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Pharmacokinetics of Single Doses of Methadone and Buprenorphine in Blood and Oral Fluid in Healthy Volunteers and Correlation With Effects on Psychomotor and Cognitive Functions

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ABSTRACT **Purpose** We aimed to study the pharmacokinetics of methadone and buprenorphine in blood and oral fluid after single dose administration and investigate correlations between concentrations in blood and neurocognitive functions.

Methods A five-way, double-blind, randomized, placebo-controlled, double-dummy, cross-over study was performed to study the pharmacokinetics and neurocognitive effects of methadone (5 and 10 mg per oral) and buprenorphine (0.2 and 0.4 mg sublingual) in 22 healthy volunteers. Blood and oral fluid were collected throughout the test days and drug concentrations in both matrixes were analysed using UHPLC-MS/MS. On-road driving testing, neurocognitive computerized tests and subjective questionnaires were performed.

Results Large individual variations in concentrations of methadone and buprenorphine in blood and oral fluid, and accordingly oral fluid/blood drug concentration ratios, were observed. The mean ratio 6.5 hours after drug administration was 2.0 (range 0.49 – 7.39) for methadone after both doses. Buprenorphine was not detected above the limit of quantification in blood after 6.5 hours. No significant correlation between methadone concentration in blood and effect was found. Significant correlation were found between buprenorphine concentration in blood and standard deviation of lateral position in the driving test, and some measures of reaction time, divided attention, balance, alertness, contentedness and sleepiness.

Conclusions Concentrations of methadone and buprenorphine in blood and oral fluid showed large inter-individual variations. No concentration-effect correlations were found for methadone while low to moderate correlations were observed between buprenorphine concentration and driving, psychomotor function and subjective rating of sleep and alertness.

INTRODUCTION The current paper is a follow-up publication of our previous study (1), which suggested a relation between methadone and buprenorphine dose and neurocognitive impairment, which has, to our knowledge, not been studied previously (2). Results of blood and oral fluid measurements and the associations between methadone and buprenorphine concentrations in blood and psychomotor and cognitive performance are published separately in the present report.

Methadone and buprenorphine are widely used as analgesics and for maintenance treatment of patients with opioid use disorder. Only one previous study measured buprenorphine concentrations in plasma while testing psychomotor and cognitive effects after single dose administration in healthy volunteers (3), whereas other studies on performance did not include pharmacokinetic measurements of buprenorphine or methadone (4-10).

The use of oral fluid for detection and quantification of drugs of abuse has increased during the last decade. The relationship between drug concentrations in blood and oral fluid has been studied for several opioids and low correlations between oral fluid and blood concentrations were observed and their ratios varied widely (11-13). The latter indicates that opioid concentrations in oral fluid cannot be used to reliably estimate opioid concentrations in blood. Few studies have measured buprenorphine in oral fluid (14), none after single dose administration, while previous studies of methadone have indicated oral fluid/blood concentration ratios ranging from 0.28 to 7.2 (12, 13).

Our previous study showed that methadone and buprenorphine caused impairment of driving and related skills in opioid-naïve subjects (1). Buprenorphine produced mild impairment of driving, while more pronounced and dose dependent impairment of cognitive skills related to driving were found for both opioids, in line with previous studies (7, 9).

Establishment of concentration-effect relations for both methadone and buprenorphine are of relevance for assigning per se limits for driving under the influence. For example, Norway has implemented per se limits for methadone and buprenorphine in the Road Traffic Act. The limits corresponding to 0.02 % BAC were set at 25 ng/mL and 0.37 ng/mL, respectively.

This study aimed to answer whether drug concentrations in oral fluid, would reflect drug concentrations in blood. Furthermore, we wanted to study whether concentrations of methadone and buprenorphine in blood are correlated to the level of impairment that these compounds produce on actual driving and neurocognitive function.

MATERIALS AND METHODS The methods used have been described in detail by Strand et al. (1).

Subjects Twenty-two healthy volunteers (11 males, 11 females) aged 23-49 years (mean age 36 years) were included. Inclusion criteria were: males or females with good health based on a physical examination and the results of blood chemistry and haematology tests; age between 23 and 50 years; body mass index between 19 and 29 kg/m^2; and experienced drivers. Exclusion criteria were: pregnancy or lactation, sleep disorders; drug or alcohol abuse; use of psychoactive medication; excessive alcohol and/or caffeine use; smoking >6 cigarettes per day; intake of any opioid within 3 months before the study; and significant disease.

