the daily dose of methadone linctus. After centrifugation at room temperature for 5 min (1000 × g), plasma was transferred to clean

¹ Department of Chemical Pathology, Old Medical School, University of Leeds, Leeds LS2 9JT, U.K.
² Leeds Addiction Unit, Leeds, U.K.

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10-mL polypropylene tubes (LSL Sales Ltd., Rochdale, U.K.) and stored at −20 °C until required for analysis.

Sample treatment. Patients’ plasma specimens, quality-control samples (pooled patients’ plasma), and plasma methadone standard solution were defrosted and thoroughly mixed (5 s) before centrifugation at room temperature for 2 min (500 × g). We mixed 2 mL of sample with 0.5 mL of benzhexol (internal standard, 1 mg/L) in aqueous methanol (50/50 by vol) and 0.5 mL of sodium carbonate buffer (1 mol/L, pH 10) in 15-mL acid-washed glass conical tubes with glass stoppers (Orme Scientific, Manchester, U.K.).

n-Butyl chloride was used as the solvent extractant in our method (15). n-Butyl chloride (5 mL) saturated with water was added to the conical tubes and the contents were mechanically mixed for 15 min, before centrifugation at 4 °C for 7 min (1000 × g). The n-butyl chloride upper layer was aspirated, placed in 5-mL glass tubes, and evaporated at 50 °C in a Model GV2 refrigerated solvent trap and centrifugal evaporator (Univap—Uniscience Ltd., Ann’s Crescent, London, U.K.). We reextracted the remaining (lower) layer with a further 5 mL of water-saturated n-butyl chloride, the new upper layer being added to the residue from the first extraction and again evaporated in the glass tubes. The dried extracts were redissolved in 0.1 mL of methanol, capped, and stored at −20 °C or transferred to 1.1-mL conical vials for immediate analysis by HPLC.

Liquid chromatography. Methadone was detected by using a silica column and a nonaqueous mobile phase (16). Extracts (20 μL) were automatically injected onto the silica column in a mobile phase recycling at 2 mL/min and consisting of methanol/1,2-dichloroethane/isopropanol/aqueous (100 g/L) ammonium perchlorate (90/5/5/4/0.5 by vol). The retention time for methadone was 6.7 min and 5.5 min for benzhexol (Figure 1).

Results

Assay Validation

Day-to-day assay reproducibility was assessed by using three different quality-control samples, each made up of pooled patients’ plasma. (Insufficient individual patient’s plasma was available to make up enough quality-control material to last for the whole study.) The mean ± SD and coefficient of variation (%) for the three samples were as follows: QC-A 0.066 ± 0.005 mg/L (9.2%, n = 8), QC-B 0.069 ± 0.009 mg/L (9.8%, n = 10), and QC-C 0.051 ± 0.005 mg/L (8.1%, n = 14). Within-day assay reproducibility was 3.2% (n = 10).

Accuracy was assessed by measuring methadone in plasma to which this drug had been added over a concentration range of 0.01–0.10 mg/L. The relationship between the quantity of methadone found to that added was linear, with a regression line of \( y = -0.0004 + 0.929x \) (\( r = 0.99, P < 0.001 \)). The mean analytical recovery of methadone was 98.4% (SD 5.2%), range 90–110%, and the detection limit of the HPLC assay (signal/noise ratio of 3) was 5 ng.

Several narcotic opioids, their metabolites, and other drugs commonly co-prescribed with methadone, including benzodiazepines and tricyclic antidepressants, were injected onto the HPLC column. None of the drugs or metabolites interfered or co-migrated with the elution of methadone, or the internal standard, benzhexol.

Clinical Results

Figure 2 shows the plasma methadone concentrations obtained from all patients as a function of the dose (per kg of body weight) consumed each day. Our results show that plasma concentration (y) and methadone dose (x, mg/kg per day) were well correlated over the dosage range studied. Linear-regression analysis gave the regression line \( y = 0.002 + 0.263x \) (variance about the regression = 0.004), indicating that the methadone concentration in plasma increased on average by 0.263 mg/L for every 1 mg/kg of methadone consumed. The correlation coefficient (\( r = 0.89, P < 0.001 \)) confirms the linear relationship between plasma concentration and daily dose of methadone for the dosage range studied.

