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Effect of intramuscular methadone on pharmacokinetic data and thermal and mechanical nociceptive thresholds in the cat.

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Abstract

Objectives

The study assessed simultaneous pharmacokinetics (PK) and thermal and mechanical antinociception after intramuscular methadone (0.6 mg/kg) in 10 cats.

Methods

Thermal (TT) and mechanical (MT) threshold testing and blood collection were conducted at baseline and up to 24 hours after administration. Methadone plasma concentrations were determined by liquid chromatography - tandem mass spectrometry (LC-MS) and PK parameters were

estimated by a non-compartmental method. TT and MT were analysed using ANOVA (P<0.05). Time of maximum plasma concentration (T_{max}), time of onset of antinociception and time of reaching cut out threshold (TT 55 °C; MT 30N) were determined.

Results

TT and MT increased above baseline from 20 to 240 minutes and 5 to 40 minutes respectively after intramuscular administration (P<0.005). Maximum delta T (measured as TT minus baseline threshold) (mean ± 95%CI) was 7.9 (4.3-11.6) °C at 60 minutes and maximum delta F (measured as MT minus baseline threshold) was 4.2 (1.6-6.7) Newtons at 45 minutes. Intramuscular methadone concentration-time data decreased curvilinearly, and gave a clearance estimate of mean 9.1 ml/kg/min (range 5.2-15.7) with median T_{max} at 20 minutes (range 5-360).

Conclusions

Intramuscular data followed classical disposition and elimination in all cats. Plasma concentrations after intramuscular administration were associated with antinociceptive effect including negative hysteresis.

Relevance

These data can be used for devising dosing schedules for methadone in clinical feline practice.

Key words

cat, antinociception, methadone, pharmacokinetics, thermal and mechanical threshold.

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Introduction

Methadone is structurally unrelated to other opium-derived analgesics and exists as a racemic mixture. Each enantiomer has a separate mode of action; the d-isomer noncompetitively antagonizes the NMDA receptor and inhibits norepinephrine reuptake; the l-isomer is a μ-opioid receptor agonist [1]

There is only one report of the kinetics of methadone data in the cat [2] and no data enabling the relationship between methadone pharmacokinetics and dynamic endpoints to be assessed. While administration of drugs IV is the most reliable route to ensure full uptake this is not always practical in a clinical setting, for example, when used as anaesthetic premedication where IV access may not be possible. The present study evaluated the kinetics and dynamics of a single dose of intramuscular (IM) methadone (0.6 mg/kg) in the cat. Thermal and mechanical thresholds were measured at the same time as blood sampling to 24 hours post-drug administration. The study was undertaken in order to provide registration data for an indication for use of IM racemic methadone in the cat.

Methods

All studies were conducted after approval by the local institutional review board and according to UK Home Office licence; in-life data were collected in September 2009.

Pharmacokinetics of methadone

Twelve adult neutered cats (3 male, 9 female; identified as M to X; mean weight 4.4, range 2.9-6.1kg; aged 34 months) received methadone (0.6 mg/kg) administered IM into the quadriceps femoris muscle. The day prior to their testing procedure, the cats were anaesthetised with sevoflurane delivered in oxygen (induction by face mask followed by endotracheal intubation for maintenance). Over-the-needle catheters were placed in both cephalic veins for ease of access in case non protocol intravenous administration of substances was required during the study and as a back-up for/ as the main route for blood sampling. A modified Seldinger technique was used to place 5.5cm intravenous (IV) catheters in the jugular vein in 6 cats (cats O, Q, S, U, V, X) and saphenous vein in 2 cats (cats M,W), the remaining 4 cats (N,P, R, T) were blood sampled using the second cephalic catheter. Once the catheter was securely fixed the cats were returned to their holding cages for recovery from anaesthesia. On the day of testing, blood samples were withdrawn from the (IV) (jugular, saphenous or cephalic) catheter before and at 5, 20, 40, 60 minutes, and then at 2, 3, 4, 6, 8, 12 and 24 hours after drug dosing. The volume of blood taken was adjusted for each individual cat, such that over the 24 hour sampling period, the total blood volume taken was < 10% blood volume. An equal volume of 0.9% saline was injected after each sample was withdrawn. Blood was taken into lithium heparin tubes, and centrifuged (2000g) for 10 minutes. Plasma was separated and stored at -20°C for a maximum of 4 months.