Design and treatments The acute effects of two single doses of buprenorphine (0.2 and 0.4 mg sublingual) and methadone (5 and 10 mg per oral) were studied in a five-way, double-blind, randomized, placebo-controlled, double-dummy, cross-over study.

Procedure Blood samples were collected 1, 2, 3.5 and 6.5 hours after drug administration, while the highway driving test was performed after 4 hours and the neurocognitive tests and subjective evaluations after 2 and 6 hours (morning and afternoon condition).

Pharmacokinetic assessments and drug analysis The analytical results in blood and oral fluid samples for two subjects were excluded from the data analysis due to incorrect labelling of the samples. Blood was collected using 5 ml Vacutainer® tubes containing Sodium Fluoride (20 mg) and Sodium Heparin (143 IU) (BD Diagnostics, Franklin Lakes, NJ, USA). Drug concentrations in whole blood samples were determined using UHPLC-MS/MS (15); the method was slightly modified for the determination of methadone and buprenorphine by adding relevant calibration standards. The limit of quantification (LOQ) in blood was 0.15 ng/mL for methadone and 0.09 ng/mL for buprenorphine, while the limit of detection (LOD) was 0.05 ng/mL for methadone and 0.009 ng/mL for buprenorphine.

Oral fluid was collected using Quantisal™ Oral Fluid Collection Device (Abbot, Lake Bluff, IL, USA). Concentrations of methadone and buprenorphine in oral fluid were determined using ultra-high performance liquid chromatography - tandem mass spectrometry (UHPLC-MS/MS) after 96-well supported liquid extraction (16). The LOQ in oral fluid was 0.31 ng/mL for methadone and 0.47 ng/mL for buprenorphine, while the LOD was 0.046 ng/mL for methadone and 0.07 ng/mL for buprenorphine. The oral fluid samples were diluted with 3 ml preservative buffer that was present in the Quantisal device. Therefore, the samples were weighed to determine the amount of oral fluid collected in order to calculate concentrations in neat oral fluid, which were calculated by multiplying the concentration of drug in oral fluid by the oral fluid weight (in g) + 3 g, and divide this by the oral fluid weight.

All data was included in the calculation of median values for blood and oral fluid concentrations shown in Figure 1. An outlier was defined as any value exceeding the third quartile plus the interquartile range × 1.5 or being less than the first quartile minus the interquartile range × 1.5.

Driving test, neurocognitive tests and subjective evaluations The on-road driving test was performed on a 100 km primary highway segment in normal traffic with duration of approximately one hour (17). Participants were instructed to drive with a steady lateral position within the right traffic lane at a constant speed of 95 km/h (60 mph) and a specially instrumented vehicle was used to measure standard deviation of lateral position (SDLP) in centimetres and mean lateral position (MLP) (18). A battery of computerized tests was performed: The Psychomotor Vigilance Task (PVT); The Critical Tracking Task (CTT); The Divided Attention Test (DAT); The Useful Field of View Test (UFOV); The Digit Symbol Substitution Test (DSST); Postural Balance test (PBT); and The Determination Test (DT/S1) of the Vienna Test System. A clinical test of impairment was performed, consisting of tests of balance and an overall impression of the subject (19). Subjective evaluations were assessed by visual analogue scales (Bond and Lader) (20) and the Karolinska sleepiness scale (21).

Statistics Bivariate correlations were used to evaluate associations between opioid concentrations in blood and opioid induced changes in driving and neurocognitive performance (concentration in blood x performance). The assumption was a linear concentration-effect relationship. The concentrations measured 3.5 hours after administration were used to calculate correlation with the driving test, while the concentrations measured after 2 and 6.5 hours were used to calculate correlation with the neurocognitive tests and subjective evaluations performed in the morning and afternoon, respectively. Correlations were calculated for the morning and afternoon separately due to the risk of acute tolerance to the drug effects. To calculate drug induced change, performance scores during placebo were subtracted from performance scores during treatment with methadone and buprenorphine. No correlation to oral fluid concentrations was calculated due to few correlations with blood concentrations. The statistical analyses were conducted using SPSS® Statistics version 25 (IBM Corporation, Armonk, NY, USA).