Of the 31 patients studied, five had anomalous plasma
methadone concentrations. Three patients (A, B, and C) had unusually high plasma methadone concentrations. Two of these (A and B), both maintained on 50 mg of methadone/day, supplemented their prescribed dose with methadone obtained illicitly and were excluded from Figure 2. There was no apparent reason for the increased concentration in plasma in the third patient (C), who had high concentrations after being prescribed 60 mg and subsequently 80 mg of methadone/day. In addition, two patients had low plasma methadone concentrations: one while receiving 50 mg of methadone/day (D), and the second, taking 36 mg of methadone/day (E). Patients C, D, and E are included in Figure 2 because there was no apparent reason for their anomalies. The plasma methadone data collected from these five patients is highlighted in relation to the mean plasma concentrations from all of the other patients in the study (Figure 3). Linear-regression analysis applied to the mean values by least squares gave a regression line of \( y = 0.005 + 0.275x \) (\( r = 0.89, P < 0.001 \)).

Previous studies involving fewer patients were conducted at higher dosage concentrations (7). Figure 4 shows the mean plasma concentrations reported in the literature for different doses of methadone, compared with the more extensive data obtained in our studies. Our data, represented by mean values (calculated from absolute data without the body weight conversion), gave a regression line of \( y = -0.0385 + 0.0055x \) (\( r = 0.92, P < 0.001 \)).

Discussion

Patients are prescribed methadone on the strength of their opioid dependence. The clinician aims to provide a dose that will protect the addict both from the extremes of a "high" (euphoric feeling sought by heroin misusers) and from the discomfort of withdrawal (17). Earlier work reported a poor correlation between the dose of methadone, plasma concentration, and treatment outcome (12). Individual variation in the kinetics of methadone was thought to be the reason for this, and clinicians were led to believe that measurement of methadone in plasma would not help in patient management. With our larger population and more sensitive assay, we find that, in fact, there is a good linear relationship between plasma concentration and methadone (dose per kilogram of body weight) under steady-state conditions. These data enable the clinician to predict the dose required to give a certain plasma concentration and to assess, independently of the patient's self-reporting, the likelihood of opioid withdrawal. A further investigation (18) found that patients were most likely to experience withdrawal symptoms when their plasma methadone concentration fell below 0.05 mg/L.

Variation in plasma concentration between patients

**Fig. 2.** Plasma methadone concentrations from 29 patients under steady-state conditions as a function of dose (corrected for body weight)

**Fig. 3.** The relationship between mean plasma concentration and methadone dose (mg/kg per day), highlighting the five patients with anomalous concentrations of methadone in plasma: patient A (0.64 mg/kg daily), patient B (0.83 mg/kg daily), patient C (0.96 and 1.33 mg/kg daily), patient D (0.51 mg/kg daily), and patient E (0.48 mg/kg daily)

**Fig. 4.** The relationship between plasma concentration and methadone dose, highlighting other independent studies measuring plasma concentrations in methadone-treatment patients

Other studies (number of patients, methadone dose mg/day, length of time of receiving methadone before study, and reference): □ = 16, 60 mg, 10-24 days; □ = 7, 60 mg, 0.5 day; □ = 1, 40 mg, 0.5 day; □ = 11, 60 mg, 3 months (14). □ = 1, 80 mg, five years (8). □ = 6, 80 mg, nine days; □ = 40, 11 days (5). □ = 9, 100 mg, several months (4). □ = 5, 100 mg, six weeks to a year (7)
on the same fixed dose of methadone was small in most cases and was partly due to the fact that some patients maintained on a fixed dose of methadone had significantly lower plasma concentrations at the end of the study than at the beginning (19).

The data in the literature are generally in good agreement with ours. Plasma measurements in the literature were collected at the end of a dosing interval (24 h), before a subsequent dose, and after initial dosing. However, the time spent on methadone by these patients before sample collections varied greatly (range, one day to five years). Plasma concentrations that were lower (than in our subjects) for the same methadone dosage were collected from patients who had been receiving methadone for only a short time, and who may not have reached steady-state (Figure 4).

It is apparent from our results, however, that certain patients had anomalous concentrations of methadone in plasma, either unexpectedly high or low for the given dose. There are no well-established reasons to explain why some patients should have unexpectedly high methadone concentrations in plasma. Supplementation with illicit methadone accounted for the high methadone concentrations in two of the three patients in our study (A and B in Figure 3); this was discovered by monitoring compliance in all of our subjects by using a low-dose phenobarbital marker (20) at concentrations too low to cause enzyme inductions (21).