Plasma methadone concentrations were measured at a commercial assay laboratory (Quotient Bioresearch Ltd, Cambs, UK) using liquid chromatography - tandem mass spectrometry (LC-MS). In the concentration range 0.5 to 150 ng/ mL, the inter- and intra-assay coefficients of variation were between 0.8 and 4.3%. Plasma methadone concentrations were linear over the range 0.5 to 150 ng/ mL. The limit of detection for a 1 mL sample was 0.5 ng/ mL.

The maximum plasma concentration (C_{max}) and time of maximum concentration (T_{max} were observed values. Pharmacokinetic parameters were determined by a non-compartmental method [3]. Drug-concentration time profiles were subjected to nonlinear least squares regression of at least three plasma concentrations to calculate the elimination half-life ($t_{1/2z}$). The area under the concentration-time curve (AUC) was calculated to the final concentration time point of 24 hours (Ct) by the linear trapezoidal rule. The residual area to infinity was calculated as Ct/kz where z is the terminal or elimination rate constant. From these values, other kinetic parameters were calculated: apparent clearance (Cl/F) and apparent volume of distribution during the elimination phase (V_z/F) (which are given as Cl/F and and V_z/F because we do not know the bioavailability (F) after intramuscular dosing). Two sets of data for (Cl/F) and (V_z/F) are shown in table 1 - per kg body weight and weight independent. V_z (also known as V_{area}) was used rather than V_{dss} (steady-state volume of distribution) as it is difficult to calculate the latter for the IM route.

Pharmacodynamics of methadone

Thermal and mechanical nociceptive thresholds threshold were used to determine the dynamic effects of methadone.

Thermal threshold testing. Thermal nociceptive thresholds were measured using a remote (infra-red) controlled system [4]. The testing device (WTT1, Topcat Metrology Ltd) is attached over the cat's back with a Velcro® and elastic strap. A small heating and temperature sensing probe is held against the shaved thorax of the cat with a pressure bladder inside the strap. The starting skin temperature (SST) is recorded on the integral display and then the heater activated so that temperature increases at 0.8˚C per second. At the cat's response (skin twitch accompanied by a behavioural response such as head turn, body shift) heating is stopped immediately and the temperature at the response is held on the display and recorded as the thermal nociceptive threshold (TT). The probe rapidly returns to the starting skin temperature after the heating is stopped. There is a safety cut out of 55˚C.

Mechanical threshold testing. Mechanical nociceptive thresholds were measured using a silent, pneumatic system (MTT1, Topcat Metrology Ltd) which employs a rolling diaphragm actuator [5] attached to a forelimb using a Velcro® and elastic bracelet. The actuator was placed on shaved skin on the dorso-lateral aspect of the forelimb between carpus and elbow. Three hemispherical tipped pins (2.5 mm diameter) held in the actuator were advanced against the skin at 2 N per second by increasing the pressure in the actuator manually, using an air filled syringe. A cage mounted set of LED lights guides the rate of inflation (red = too fast, green = too slow, lights off = correct rate). Inflation is terminated as soon as the cat's response is seen (leg shake, biting at the leg, picking up the leg) and the force (N) applied by the actuator at this point is held on the display and recorded as the mechanical nociceptive threshold (MT). There is a safety cut out of 30N.

Four baseline thresholds were recorded at 15 minute intervals before treatment. The mean of these four readings was taken as the baseline TT or MT. Thereafter TT and MT were measured at each sampling point to 24 hours post-drug administration. The increase in threshold at the various

time points following methadone administration was determined as delta T (calculated as the TT minus the pre-treatment TT for each cat) and delta F (MT minus the pre-treatment MT).

The same investigator (LS) performed all the TT and MT tests. There was no blinding as this was a single dose study.

