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Codeine and Morphine Blood Concentrations Increase During Blood Loss*

ABSTRACT: During extensive blood loss, a plasma volume refill will take place by transfer of extravascular fluid into the circulation. Drugs present in this fluid may follow and cause a rise or a drop in blood drug concentration, depending on their levels and accessibility in the restoration fluid. This study explored the possible changes of codeine, and its metabolite morphine, in whole blood during a standardized exsanguination in the rat. Three doses containing 5 mg codeine were given orally. In eight rats, blood loss was accomplished by slowly withdrawing 0.8 mL blood at 10 min intervals during 70 min. In control rats, blood was withdrawn only at 0 and 70 min. At 70 min, the final/initial codeine and morphine concentration ratios were 0.70 ± 0.38 and 0.88 ± 0.47, respectively, in controls, but increased to 1.28 ± 0.44 ($p=0.014$) and 1.41 ± 0.34 ($p=0.021$), respectively, in exsanguinated rats. It is concluded that blood loss can affect blood drug concentrations.

KEYWORDS: forensic science, forensic toxicology, pharmacokinetics, blood loss, hemorrhage, codeine, morphine, redistribution, postmortem, rat

During extensive blood loss, a number of compensatory mechanisms come into action to maintain a sufficient blood pressure. Hence, via an activation of the sympathetic nervous system, the heart rate will increase, and selected peripheral vessels will be constricted, resulting in a reduced circulatory compartment and a decreased glomerular filtration (1). If the blood loss continues, a plasma volume refill will take place by transfer of extravascular fluid into the blood circulation (1–3). The difference in pressure (hydrostatic+osmotic) between the intracapillary compartment and the surrounding tissue will determine the rate of fluid influx (Fig. 1). Drugs present in this fluid may follow and cause a rise or a drop in blood drug concentration, depending on their levels and accessibility in the restoration fluid. Hence, the drug concentrations found in the blood of a trauma victim may differ from the concentrations present at the time of trauma (e.g., an automobile accident). Such a "post-traumatic" change in blood concentrations can be of importance for the interpretation of the concentrations of both living and deceased subjects, in terms of incapacitation, or for an evaluation of statements about amount taken (e.g., dose according to prescription, or not) of certain drugs. The possibilities of a clinical effect of such a redistribution of drugs should also be considered, particularly if this results in a substantial increase (or decrease) in the transport of the drug to its effector organs. It has previously been shown that the pharmacokinetics and pharmacodynamics of certain drugs are altered when given to hypovolemic animals (4–6). However, no studies have been conducted on the influence of blood loss on the pharmacokinetics of drugs pre-existing in the body at the time of trauma, which is a setting more relevant to the forensic casework. This study was carried out to evaluate the possible changes of codeine, and its metabolite morphine, in whole blood during a standardized exsanguination in the rat.

Material and Methods

Animals

Female Sprague-Dawley rats (B&K Universal AB, Sollentuna, Sweden) weighing 250–300 g were used. All animals had free access to standard laboratory pelleted chow containing 14.5% crude protein (R70; Lactamin AB, Vadstena, Sweden) and tap water *ad libitum*. All rats were housed in groups of two animals in macrolone cages under climate-controlled conditions for regular in-door temperature and humidity. The rats were kept in a constant 12:12 h light:dark cycle synchronous with daylight.

Ethics

All experiments were performed in strict accordance with the guidelines and with the consent of the Animal Ethics Committee, Linköping, Sweden (Permit No. 64-99).

Experimental Procedure

All rats were given a 0.3 mL suspension containing 5 mg codeine (codeine phosphate semihydrate; Kodein Recip; Recip AB, Årsta, Sweden) orally through a feeding tube during a brief CO_2 anesthesia. Three doses were administered at 45 min intervals (corresponding to t½ for codeine in the rat) to build up the tissue concentrations. The drug administration scheme and surgery procedures are illustrated in Fig. 2. The animals were fasted for several hours before the experiments. Anesthesia was induced and maintained using isofluorane (Forene®; Abbott Scandinavia AB, Solna, Sweden), and the body temperature was carefully monitored at 37.5 ± 1°C using a servo-controlled heating pad and a heating lamp. The left femoral artery was dissected free and a thin, own-

