Study of free/total morphine-ratio in blood after heroin intake as a tool to predict the outcome of heroin-intoxication

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ABSTRACT

The time course of free/total morphine ratio (f/t M-ratio) after i.v. heroin administration in both healthy people and patients with liver or renal insufficiency was pharmacokinetically simulated. In the 502 cases of heroin consumption investigated in this study, the morphine blood levels and f/t M-ratio were registered, arranged, and statistically analysed.

Total morphine blood levels in survivors and non survivors were 0.010-3.900 µg/ml (with 0-0.275 µg/ml of free morphine) and 0.010-8.570 µg/ml (with 0-2.200 µg/ml of free morphine), respectively. Therefore the respective ranges of f/t M-ratio-levels in survivors and in lethal cases were 0-0.786 and 0-0.935.

Based on this simulation, the f/t M-ratio after heroin injection was determined by the time elapsed since the last dose and individual pharmacokinetics of heroin and its metabolites. This ratio is independent to the heroin dose.

Keywords: Heroin; Morphine; Lethal intoxication; Pharmacokinetic simulation

INTRODUCTION

The ratio value of f/t M-ratio in blood can be used to predict a fatal outcome after illicit heroin intake [1]. To better use this ratio, it is necessary to understand the influence of the opiate's pharmacokinetics on the time course of this ratio after heroin consumption. A review of the literature shows that there was a large intra- and inter-individual variation in all pharmacokinetic parameters of morphine and its glucuronides [2]. This large variation

contributes to differences in the time course of f/t M-ratio after heroin intake.

In the present study, the time course of f/t M-ratio in different subject groups (healthy subjects, and patients with liver cirrhosis or kidney failure) was pharmacokinetically simulated. For positive opiate-findings, the distribution of blood levels of free and total morphine and f/t M-ratio in heroin-related deaths and in cases surviving heroin intoxication were evaluated. The aim of this study was to simulate the influence of inter-individual variations in pharmacokinetics of morphine and its glucuronides on the time course of the f/t M-ratio after heroin injection, with a view to better predict the outcome of heroin-intoxication.

MATERIALS AND METHODS

A Case material

All toxicological cases submitted by local authorities to the Department of Legal Medicine in Göttingen from January 1997 to December 2002 were identified. In 502 cases (370 cases of heroin-related deaths and 132 cases surviving heroin intoxication) investigated in the present study, morphine in blood levels were detected. The free morphine levels, total morphine levels and f/t M-ratio in blood were then analysed statistically.

B. Chemical-toxicological analyses

The toxicological examinations of specimens involved an immunological screen for opiate, methadone, cocaine metabolites, benzodiazepines, cannabinoids, tricyclic anti-depressants and barbiturates by using Dade-Behring, Emit® II Plus reagents and the Viva analyzer (Syva Company). For the screening of amphetamines, MTP-Drug-immunoassay from Mahsan® Diagnostika (Mahsan Diagnostika Vertriebsges. mbH, Reinbek, Germany) was used. Samples with positive results were verified and quantified by specific methods (GC/MS).

Opiates in specimens separated and isolated from CSDAU-203-column (United Chemical Technologies Inc., Horsham, PA USA) were derivatized with pentafluoropropionic anhydride (PFPA). These derivates were examined by using of GC/MS (Shimadzu). The level of free and total morphine was determined before and after acid hydrolysing the specimens. In hydrolysis, 0.5 ml of 37% hydrochloric acid was added into 1 ml specimens and

incubated at 120°C for 45 minutes. The efficiency of hydrolysis was 95%.

C Simulation of plasma concentration-time profiles of opiates after intravenous ingestion of heroin

The simulation was based on the model of metabolite formation pharmacokinetics from Weiss [3]. In this case, a bi-exponential disposition model was used for disposition functions of heroin and its metabolites in the human body.