Chronic liver disease, usually the result of acute viral hepatitis, has been used to explain abnormal methadone kinetics (22), but evidence to support this is conflicting (23, 24). However, a recent report suggests that liver disease can reduce the clearance of methadone, allowing methadone to reach higher than expected blood concentrations (25). Increased hematocrit may also change the distribution of methadone within the blood. Because methadone is partially excluded from erythrocytes (26), patients with higher than normal hematocrit might tend to have higher concentrations of free methadone in plasma (27). High plasma concentrations could also persist if a physiological disturbance caused the release of methadone from its binding sites. The high plasma concentrations in the third patient (C) could not be adequately explained by any of these phenomena.

Many more circumstances have been associated with unexpectedly low plasma methadone concentrations (28). Early work suggested that patients whose plasma concentrations of drug rapidly decline several hours after a single methadone dose (excessive clearance) may have low concentrations of methadone at steady-state (29). Data from plasma concentration vs time were available for one of our patients with low plasma concentrations (E); however, his methadone clearance was 2.87 mL/min per kilogram, similar to the clearance rates reported for other individuals (30).

An important cause of low plasma methadone concentrations could be the induction of methadone-metabolizing enzymes by other drugs prescribed during methadone treatment. Certain drugs such as rifampicin used for treating tuberculosis (31) and the anticonvulsant phenytoin (32) can cause the onset of signs of opioid withdrawal, owing to low concentrations of methadone in plasma. Furthermore, HIV-infected patients who participated in a levomethadone maintenance program showed sustained symptoms of underdosage after treatment with Zidovudine (azidothymidine; AZT) (33).

Only one of the eight patients known to be HIV-seropositive in our study had low plasma concentrations (E), and he received only the non-enzyme-inducing antibiotic, flucloxacillin. The other seven received similar antibiotic prescriptions, but their methadone concentrations were not abnormally low for their respective dosage schedules.

Many combinations of drugs are prescribed in methadone-treatment programs (34). For instance, chlorpromazine, amitriptyline, and thioridazine are sometimes used for the relief of anxiety and agitation and to control vomiting, whereas benzodiazepines are used to help stabilize sleep patterns and to provide symptomatic relief of anxiety and severe muscle cramps. The benzodiazepines (especially diazepam), which also have known abuse potential (35), are thought to interfere with the normal metabolism of methadone, whereas some are able to modify receptor binding sites (36). Two patients (A and B) who supplemented their methadone dosage misused and had some degree of dependence on benzodiazepines. We do not know if their use of benzodiazepines played any part in their need to supplement their methadone prescription.

Amitriptyline—also prescribed to some of our patients—increases the plasma concentration of alpha1-acid glycoprotein (37), the main protein binding methadone in blood. Protein binding of methadone may increase under these circumstances and reduce the unbound fraction of the drug in plasma (38). One of our patients (D) who was prescribed amitriptyline (75 mg/day) during the study had very low concentrations of methadone in plasma.

The drug interaction of greatest concern in methadone treatment, and the one most difficult to assess, is that between alcohol and methadone. Scientific data indicate that methadone and alcohol appear to enhance the metabolism of one another in a reciprocal fashion (39). Many of the patients in our study consumed alcohol socially (once or twice a week or during the weekend), but this was not possible to quantify. Anecdotally, excessive consumption the previous evening may explain the withdrawal symptom complaints some patients made when they attended the clinic in the morning for their daily dose. Both of the patients with low plasma methadone concentrations (D and E) had histories of alcohol abuse. In one case (E), an attempt to reduce alcohol consumption was made by prescribing disulfiram (Antabuse, 200 mg/day), which is another drug found to cause abnormally low concentrations of methadone in plasma (40).

Possibly, drug interactions such as those we have described contribute to the variation in plasma metha-
done concentrations observed between patients on the same fixed dose.

As this study has shown, plasma methadone concentrations obtained at steady-state correlate well with dose per kilogram of body weight, and this relationship can be used to confirm that individuals complaining of withdrawal symptoms do indeed have abnormally low drug concentrations in plasma. Blood sampling would help to detect these individuals and would enable dosages to be amended to suit. The dose of such an individual could be optimized empirically, by blood sampling, and then be fixed once the plasma concentrations fall consistently within the range of values (observed by linear regression) for similar patients. We also recommend that methadone concentrations in plasma should be used to assess patients who are simultaneously taking drugs known to be inducers of microsomal enzymes. These patients form a subgroup that may require higher daily doses of methadone.

References

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