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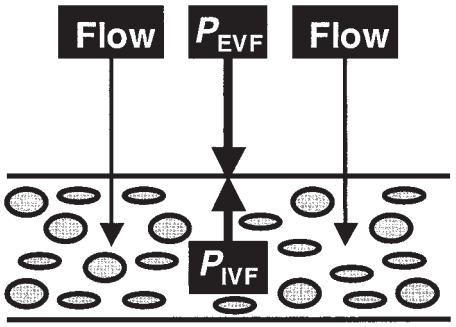

FIG. 1—*The difference in concentration of a drug within and outside the blood vessels are decisive for the possible changes in blood drug concentration when the extravascular fluid is drawn into the circulation during blood loss. The difference in pressure (hydrostatic +osmotic; thick arrows) between the intracapillary compartment (P_{IVF}) and the surrounding tissue (P_{EVF}) will determine the rate of fluid influx (thin arrows).*

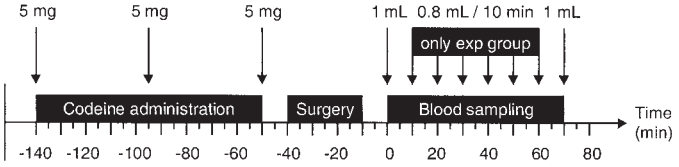

FIG. 2—*The experimental design. For details, see text.*

designed, heparinized polypropylene catheter was inserted. Blood loss was accomplished in eight rats by slowly withdrawing 0.8 mL blood at 10 min intervals, except for the first and 8th samples when 1.0 mL was withdrawn to allow for hematocrit measurements. Assuming a blood volume of 60 mL/kg body weight in the Sprague-Dawley rats (7), this equals a blood loss of approximately 40%. Control rats were subjected to the same protocol, but blood was withdrawn only at 0 and 70 min. The samples collected at 0, 10, 30, 50 and 70 min were analyzed for codeine and morphine using a gas chromatographic-mass spectrometric (GC-MS) method, described below. The ratio between the final and the initial blood concentrations for codeine and morphine was calculated for each animal, and was used to compare experimental (exsanguinated) rats with the controls. The hematocrit was analyzed in triplicates in the initial and final blood samples. The animals were sacrificed by puncture of the abdominal aorta. This blood was collected, and the plasma prepared was analyzed regarding the albumin content (for details; see below).

Analysis of Codeine and Morphine

Concentrations of free (non-glucuronized) codeine and morphine in whole blood were determined with a routine GC-MS method based on a previously described method (8) with slight modifications. Briefly, after solid-phase extraction with Bond Elute Certify cartridges, the opiates and deuterium-labeled internal standards were determined on a FISON GC-MS system in selected-ion monitoring mode. Ions monitored for the analytes were m/z 282.2 and 445.2 for codeine; m/z 448.2 for codeine-D_3; m/z 361.2, 414.2, and 577.2 for morphine; and m/z 417.2 for morphine-D_3. The blood aliquots were measured by weight and drug concentrations were reported in mass/mass units (μg/g). The limit of quantitation for both codeine and morphine was 5 ng/g. The calibration curve was constructed up to a maximum concentration of 0.4 μg/g.

Analysis of Plasma Albumin

Albumin in plasma was measured by an in vitro diagnostic reagent system (COBAS® INTEGRA Albumin; Roche Diagnostics Scandinavia AB, Bromma, Sweden) used on a COBAS® INTEGRA 700 (Roche Diagnostics Scandinavia AB). This method is based on the bromcresol green assay (9) with some modifications. Briefly, albumin at pH 4.3 is sufficiently cationic to bind the anionic dye bromcresol green to form a blue-green colored complex. The intensity of the blue-green color is directly proportional to the concentration of albumin in the sample. The concentration was determined by monitoring the increase in absorbance at 629 nm.

Statistics

All statistical analyses were performed using the computer software StatView® for Windows Version 5.0 (SAS® Institute Inc., Cary, NC). Drug concentration ratios, hematocrit, and protein concentrations determined at the end of the experiment were compared between the experimental group and the control group using the unpaired two-tailed Student's *t*-test. The hematocrit levels determined at the start and at the end of the experiment within the two groups were compared using the paired two-tailed Student's *t*-test. A value of $p<0.05$ was considered statistically significant.