RESULTS **Pharmacokinetics** Mean concentrations of methadone and buprenorphine in whole blood and neat oral fluid are shown in Figure 1. After administration of methadone, concentrations in blood were above the LOQ of 0.15 ng/mL in all samples, except from one subject, who did not have methadone concentrations above the LOD 1 hour after both doses. For buprenorphine, only 36% of the blood samples had concentrations above the LOQ of 0.096 ng/mL. Only one subject had a concentration of buprenorphine in blood above the LOQ 6.5 hours after administration of 0.4 mg buprenorphine. Nine subjects did not have concentrations of buprenorphine in blood above the LOQ at any sampling time after administration of the low dose. After the high dose all subjects had buprenorphine concentrations in blood above the LOQ in two or more samples. Estimated concentrations below the LOQ, and above the LOD, are included in Figure 1.

Concentration ratios in oral fluid/blood A box-plot of all oral fluid/blood concentration ratios for methadone at the different times of sampling is shown in Figure 2. Only concentrations exceeding the LOQ were used to calculate oral fluid/blood ratios. Reliable oral fluid/blood ratios for buprenorphine could not be calculated due to oral residual drug.

Driving test, neurocognitive tests and subjective evaluations There was a significant correlation between the concentrations of buprenorphine in blood and changes in SDLP (r=.477, p=0.002), see scatter plots in Figure 3, and MLP (r=.323, p=0.039). There was a significant correlation between concentrations of buprenorphine in blood and the following measures in the morning: PVT reaction time (r=.369, p=0.016), PVT lapses of attention (r=.327, p=0.035), DAT reaction time (r=.335, p=0.032), DSST (r=-.342, p=0.027), PBT eyes open (r=.428, p=0.005), PBT eyes closed (r=.383, p=0.013) alertness (r=.573, p=0.000), contentedness (r=.417, p=0.006) and sleepiness (r=.408, p=0.007). During the afternoon a significant correlation was found between buprenorphine concentrations and the number of correct signal detections in the DAT (r=-.335, p=0.040) and alertness (r=.358, p=0.022).

Methadone concentrations significantly correlated with DSST performance (r=-.371, p=0.022) in the afternoon.

DISCUSSION Our study found large individual variations in blood and oral fluid concentrations for both drugs. Mean concentrations of methadone in blood were lower than previously reported concentrations after similar doses in healthy volunteers (22). Previous studies where buprenorphine has been administered to healthy volunteers have in most cases been with higher doses (4 and 8 mg sublingual) than those in the current study (23-25). If using their data to estimate the expected drug concentrations in blood after administration of 0.2 and 0.4 mg doses, the extrapolated concentrations of buprenorphine would be higher than those found in our study. This could indicate some type of saturation pharmacokinetics for buprenorphine when higher doses are administered.

Our findings indicate that the doses given caused detectable concentrations of buprenorphine in oral fluid up to approximately 6.5 hours after administration. A previous study reported that after acute sublingual administration of buprenorphine saliva and plasma levels were substantially elevated during the first twelve hours (14). The large inter-individual variations in blood and oral fluid concentrations also caused wide ranges of the oral fluid/blood concentration ratios. The oral fluid/blood ratios for buprenorphine were more than 1,000 times higher than those of methadone and extremely large during the first hours. This is most likely due to presence of residual buprenorphine in the oral cavity after sublingual administration. Buprenorphine concentrations in blood were below the LOQ after 6.5 hours, at a time where one could expect a steady state between blood and oral fluid, thus a reliable oral fluid/blood concentration could not be calculated at equilibrium. A mean oral fluid/blood concentration ratio for methadone of 0.7 has previously been reported (12) and oral fluid/plasma concentration ratios between 0.5 and 7.2 (13). The ratios for methadone 6.5

hours after drug administration, at a point where steady state between oral fluid and blood was probably achieved, were similar to these ratios.

The curve for methadone in oral fluid shows a "dip" at +3.5 hours after drug administration, around lunch time. This effect has previously been described after intake of zopiclone (26), and it has been suggested that the intake of a meal will increase saliva secretion and thereby dilute the oral fluid sample. This effect was however not observed for buprenorphine.