Pupil dilation, sedation scores, monitoring of adverse events Pupil dilation and sedation were recorded at each study time-point with 4 point scores:

Pupil dilation: 1 = constricted, 2 = normal, 3 = partially dilated, 4 = fully dilated.

Sedation: 0 = none, 1 = mild (cat was relaxed, but could be roused and could walk with no ataxia), 2 = moderate (cat was in sternal or lateral recumbency, but could be roused and had obvious signs of ataxia), 3 = no response to stimulation.

Any other behaviours or adverse events were recorded as and when they occurred.

Statistical analyses

Repeated measures ANOVA was performed on the mechanical and thermal dynamic data with post hoc Dunnett's test; p values less than 0.05 were deemed to be statistically significant.

Three indices of methadone's antinociceptive effect were determined:

. time to achieving peak plasma drug concentration (Tmax)

. time to onset of antinociceptive effect of the drug and the related methadone plasma concentration (Using a conventional method to define biological references ranges [6], antinociceptive effect in our model was defined as the baseline threshold (mechanical or thermal) plus 2 SD, all calculated from the grouped data.)

. time to first achieving the cut-off temperature or mechanical pressure.

These data are presented as median and range values unless otherwise stated.

AUCs for plasma drug concentration and dynamic endpoints delta T and delta F were calculated using the linear trapezoidal method, and were compared against each other using regression analysis.

Results

Prior to dosing, most cats had mildly constricted or normal diameter pupils. Administration of IM methadone resulted in profound thermal antinociception with no side-effects. It caused pupillary dilation which persisted for up to 12 hours, but no detectable sedation. Due to various issues full data from 2 cats (O and X) could not be collected; for this reason although 12 cats started the study, only data from 10 cats are presented.

Kinetics of methadone in the cat

Unless stated otherwise all data presented are mean (SD).

Following the 0.6 mg/ kg IM dose of methadone, plasma concentration T_{max} occurred at 5 to 120 minutes post dosing in 9 of the cats (median 20 minutes) as presented in Table 1. The exception was cat U which is discussed below.

The C_{max} in the 10 cats was 105 (28) ng/mL (range from 54 to 139 ng/mL). The sensitivity of the assay allowed all cats to have measurable plasma methadone concentrations to the last sample point of 24 hours. The AUCs to 24 hours and to infinity were 52803 (14885) and 74464 (25142) ng/mL/min respectively.

Examination of the derived kinetic parameters showed a normal distribution. The estimates for systemic clearance (Cl) and volume of distribution during the elimination phase (V_z) are shown as a ratio of parameter divided by bioavailability (F) as an estimate for the latter was not measured or cited in the literature following IM dosing. The disposition data for the 10 cats are shown in Table 1 including the location of the intravenous catheter for blood smapling. There were no apparent differences seen using one way ANOVA for the main PK values between animals where blood sampling had been via the cephalic, jugular or saphenous veins. However the present study was not either aimed at or powered to examine this variation.

Dynamics of methadone in the cat

The delta values for MT and TT (i.e. threshold minus baseline) are shown in Figures 1a and b. There was no clinically significant change in the SST over the time period 0-24 hours (variation by less than 1 ºC). The group pre-treatment TT was 43.4 (2.0) ºC. After intramuscular methadone dosing, the TT increased significantly from 20 minutes to 4 hours. The peak TT was 54.9 (0.3) ºC. The maximum threshold (safety cut out) of 55ºC was reached in nine cats at 22 (33) minutes after treatment and remained at this value for periods between 20 minutes and 2 hours in these cats.

The maximum delta T values in the individual cats ranged between 8.7 and 14.8 ºC and occurred between 33 and 150 minutes after dosing. Where cats remained at the same delta T for more than one time point, then the closest study time point to the average was taken. The plasma methadone concentration at the time of maximum delta T ranged between 46.7 and 104.0 ng/mL. Thermal threshold T_{max} values are difficult to estimate in the cats that spent a period of time with thresholds that exceeded the cut out temperature. Individual T_{max} values (where there is a range, this indicates the time spent at cut out) were: M 40 minutes; N 40; P 20-120; Q 40-60; R 120; S 20-180; T 40-240; U 120; V 60; W 5-60.