Results

The results are displayed in Table 1 and Fig. 3. The average body weight was equal in experimental rats and in controls. The mean initial blood concentration of codeine and morphine did not differ between the groups. The control rats showed a decrease in codeine and morphine concentrations, as could be inferred from the contin-

TABLE 1—*The results in summary. All values are means ±SD.* N = *8 in each group, except for plasma albumin (exp,* N = *4; ctrl,* N = *5).*

	Exp	Ctrl	*p* (exp vs. ctrl)
Codeine initial (μg/g)	0.15 ± 0.07	0.15 ± 0.11	0.957
Codeine final (μg/g)	0.20 ± 0.13	0.09 ± 0.06	0.040
Morphine initial (μg/g)	0.23 ± 0.13	0.26 ± 0.24	0.786
Morphine final (μg/g)	0.33 ± 0.21	0.17 ± 0.06	0.053
Hct initial (%)	44.0 ± 0.8	43.5 ± 1.3	0.365
Hct final (%)	33.9 ± 2.2	41.8 ± 2.3	<0.0001
Plasma albumin (g/L)	23.5 ± 1.9	25.7 ± 1.0	0.059
Body weight (g)	265 ± 11	271 ± 18	0.439

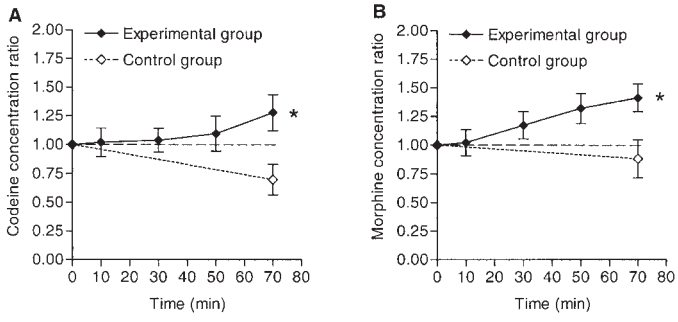

FIG. 3—*The ratios of blood concentrations of codeine (A) and morphine (B). The ratios were calculated for each rat by dividing the drug concentrations at 10, 30, 50 and 70 min by the initial drug concentrations at 0 min. Values are means ±SEM,* N=*8 for each time point. *=significantly different from controls; (A)* p=*0.014, (B)* p=*0.021.*

uing metabolism. In contrast, the concentrations of both codeine and morphine gradually increased in rats subjected to blood loss. The final/initial blood concentration ratios for codeine increased to 1.28 ± 0.44 in experimental rats as compared to a drop to 0.70 ± 0.38 in control rats, $p = 0.014$. Similarly, the final/initial blood concentration ratios for morphine averaged 1.41 ± 0.34 in experimental rats, but dropped to 0.88 ± 0.47 in controls, $p = 0.021$. The hematocrit was equal in both groups before surgery. The drop in hematocrit among exsanguinated rats was substantial (42% to 34%), $p < 0.0001$. A slight decrease was also noted among the controls (44% to 42%), however, this difference was not statistically significant. The time between the last dose and the initial blood sample averaged exactly 51.13 min in both experimental rats and controls. The time for fasting before the experiment was intentionally modified so that certain rats in both groups were kept fasting for a longer time, and others at shorter times. There was, however, no correlation between the time of fasting and the blood concentrations of codeine or morphine, or the final-initial blood concentration ratios. The average fasting time was 4.7 and 4.6 h in experimental rats and controls, respectively. Plasma albumin concentrations were somewhat lower among experimental rats as compared to controls, but the difference did not reach statistical significance.

Discussion

We investigated the effect of standardized blood loss on the blood concentrations of codeine and morphine in the rat. We found that the concentrations gradually increased in the experimental rats, but dropped in the control rats, as can be expected due to the continuing metabolism. The final/initial concentration ratios of codeine and morphine were significantly higher in experimental rats than in controls (1.8 and 1.6 times, respectively). The concentrations of morphine increased somewhat more in experimental rats and decreased less in control rats, as compared to the changes in codeine concentrations. This is also a reasonable finding considering the on-going conversion of codeine to morphine during the experiment.