For intravenous administration of heroin, the plasma concentration-time-profile of heroin in the bi-exponential disposition model can be written as:

$$[H](t) = D_H^{iv} \sum_{i=1}^{2} \alpha_{i\,H}\, e^{-\lambda_{iH}} \qquad (1)$$

where *[H] (t)* denotes the concentration-time profile after i.v. dose of heroin $D^{iv}; H$ and parameter α_{iH} and λ_{iH}, defined as the disposition parameters of heroin. The Laplace transformation of eq. 1 is given by:

$$[H](s) = D_H^{iv} \sum_{i=1}^{2} \left(\frac{\alpha_{i\,H}}{(s + \lambda_{i\,H})} \right) \qquad (2)$$

According to the model of metabolite formation pharmacokinetics from Weiss [3], the input function of 6-acetylmorphine (6-AM) generated from heroin can be written as:

$$I_{6\text{–}AM}(s) = F_{H\_6\text{–}AM}\; CL_H\; \Psi_{H\_6\text{–}AM}(s)\,[H](s) \qquad (3)$$

where CL_H is the total clearance of heroin, $F_{H\_6\text{-}AM}$ denotes the fraction of heroin metabolized to the 6-AM and $\psi_{H\_6\text{-}AM}\ (s)$ is the transit time density corresponding to the formation of 6-AM from heroin. In this study the transit time density $\psi_{p\_m}(s)$ corresponding to formation of the primary metabolite *m* from parent drug *p* was neglected.

The time course of 6-AM concentration generated after i.v. administration of heroin can be written as:

$$[6\text{–}AM](s) = I_{6\text{–}AM}(s) \sum_{i=1}^{2} \left(\frac{\alpha_{i\ 6\text{–}AM}}{(s + \lambda_{i\ 6\text{–}AM})} \right) \qquad (4)$$

The parameters of disposition functions of heroin and 6-AM were calculated on the basis of the estimated mean concentration values. The mean values of plasma concentration-time profiles of heroin and 6-AM were taken from the literature [4]. During the simulation, it was assumed that there are no significant changes of the intra- and inter-individual disposition parameters of heroin and 6-AM.

The input function of morphine (M) generated from 6-AM is:

$$I_M(s) = F_{6\text{–}AM\_M}\; CL_{6\text{–}AM}\; [6\text{–}AM](s) \qquad (5)$$

where $CL_{6\text{-}AM}$ is the total clearance of 6-AM, and $F_{6\text{-}AM\_M}$ denotes the fraction of 6-AM metabolized to morphine. The concentration-time profile of morphine can be described as:

$$[M](s) = I_M(s)\sum_{i=1}^{2}\left(\frac{\alpha_{i\ M}}{(s+\lambda_{i\ M})}\right) \qquad (6)$$

The $F_{H\_6\text{-}AM}$ was calculated from the fraction of heroin excreted unchanged in urine, $F_{Exr\ H,}$ after i.v. administration of heroin, $F_{H\_6\text{-}AM} = 1 - F_{Exr\ H}$. The mean (± S.E.) urinary excretion of heroin and 6-AM after i.v. administration was 0.05 ± 0.01% and 1.7 ± 0.3% of the administered heroin dose, respectively [5]. Based on the urinary excretion of heroin and 6-AM, for the simulation $F_{H\_6\text{-}AM} = 0.99$ and $F_{6\text{-}AM\_M} = 0.98$ was chosen.

The input functions of M3G, $I_{M3G}(s)$, and M6G, $I_{M6G}(s)$, generated from morphine are:

$$I_{M3G}(s) = F_{M\_M3G}\ CL_M\ [M](s) \qquad (7)$$

$$I_{M6G}(s) = F_{M\_M6G}\ CL_M\ [M](s) \qquad (8)$$

where CL_M is the total clearance of morphine, F_{M_M3G} denotes the fraction of morphine metabolized to the M3G, and F_{M_M6G} denotes the fraction of morphine metabolized to the M6G. The concentration-time profile of morphine glucuronides can be described as follows:

$$[M3G](s) = I_{M3G}(s)\sum_{i=1}^{2}\left(\frac{\alpha_{i\ M3G}}{(s+\lambda_{i\ M3G})}\right) \qquad (9)$$

$$[M6G](s) = I_{M6G}(s)\sum_{i=1}^{2}\left(\frac{\alpha_{i\ M6G}}{(s+\lambda_{i\ M6G})}\right) \qquad (10)$$

The mean of disposition functions-parameters of either morphine or M6G regard to the healthy subject were taken from literature [6]. During the simulation, it was assumed that the disposition parameters of M3G are equal to the one of M6G. Literature shows that after i.v. administration of morphine in humans, 8-13% of the morphine dose was excreted unchanged in urine, 43-55% of dose was excreted as M3G, and 8-12% of dose as M6G [7,8]. During this simulation F_{M_M3G} = 0.55 and F_{M_M6G} = 0.10 were chosen.