Mechanical thresholds varied significantly over time with MT significantly raised at 5, 20 and 40 minutes compared to baseline. The pre-treatment MT was 8.4 (1.6) N. The largest delta F ranged in the individual cats between 3.2 and 13.2N, and occurred between 5 and 240 minutes after dosing. The plasma methadone concentration at the time of maximum delta F ranged between 23.4 and 139.0 ng/mL.

The AUC (of delta T over time) for the period 0-1440 minutes was 3772.8 (1436.9) ºC.min for the intramuscular dosing; while the corresponding AUC (of delta F over time) for the same period was 1769.3 (1375.6) Newtons.min. Correlation analysis between the AUC (of

plasma methadone concentration over time) and AUC (of antinociceptive effects over time) to 1440 minutes (24 hours) post-dosing indicated significance for the delta T vs. concentration measurement (r = 0.8573) but not for delta F vs. concentration (r = -0.4156). There was no significant correlation between the AUCs for the two antinociceptive measures (r = -0.2086).

Kinetic-dynamic relationships

When the measures delta T, delta F and plasma methadone concentration were plotted together against time (Figure 2), there was little difference between kinetic and dynamic profiles. There did not appear to be any significant separation between drug concentration and effect - although the T_{max} (median) was 20 minutes for concentration; 40 min for MT and for TT.

As with most drug responses the plasma concentration-time profile and the effect-time relationship were not in phase and there was an anticlockwise hysteresis loop (Figure 3).

Both stimulus modalities also showed a dip in response between 360 and 720 minutes (Figure 2).

The plasma drug concentrations associated with the onset of antinociception baseline plus 2 SD ranged between 39.2 and 124 ng/ml for temperature; and 23.4 and 139 ng/ml for mechanical pressure. The comparable values for the offset of antinociception were 13.9-105 ng/ml and 15.1-102 ng/ml respectively.

Discussion

Methadone is a synthetic full agonist opioid, but studies have revealed that it also acts on *N*-methyl-D-aspartate receptors [7] and it has been used as an alternative to morphine and hydromorphone in human patients with severe pain. Clinically, in cats, methadone is used for analgesia often administered as part of anaesthetic premedication where it can assist in the production of sedation in combination with tranquilizers or sedatives. Most comparable clinical studies have used feline ovariohysterectomy as a surgical model with methadone administered with the premedication and pain/ analgesia scored by a variety of methods including wound palpation and behavioural and physiological observations. An early study examined use of methadone 0.5 mg/kg IM where analgesia was reported for 1.5-6 hours from administration of methadone[8]; a later study with 0.6 mg/kg IM at premedication reported that 18/19 cats has adequate analgesia for the entire study period of 4 hours after surgery and only a single cat required rescue analgesia after 90 minutes[9]; a third study using 0.5 mg/kg IM reported good analgesia for the study period of 6 hours in 6/8 cats with 1/8 requiring rescue analgesic at 4 hours and 1/8 at 5 hours [10]. Pre-anaesthetic sedation with acepromazine - methadone combination was reported to be poor but similar to that seen with acepromazine - butorphanol combination [10].

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The current study used threshold testing tools to assess antinociception rather than a surgical stimulus to assess pain/ analgesia. The physiological processes behind a thermal stimulus applied to the thoracic skin or a mechanical stimulus applied to a forelimb are not the same as the pain caused by surgery so their clinical relevance could be questioned. However nociceptive threshold testing tools are widely used and accepted for pain/ analgesia research in laboratory species and in human volunteers. Our study has demonstrated clear differences between the

effects of methadone on the two stimulus modalities of heat and pressure; significant effects on mechanical thresholds were much shorter acting than those on thermal thresholds. This is not unknown in analgesia research and may be due to a number of factors including device design and / or different physiological pathways for the two stimuli. Since the thermal threshold testing tool has already demonstrated similar thermal antinociceptive profiles compared to the well documented clinical analgesic profile of commonly used opioid analgesics such as buprenorphine we would suggest that, for whatever reason, the thermal threshold data are more likely to be similar to the clinical profile of methadone than the mechanical thresholds.