Previous studies have focused on hypovolemic animals, i.e., the drugs have been administered after blood loss in order to mimic treatment of hypovolemic patients. De Paepe et al. (4) thus found that the same doses of morphine resulted in significantly higher plasma concentrations in hypovolemic rats. Further, studies on the electroencephalographic effect of the antiepileptic etomidate showed that hypovolemic rats required significantly lower doses to achieve the desirable effect (5). The present study explored the effect of blood loss in animals with pre-existing concentrations of codeine and morphine in different body compartments, thus trying to simulate a situation where a person on a medication is subjected to a trauma. The observed increase in blood concentrations during blood loss cannot be explained by reduced renal elimination since only a negligible portion of codeine and morphine is excreted unchanged into the urine. Further, the increase in codeine concentrations is not likely to be due to reduced glucuronization, since, in the rat, this compound is only formed to a very little extent, and instead morphine constitutes the main metabolite (10). Thus, the increased concentrations are more reasonably explained by a fluid refill from stores with higher concentrations of codeine and morphine. Experimental studies suggest that codeine is accumulated in the adrenals, brain, spinal cord, lungs and the heart in the rat (11,12). Most likely, most of the fluid transferred to the blood originates from the skeletal muscles, which constitutes a large fluid reservoir that has been shown to decrease in weight during hemorrhage (13). A certain portion of the drugs may, however, have been transferred from the gastro-intestinal canal to the blood during the blood loss; however this contribution was seemingly not important in the present experimental setting since there was no correlation between blood levels and fasting time.

The substantial refill of extravascular fluid to the blood stream was confirmed by a significant decrease in hematocrit in exsanguinated rats. However, there was also a slight drop in hematocrit in the control rats. The tapping of $1+1$ mL of blood from the controls was necessary for the analyses, but might have caused some transfer of fluid from the stores; hence the difference in blood concentration ratios between the groups might actually been even larger if this tapping could have been minimized.

The plasma albumin concentration was slightly lower in the experimental rats than in the controls, but it is difficult to determine if such a possible decrease per se affects the blood drug concentrations of codeine and morphine. The plasma protein binding for codeine is 12% and 14% in the rat and in humans, respectively (14), implying that slight changes in plasma protein concentration is not likely to result in major alteration of the whole blood concentrations of codeine.

The volume of distribution (V_D) of codeine and morphine in the rat is 4–5 and 8 L/kg, respectively (4,15,16). For drugs with higher V_D, it is likely that this pathophysiologic mechanism will cause a substantially larger increase in blood concentrations during blood loss. Conversely, the blood concentrations of drugs with a very low V_D may drop significantly when extravascular fluid with lower concentrations of the drug is drawn into the circulation during blood loss. The sympathetically mediated vasoconstriction during blood loss is selective and gradually gives more priority to the vital organs, particularly the heart and the brain (1,3). This redistribution can thus result in increased or decreased pharmacological effect, depending on effector organs involved. In other words, a toxic effect, or a loss of therapeutic effect may result from blood loss in subjects on controlled, chronic medication with different drugs.

In the present study, three doses of codeine were administered to build up concentrations in the different tissue stores, however it is likely that these stores were not maximally filled up. Hence, blood loss in animals subjected to a chronic dosage over several days might have caused even larger alterations in the blood concentrations. The blood concentrations achieved were possibly in the toxic range, but obviously not lethal since the rats did not show any signs of respiratory depression. The use of codeine allowed for detection of both codeine and morphine, which can be analyzed simultaneously. The concentrations detected were all well above the limit of detection for the GC-MS method used, but a few samples slightly exceeded the calibration interval, and were reanalyzed after dilution. It should be pointed out that codeine is only to a minor extent metabolized to morphine in man (16), hence the results in this respect should not be applied to the situation in humans.

A rapid bleeding leading to death within a few minutes is not likely to result in any significant changes in blood concentrations of drugs, but during prolonged hemorrhage fluid transfer from extravascular tissue to the blood will follow. This phenomenon has been considered to start relatively late, but Lundvall and Länne (17) have shown that experimental hypovolemia in humans may cause a transfer of about 500 mL in 10 min. It can be assumed that the alterations in blood concentrations will be most pronounced for drugs with a very high or very low V_D. It is possible that factors such as the pKa, lipophilicity and degree of protein binding also can influence the outcome. It is interesting to note that even the distribution of a small molecule such as ethanol seems to be affected by blood loss (18).

The findings of the present study may imply that: 1) the drug concentrations after blood loss can be different from the drug concentrations at the time of trauma, and hence lead to erroneous conclusions e.g., in terms of incapacitation, or insufficient effect of certain drugs; and 2) the redistribution can lead to increase or decrease in pharmacological effect of the particular drug. Further studies using substances with higher, or lower V_D, are encouraged to further explore the influence of hemorrhage on pharmacokinetics and pharmacodynamics.

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