Studies of the pharmacokinetics of morphine and its glucuronides in cases of patients with renal or liver insufficiency showed an alteration of their disposition parameters [9-13]. To obtain the altered dispositions parameters (□ᵢand □ᵢ), the disposition function was reparameterized as a two-compartment model, using standard equations (for details, see [6]). It was assumed that there were no changes of

values of the transfer constants between compartments (k_{12} and k_{21}) of either morphine or morphine glucuronides regarding to healthy subjects, kidney failure, and liver cirrhosis. The k_{12} and k_{21} of either morphine or morphine glucuronides were obtained from Lötsch [6].

The reparameterization of disposition functions of morphine and its glucuronides regarding to patients with renal insufficiency was based on the results of studies by Osborne [9] and by Hanna [13], respectively. The reparameterization of disposition functions of morphine in regard to patients with liver insufficiency was based on results by Mazoit [10] and by Hasselström [12]. It was assumed that the disposition functions of morphine glucuronides were the same for patients with liver cirrhosis and the healthy subjects.

The detection limit of opiates in this simulation was set at 1 ng/ml. The time course of f/t M-ratio was calculated from the simulated plasma concentration-time profiles of opiates after intravenous ingestion of 200 mg heroin. The simulation showed the total morphine is the sum of concentrations of heroin, 6-AM, morphine, and morphine glucuronides. The time course of f/t M-ratio can be written as:

$$f/t\,M-ratio(s)=\frac{[M](s)}{[H](s)+[6-AM](s)+[M](s)+[M3G](s)+[M6G](s)} \quad (11)$$

For the whole pharmacokinetic simulation, Scientist 2.0-Software (MicroMath Inc., Salt Lake City, UT) was used.

RESULTS AND DISCUSSION

Results

A Distribution of concentrations of morphine and the ratio of f/t M-ratio in blood after heroin consumption.

The variability of blood levels of free and total morphine are summarized in Table 1. These displayed a large area overlap of morphine-blood levels and also f/t M-ratio between survivors and non-survivors. The frequency distribution of f/t M-ratio is illustrated in Figure 1.

Table 1: Median and range of morphine levels in the blood after heroin or morphine intake

Group	N	Free morphine (µg/ml)		Total morphine (µg/ml)		f/t M- ratio	
		Median	Range	Median	Range	Median	Range
All cases							
Lethal intoxication	132	0.165	0 - 2.200	0.510	0.010 - 8.570	0.300	0 – 0.935
Survived intoxication	370	0.020	0 - 0.275	0.280	0.010 - 3.900	0.062	0 – 0.786
Total	502						
Intoxication only by opiate							
Lethal intoxication	12	0.200	0 - 0.760	0.420	0.070 - 1.740	0.404	0 – 0.621
Survived intoxication	90	0.025	0 - 0.275	0.348	0.012 - 2.200	0.070	0 – 0.300
Total	102						
Multiple drug intoxication							
Lethal intoxication	120	0.160	0 - 2.200	0.525	0.010 - 8.570	0.300	0 – 0.935
Survived intoxication	280	0.020	0 - 0.255	0.268	0.010 - 3.900	0.058	0 – 0.786
Total	400						

N = number of cases.