Comparison of the time-courses of kinetic and dynamic effects of the opioid show a lag time between increasing plasma concentrations and increase in observable effects. This is in keeping with the site of action of methadone being at opioid receptors in tissues rather than in plasma (Figure 3); with the lag being the time taken for methadone to move across the blood brain barrier to opioid receptors in the central nervous system. Many other drugs demonstrate a similar 'out of phase' concentration-time and the effect-time relationship.

Much less individual variation relating to drug disposition was seen in this study compared to previous studies of other opioids (buprenorphine, morphine and pethidine) in cats [11, 12]. Maximum plasma concentration (C_{max}) and time of maximum plasma concentration (T_{max}) were very consistent for all cats except for animal U. In this cat there was a late peak in concentration at 360 minutes which could indicate slow absorption or perhaps injection into a poorly vascularised area of the body. The quadriceps muscle was used as the site of injection and should be well

vascularised but this cat was noticeably fatter than the other cats and hence injection of drug into fat tissue might be associated with a reduced rate of absorption.

Median plasma T_{max} was at 20 minutes. The absorption was less rapid than that seen in a presently unpublished parallel study in dogs (range 5-15 minutes) although prolonged absorption time was seen in dogs when administered a higher dose of 0.5mg kg-1 compared to 0.3 mg kg-1. Other studies with opioids in cats have demonstrated the plasma T_{max} after IM injection were 15, 3, and 10 minutes for morphine, buprenorphine and pethidine respectively [11] and 21 minutes for butorphanol [13].

Racemic methadone was administered at 0.6 mg/kg IM in the current study and as this was the only route of administration and the cats were not also administered an IV dose it was not possible to calculate bioavailability (F) which is why our data has been corrected for F. At the time of analysis we were not aware of directly measured data for the bioavailability of methadone after IM administration in the cat. Recently published data [14] gives a value for F of 44.2% after buccal dosing; with the kinetics for a 0.3 mg/kg IV dose being quoted as clearance 7.2 mL/kg/min and Vz 2.4/kg. Using the area under the curve in their study and applying it to our data, the estimated bioavailability in our study would be between 80 and 85%. From a study in cats where 0.3 mg/kg methadone was administered IV [2], using their presented data we have calculated the clearance as 4.3 mL/kg/min and $t_{½}$ as 278.4 minutes (4.6 hours). The PK values from these 2 studies are comparable with the values we have reported here (Cl/F = 9.1 (3.3) mL/ kg/min and Vz/F = 7.8 (2.7) L/ kg; $t_{½}$ = 627.9 minutes (212.2)).

Although Hedges and colleagues[15] found differences in the disposition after buccal administration of buprenorphine in cats depending on the site of blood sampling, we have not found any significant differences in the systemic clearance of methadone when comparing data from the three sites used for blood sampling in this study. However the sample size of each group of cats was small, and to examine it further a properly powered study would be needed. There are also differences in the route of drug dosing (intramuscular vs buccal) between the two studies.

The plasma methadone pharmacokinetics reported here for the cat resembles those seen in dogs. After IV administration of 0.45 mg/kg racemic methadone base to greyhounds [16], systemic clearance was 56.0 (9.4) mL/kg/min and Vz was 7.8 (1.9) L/ kg; after administration of 1 mg/ kg IV to beagles, total body clearance was 24.1 (9.8) mL/kg/min and Vz was 3.7 (1.1) L/ kg [17].