Methadone levels in blood did not correlate with the behavioural outcome measures. Some low to moderate correlations were found for buprenorphine, mainly in the morning around the time of maximum concentration of buprenorphine in blood. The tests where a concentration-dependent effect was found were not always coinciding with those where dose-dependent effects were found (1), even though the higher doses of methadone and buprenorphine caused higher mean concentrations in blood as compared to the lower doses. The main explanation for this discrepancy is probably that the degree of impairment also depends on factors other than drug concentrations in blood, i.e. drug transport across the blood-brain barrier (27, 28) and the activation of opioid-receptors (29).

We conclude that large inter-individual variations were observed in both blood and oral fluid concentrations after single dose administrations of methadone and buprenorphine. Furthermore, no correlation between methadone concentrations in blood and effects on driving performance, neurocognitive function and subjective ratings were found. Low to moderate correlations between buprenorphine concentrations and measures of driving, reaction time, divided attention, balance test, alertness, contentedness and sleepiness were found.

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Figure 1 Mean (SD) concentrations of methadone and buprenorphine in oral fluid and whole blood (ng/mL) after administration of 5 or 10 mg methadone per oral (po) and 0.2 or 0.4 mg buprenorphine sublingual (sl).

_____ Oral fluid

- - - - - - Blood

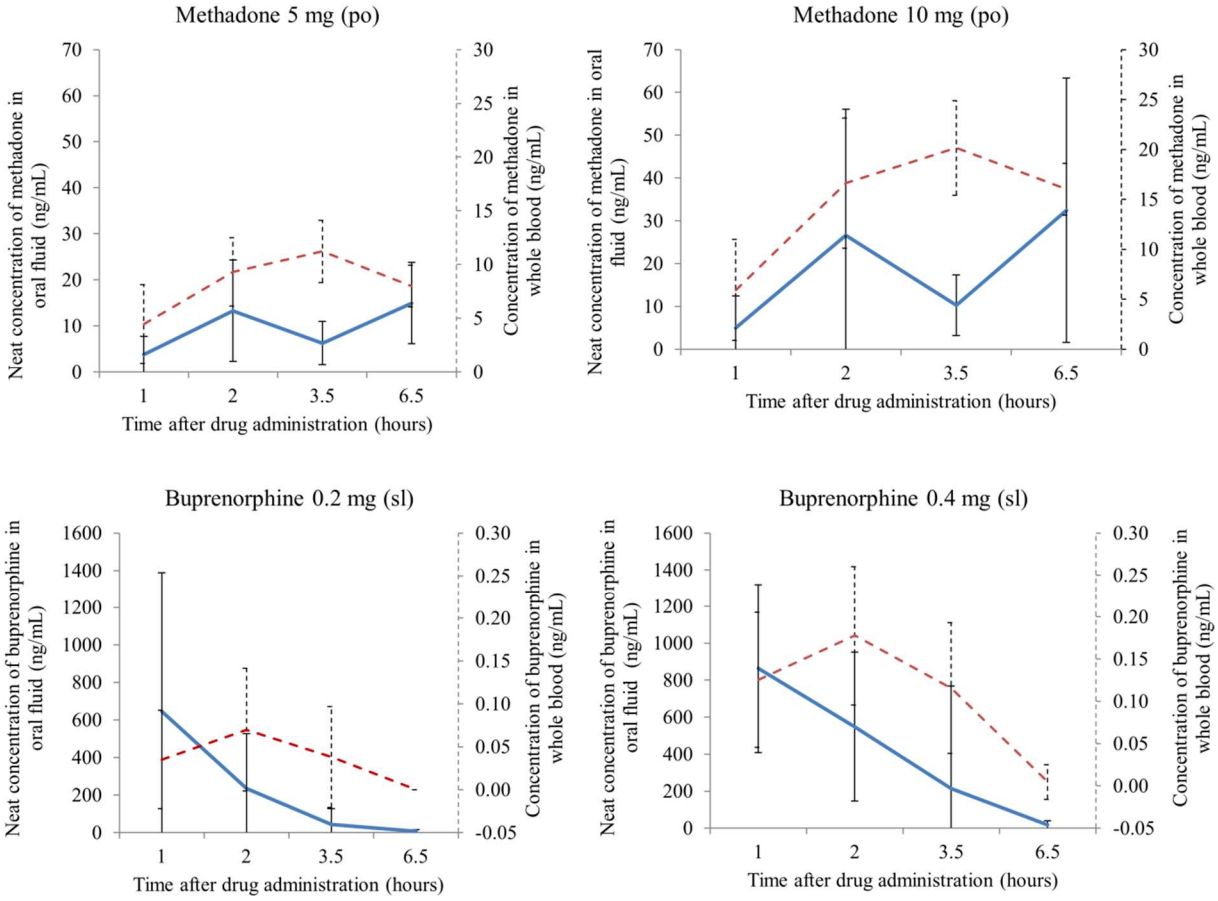

Figure 2 Boxplots showing rations between concentrations of methadone in oral fluid compared to whole blood.

× = maximum outliers

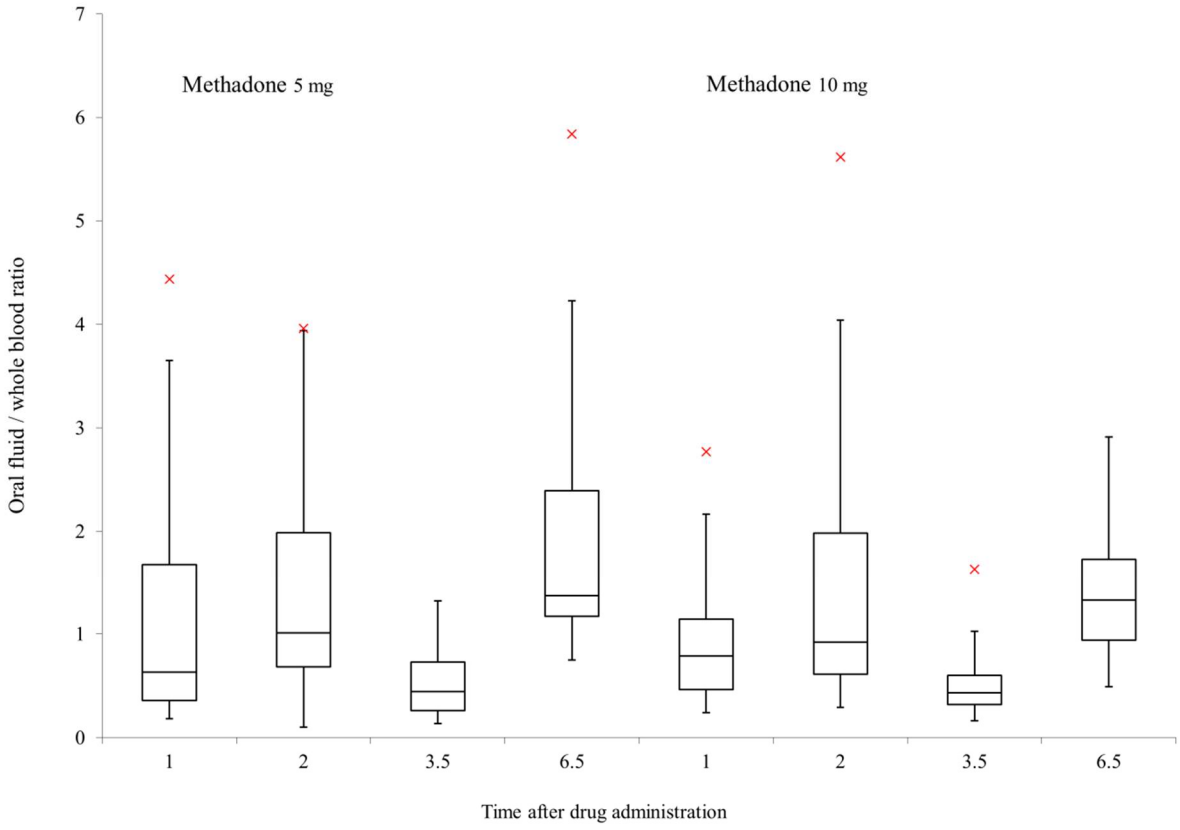

Figure 3 Scatter plot showing the relationship between differences in standard deviation of lateral position (SDLP) from placebo and concentrations of buprenorphine and methadone in whole blood (ng/mL). A significant correlation was found between concentration of buprenorphine in blood and change in SDLP as compared to the placebo condition.