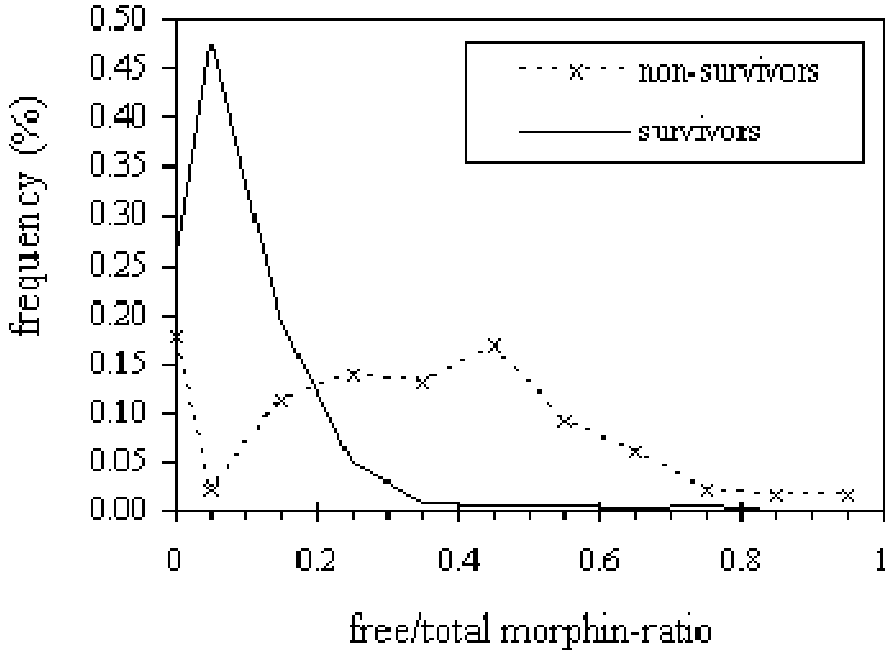

Figure 1: Frequency distribution of the f/t M-ratio in blood in cases of survivors (N=370) and of non-survivors (N=132). In cases of survivors after heroin intake, the median f/t M-ratio was 0.062 (range, 0-0.786) and 0.300 for all cases of non-survivors (range, 0-0.935), respectively. An overlap is observed for the f/t M-ratio in the two groups.

By applying a cut off value of 0.12 for the f/t M-ratio as described by Meissner [1], results of the present study showed 80.5% cases of heroin intoxication survivors. In addition, the 28% death cases found had a ratio below the cut off value (0.12).

B. Simulation of time course of f/t M-ratio in blood after intravenous ingestion of heroin

The parameters of the disposition functions of heroin and their metabolites were used in the simulation are summarized in Table 2. The simulated time course of f/t M-ratio after i.v. administration of heroin in healthy subjects, patients with kidney failure, and patients with liver insufficiency are illustrated in Figure 2.

Table 2: Pharmacokinetic parameters of heroin and its metabolites, which were used at the simulation

Parameters	Healthy subjects				Liver insufficiency	Renal insufficiency	
	H[a]	6-AM[a]	M[b]	MG[b]	M	M	MG
□1 (l^{-1})	0.003	0.032	0.058	0.082	0.026	0.054	0.059
□z (l^{-1})	0.307	0.002	0.001	0.039	0.002	0.002	0.054
□1(min^{-1})	0.641	0.577	0.280	0.071	0.170	0.221	0.059
□z (min^{-1})	0.315	0.030	0.0053	0.0085	0.0019	0.0048	0.00057
$t_{1/2\,z}$ (min)	2.2	23	135	83	369	142	1210
CL (ml/min)			2224	178	798	1344	10.6
V_c (l)			35	8	35	17	8

□¡and □i = parameters of the disposition functions, $t_{1/2\,z}$ = terminal half-life, CL = total body clearance, H = Heroin, M = Morphine, MG = morphine glucuronides, [a]= was calculated from published mean concentration-time-cuves by Bourquin [11]. [b]= were obtained from Lötsch [13]. In this simulation, it assumed that there are no significant changes of the disposition parameters of heroin and 6-AM regarding to three subjects groups.

The reparameterization of disposition parameters of morphine in patients with liver insufficiency was based on the results of pharmacokinetic study of morphine in patients with liver cirrhosis [10, 12]. For this subject group, it was assumed that there was no significant change in disposition parameters of morphine glucuronides.

The reparameterization of disposition parameters of morphine and its glucuronides in patients with renal failure was based on the results of opiate's pharmacokinetic studies in patients with renal insufficiency [9, 13].