When reviewing the literature in humans it becomes clear that while there are many published studies, most publications relate to aspects of its use is as a heroin replacement substance rather than a first line analgesic and therefore the number of pharmacokinetic studies of methadone in healthy, non-opiate users are limited. There is also a difficulty in making direct comparisons as most animal studies report values adjusted for bodyweight whereas those in humans do not. Clearance (Cl) adjusted for bioavailability (Cl/F) after oral administration in opiate-naive humans was 115 mL/min (data reported as 6.9 L/ hour) and half-life estimates were 33-46 hours [18]. In these individuals, weight as a covariable had no significant relationship to Cl/F and using the median bodyweight, the calculated Cl/F would be about 2 mL/kg/min. Another study [19] reported mean clearance after a 5mg IV dose as 8.3 L/ hour (138 mL/ min) and after a 10mg oral dose Cl/F = 9.8 L/ hour (163 mL/ min); adjustment for

mean volunteer weight (84kg) gives a Cl (IV) of 1.6 mL/kg/min and Cl/F (oral) 1.9 mL/kg/min. The estimate for oral bioavailability in this study was calculated as 86% making Cl (oral) = 2.2 mL/kg/min, and the half-life was reported as 32 and 31 hours in the IV and oral groups respectively. Terminal half lives in man, however, are substantially greater than in the cat, for example after effectively a 0.05 mg/ kg dose in humans, the $t_{½}$ was 32 hours whereas after 0.6 mg/kg in the cat the $t_{½}$ was 10 hours, this might be due to the large V_d in humans.

A number of studies have investigated plasma concentration with respect to the antinociceptive or analgesic action of methadone in human subjects; they appear to be similar for acute pain stimuli such as antinociceptive tests and surgery although much higher concentrations were required for patients with chronic pain [20, 21]. Postoperative minimum analgesic concentrations in humans were 30-33 ng/ml [22] whereas the EC_{50} (half maximal effective concentration - the concentration of a drug which induces a response halfway between the baseline and maximum) in 8 chronic pain patients (5 with cancer) was 290 ng/ml [20]. Our study findings of plasma methadone concentrations for onset (39.2 to 124 ng/ml) and offset 13.9-105 ng/ml) of thermal antinociception and onset (23.4 to 139 ng/ml) and offset (15.1-102 ng/ml) of mechanical antinociception are similar to those required in humans for acute pain stimuli.

Pharmacokinetics can be defined as what the body does with an administered drug and pharmacodynamics as what the drug does to the body. The aim when investigating potentially useful clinical analgesics is to determine the optimum plasma concentration associated with analgesia, PK and PD data may then be used to determine optimum route of administration, dose and dosing interval in order to maintain this analgesic

plasma concentration. In this study, we have shown that the kinetics of a single dose of IM methadone in the cat are similar to that reported in the dog. Drug uptake is rapid in the cat, but systemic clearance for both species is greater than that reported for man. We have also determined analgesic plasma concentrations of methadone. These data may be used in combination to suggest that analgesia from an intramuscular dose of methadone at 0.6 mg/kg would be expected to provide four hours of analgesia and that the target plasma concentration for onset of analgesia lies between 40 and 124 ng/ml.

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Table 1: Pharmacokinetic parameters for 10 cats after IM administration of 0.6mg/kg methadone. Clearance and volume of distribution are shown as both body weight dependent and independent. Sampling route, Cep = cephalic; Jug = jugular; Sap = saphenous

cat	sampling route	Cmax ng/ml	Tmax min	Cl/F ml/min	Cl/F ml/kg/min	t ½ z min	Vz/F litres	Vz/F l/kg	AUC24 ng/mL/min
N	Cep	132	20	34	7.9	350	17	4	62446
P	Cep	92	20	54.2	10.1	412	32	6	53642
R	Cep	122	5	26.4	6.3	824	31	7	68647
T	Cep	125	5	30.2	7	416	18	4	41746
Q	Jug	100	120	27.9	5.2	906	36	7	74022
S	Jug	71	40	50.2	12.5	549	40	10	38990
U	Jug	54	360	40.3	8.6	894	52	11	44206
V	Jug	139	40	25.8	6.1	801	30	7	69644
M	Sap	92	20	45.4	15.7	513	34	12	32319
W	Sap	121	20	43.4	11.1	613	38	10	42370
	mean	105	65	37.8	9.1	628	33	8	52803
	SD	28	109	10.3	3.3	212	10	3	14885
	median	111	20	37.1	8.2	581	33	7	48924