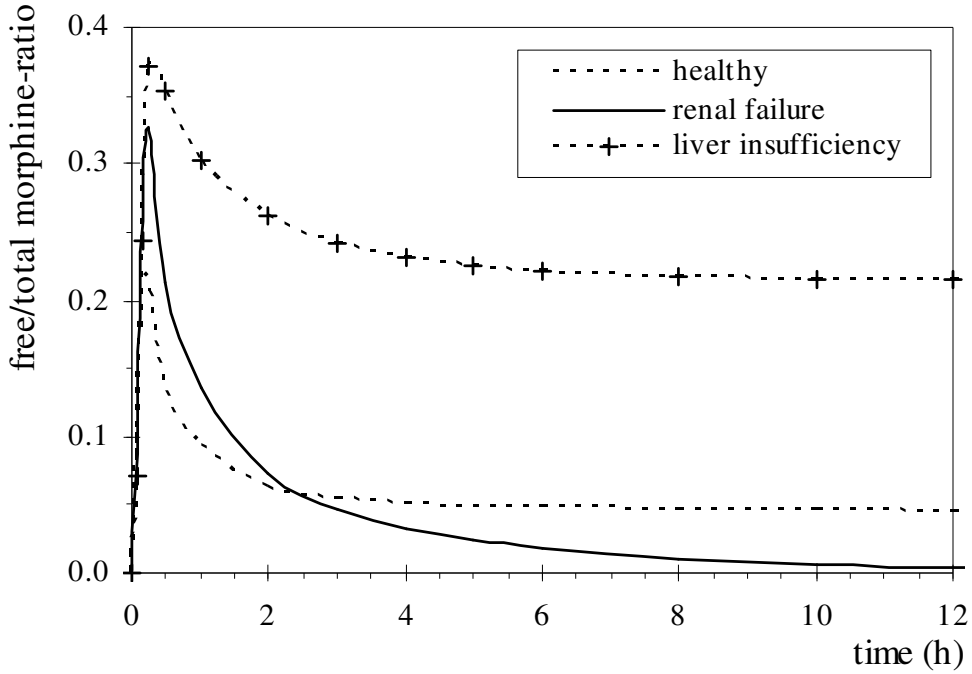

Figure 2: The simulated time course of the f/t M-ratio in blood after i.v. administration of heroin in healthy subjects, and those with renal failure, and liver insufficiency.

The simulation showed a different time course of f/t M-ratio in the three subject groups. The highest ratio-values were obtained in patients with lever insufficiency, followed by the other subject groups. Emphasis of morphine clearance in these patients prolonged morphine in the body and increased free morphine concentration. After time ingestion of one half live time of morphine, patients with kidney failure showed the lowest ratio-values. Prolonged morphine glucuronides half live in kidney failure patients accelerated reducing the ratio-values in the terminal phase. According to this simulation, a different time course for f/t M-ratio in blood was found in healthy subjects, and those with renal failure, and liver insufficiency. In patients with lever insufficiency, the highest value of this ratio as function of time was obtained when compared to other subject groups. The variation in pharmacokinetics of morphine and its glucuronides contributed a significant

change in time course of this ratio. The peak of f/t M-ratio was reached during the first hour after the administration of heroin.

DISCUSSION

In the simulation, it was assumed that the pharmacokinetics of heroin and 6-AM have a little influence on the time course of f/t M-ratio. This assumption was based on the relatively rapid elimination-rates of heroin and 6-AM from the body in comparison to the rates of morphine and its glucuronides. In this study, the disposition parameters of morphine and its glucuronides have been varied. These variations were based on the changes of the pharmacokinetics of morphine and its glucuronides in healthy subjects, patients with liver cirrhosis and patients with kidney failure. According to the simulation, the change in pharmacokinetics of morphine and its glucuronides contributed to a significant different value of f/t M-ratio as function of the time in blood after heroin injection.

After morphine administration to patients with renal failure, a reduction of morphine's distribution-volume of the central compartment (V_{cM}) was noticed [9]. By applying this pack to the simulation, a higher peak level of f/t M-ratio in patients with renal failure was obtained than in healthy subjects. This indicated that a change in distribution-volume of morphine had significantly influenced the f/t M-ratio. Literature shows a large variation in the distribution-volume (V_d) of morphine (0.008-1.3 l kg^{-1} for V_{cM} and 1-7 l kg^{-1} for the volume of distribution area) [2, 10, 14]. On the other hand there was no significant variation in the V_d of morphine glucuronides [6, 15, 16]. The large fluctuation of V_d of morphine can lead to a variation of f/t M-ratio-value.
Patients with kidney failure were shown to have a prolonged half-life elimination of morphine glucuronides but no significant change in rate of morphine's elimination was found [9, 13]. This prolonged of morphine-glucuronides's half-life elimination is due to the rapid decrease of f/t M-ratio in the terminal elimination stage in case of patients with renal failure (see Fig. 2).

The reduction of morphine clearance in patients with liver cirrhosis resulted in the prolongation of it's terminal half-life elimination [10-12]. During the simulation regarding to patients with liver cirrhosis, it was

assumed, that there was no impairment of the renal clearance of the opiates. Because most of the morphine glucuronides were excreted through renal clearance [6], it can be expected that there are no significant changes in the elimination rate of morphine glucuronides in patients with liver cirrhosis. The prolonged half-life elimination of morphine in patients with liver cirrhosis can conduct to a higher value of f/t M-ratio as a function of time.

Substituting eqs. 2, 4, 6, 9 and 10 into eq. 11, it was found that the time course of f/t M-ratio was determined by the time elapsed since the last dose and the individual disposition functions of heroin and its metabolites. Thus the time course of the f/t M-ratio is independent of the heroin dose.

Eighty point five percent of survivors had a ratio value lower than the cut off value (0.12). According to the simulation, a high arising frequency distribution of the f/t M-ratio under the cut off value for the cases surviving heroin intoxication is determined by the time elapsed between the last heroin intake and the blood sampling. The blood specimens of these cases in our materials were mostly coming from individuals arrested for driving under influence of drugs (DUID). This indicated that the blood sampling for the DUID suspects took place in the terminal elimination stage after heroin or morphine consumption. And this is as a major reason for a high frequency distribution of f/t M-ratio under the cut of value in cases surviving heroin intoxication of our case material.

The f/t M-ratio has been used to predict survival time in case of heroin-related death [17]. Due to the simulation, a large intra- and inter-individual variation in the pharmacokinetics of morphine and its glucuronides contribute a significant different of time course of f/t M-ratio. The post-mortem distribution of drugs and the spontaneous hydrolysis of 6-AM to free morphine can also contribute the change of this ratio after death. The variation in the pharmacokinetics and the post-mortem changes of drugs make the interpretation of a survival time on the basis of the f/t M-ratio being complicated.

Base on the results of statistical analysis of frequency distribution of f/t M-ratio in blood between cases of heroin-related deaths and cases surviving heroin intoxication, Meissner

et. al. [1] have recommended that by using a definite cut off value of f/t M-ratio can be used to predict the fatal outcome after heroin intake. In general, the degree of pharmacological effect of a drug is determined by the pharmacodynamic and the pharmacokinetic properties of the drug. The pharmacodynamic properties of the drug depend on the dose (or drug concentration at the place of action) and behaviour of the receptor. The degree of heroin intoxication does not just depend on the heroin dose but also on the individual tolerance level to the drug, the route of heroin administration, the individual pharmacokinetics of the drug (particularly pharmacokinetics of morphine) and the combination pharmacological effect of heroin with another psychoactive drugs [18].

According to the result of this study, the f/t M-ratio after heroin intake is independent to the heroin dose and this ratio is predominantly determinated by individual pharmacokinetics of morphine and its glucuronides. The individual pharmacokinetics of morphine and its glucuronides and the clinical significant result should be taken into account for better interpretation of toxicological analysis drug after heroin intake.

CONCLUSION

In the present study, we simulated the time course of f/t M-ratio after i.v. administration of heroin. The variations in the f/t M-ratio-value after heroin ingestion are predominantly determined by two factors: a) the time elapsed between the last heroin intake and blood sampling (in case of survivors) or the survival time (in case of related heroin deaths); b) the pharmacokinetics of heroin and its metabolites. This ratio is independent to the heroin dose.

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