Figure 1

Delta T (Fig 1a) and delta F (Fig 1b) threshold values for 10 cats after administration of methadone 0.6mg/kg (mean and standard deviation). Significant differences from baseline thermal threshold from 20 minutes up to and including 4 hours (1a) and from baseline mechanical threshold at 5, 20 and 40 minutes (1b)

Fig 1a

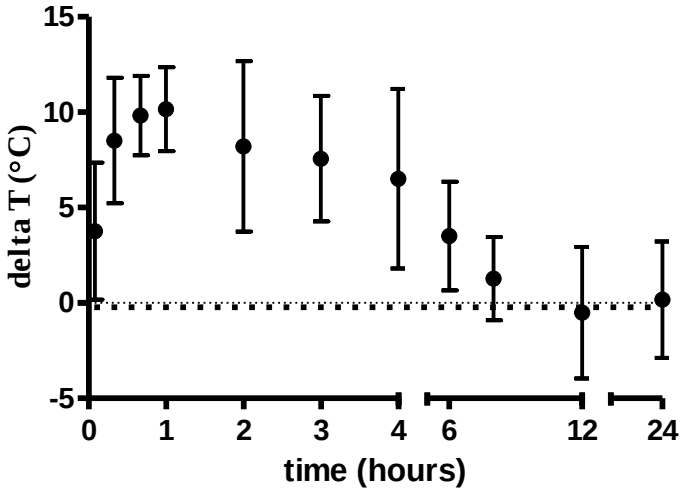
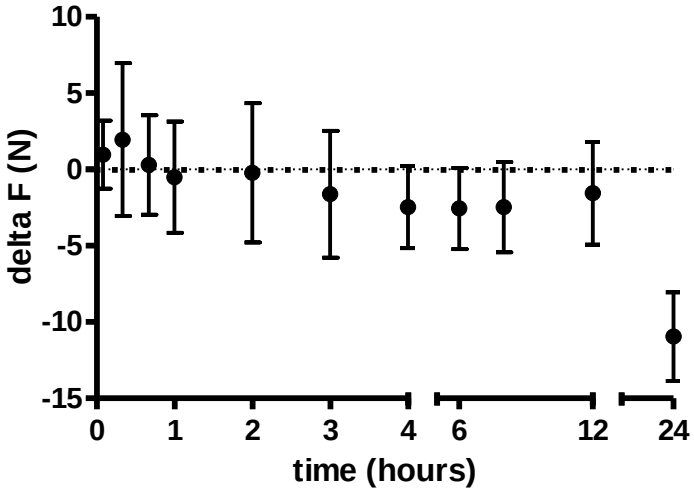


Fig 1b



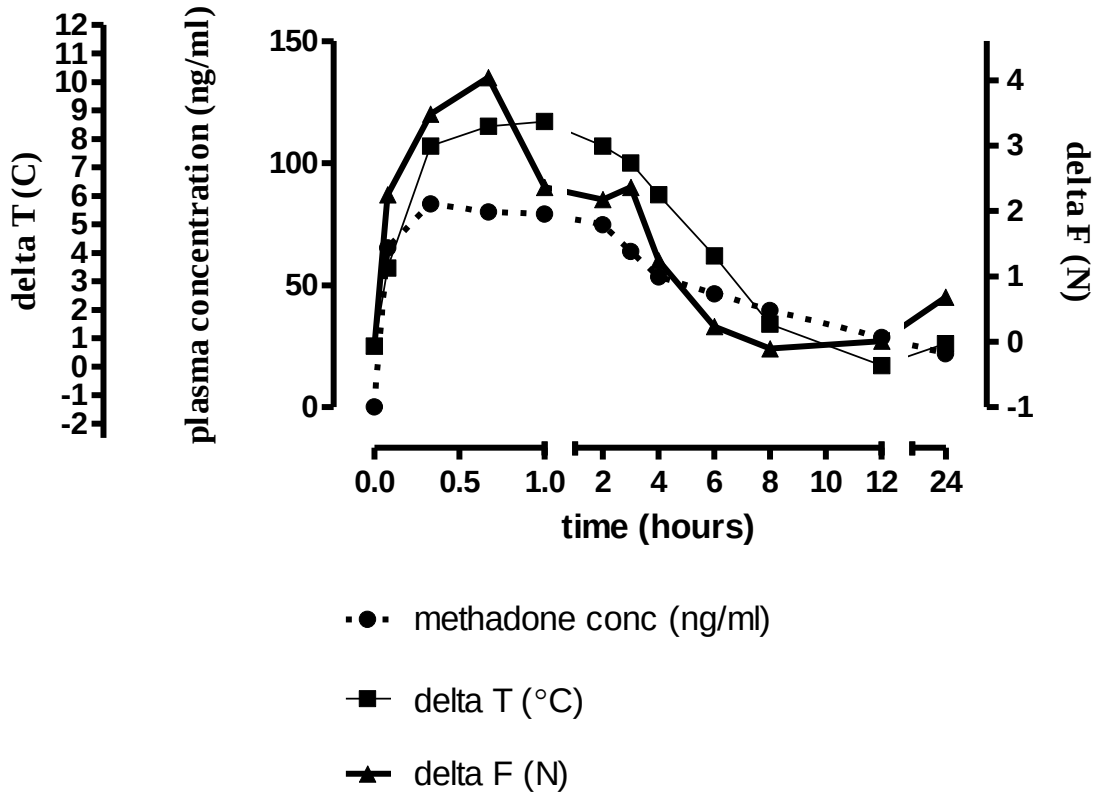


Figure 2

Methadone plamsa concentration and changes in mechanical and thermal threshold (delta T and F)

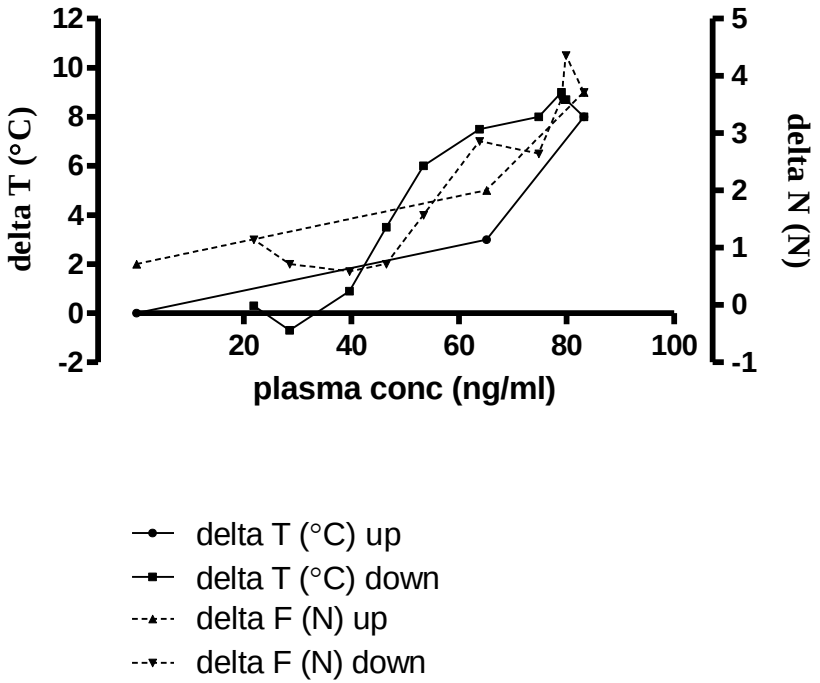

12
10
8
6
4
2
0
-2
delta T (°C)
20
40
60
80
100
plasma conc (ng/ml)
5
4
3
2
1
0
-1
delta N (N)
delta T (°C) up
delta T (°C) down
delta F (N) up
delta F (N) down

Figure 3 plasma concentration-time profile and the effect-time relationship showing anticlockwise hysteresis loop (effect data derived when concentrations were increasing are called ‘up’ and those when concentrations were decreasing were called ‘down’)