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Plasma and Cerebrospinal Fluid Concentrations of Morphine and Morphine Glucuronides in Rabbits Receiving Single and Repeated Doses of Morphine

CHRISTIAN MIGNAT, RUTH JANSEN AND ALBRECHT ZIEGLER

Department of Pharmacology, University of Kiel, Hospitalstraße 4, D-24105 Kiel, Germany

Abstract

The pharmacokinetics of morphine in plasma and the distribution of morphine and its glucuronidated metabolites within the cerebrospinal fluid were investigated in rabbits.

After single morphine dosage, the plasma AUC ratio of morphine-3-glucuronide/morphine was 11·1 compared with 0·14 for morphine-6-glucuronide/morphine. The similar elimination half-lives of morphine (107 min), morphine-3-glucuronide (122 min), and morphine-6-glucuronide (105 min) suggested the glucuronidation to be the rate-limiting step, which was substantiated by the observation that morphine-3-glucuronide becomes eliminated four times faster when applied intravenously. Both after single and repeated morphine administration, the ratios of CSF and plasma levels of the parent drug were higher than those of morphine-3-glucuronide or morphine-6-glucuronide.

These data demonstrate a poor penetration of the glucuronides across the blood-brain barrier and do not support the previously postulated accumulation of morphine-6-glucuronide in the central nervous system during chronic morphine treatment.

The main metabolic degradation of morphine includes glucuronidation to morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G), which primarily takes place in the liver. M6G is thought to contribute to the pharmacological effects of the parent drug (Abbott & Palmour 1988; Paul et al 1989; Frances et al 1992). This assumption is supported by the observation of prolonged responses to morphine in patients with renal failure: the increased sensitivity towards morphine is attributed to the accumulation of M6G (Osborne et al 1986; Milne et al 1992). Accumulation of M6G is also assumed to occur in patients with normal renal function but subjected to chronic morphine therapy (Hanks et al 1987).

A poor accessibility in the central nervous system (CNS) is expected for M6G, because the polarity of this metabolite should impair its penetration into brain. Blood-brain barrier permeability depends mainly upon the lipophilicity of the drug (Rapoport et al 1979); for glucuronides poor availability in the CNS is documented, e.g. for 7-hydroxycoumarin glucuronide (Ritschel & Hardt 1983) and for 3′-azido-3′-deoxythymidine-5′-*O*-glucuronide (Cretton et al 1991).

However, an unexpectedly high lipophilicity has recently been determined for M3G and M6G by using a reversed-phase high performance liquid chromatography (RP-HPLC) method (Carrupt et al 1991). The authors suggest a masking of the hydrophilic moiety of the molecule by a special folding of these glucuronides, enabling them to penetrate lipophilic barriers.

The present study was performed to compare the distribution of morphine and its glucuronidated metabolites into the cerebrospinal fluid (CSF) in the rabbit after single and repeated morphine administration.

Since the pharmacokinetics of morphine in the rabbit are not fully known, the investigation required a detailed analysis of the pharmacokinetic parameters of morphine and its two major metabolites in this species.

Correspondence: C. Mignat, Department of Pharmacology, University of Kiel, Hospitalstraße 4, D-24105 Kiel, Germany.

Materials and Methods

Animals

Male, chinchilla rabbits, 3–3·5 kg, were maintained in a controlled environment (20 ± 2 °C; 65% ± 15% relative humidity; 12 h light-dark cycle) and provided with standard rabbit pellets and water.

Chemicals

Morphine hydrochloride was obtained from Merck (Darmstadt, Germany). The concentrations of morphine were expressed as morphine free base. M3G, M6G, and normorphine hydrochloride were obtained from Sigma (St Louis, MO, USA). All HPLC reagents were purchased from Merck (Darmstadt).

Study design

Study A was conducted to examine the pharmacokinetics of morphine in rabbits. The animals received a single subcutaneous injection of morphine (20 mg kg^{-1}). Blood samples were taken at 5, 10 and 15 min and at 1, 1·5, 2, 2·5, 3, 3·5, 4, 5, 6, 7, 8, 9, 10, 11 and 12 h thereafter.

Study B was undertaken to investigate the distribution of morphine, M3G, and M6G across the blood-brain barrier. One group of animals received a single subcutaneous injection of morphine (20 mg kg^{-1}). Blood and CSF samples were drawn at 4 h after dosing. A second group

received chronic morphine dosage for 10 days. Morphine (10 mg kg^{-1}) was subcutaneously administered 6 times/day. Blood sampling was performed at intervals of 24 h immediately before a scheduled dose was to be given. A CSF sample was obtained together with the last blood sample at day 10.

Sample collection

Blood was sampled via a catheter in the marginal ear vein and collected into heparinized plastic tubes. During the experiments, blood samples were replaced by the same volume of saline. The plasma was separated by centrifugation and was frozen at −20°C until analysed.

To obtain liquor the cisterna magna was punctured after the animals were fully anaesthetized with ketamine (5 mg kg^{-1}). CSF samples were collected into a 1-mL disposable syringe and were frozen at −20°C until analysed.

Assay procedures

Concentrations of morphine, M3G, and M6G in plasma and CSF were determined by an HPLC method (Svensson et al 1982). The method included sample purification with a solid phase (SepPak C_{18} cartridge, Millipore Waters, Eschborn, Germany) system, separation by use of ion-pair RP-HPLC and measurement with a UV detector. The HPLC system consisted of a pump (L-6200), a column (LiChrospher 60 RP-select B column, 250 × 4 mm i.d., 5 μm particle size), an autosampler (AS-2000), and a UV detector (L-4250). The equipment was purchased from Merck (Darmstadt). The flow rate was maintained at 1·3 mL min^{-1} and absorbance was measured at 214 nm.

Normorphine was used as internal standard and quantitative determinations were obtained by relating peak areas. Calibration curves were constructed and used to determine the concentration of unknown samples. The detection limits were 5, 10 and 5 ng mL^{-1} for morphine, M3G and M6G, respectively.

Pharmacokinetic analysis

The plasma elimination half-life values ($t_{\frac{1}{2}}$) of morphine, M3G, and M6G were estimated from the β-phase of the plasma-concentration-time curve by means of a least-squares regression line fitted to the logarithm of the plasma concentration.

The area under the plasma concentration-time curve (AUC) was calculated according to the linear trapezoidal rule extrapolated to infinity by using the terminal elimination half-lives of the individual plasma concentration-time curves.

Systemic plasma clearance (CL) was determined by the ratio of dose over AUC. Volume of distribution (Vd) was calculated from CL divided by the terminal elimination rate constant.

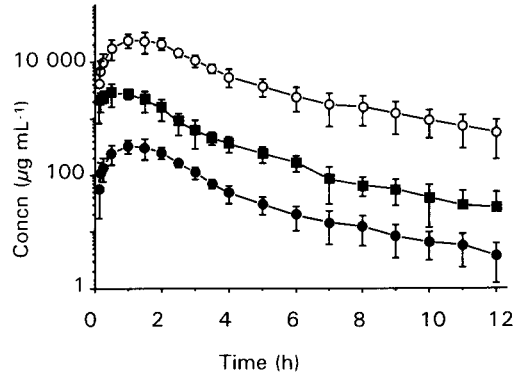

FIG. 1. Plasma concentrations of ■ morphine, ○ morphine-3-glucuronide, and ● morphine-6-glucuronide in rabbits after subcutaneous administration of 20 mg kg^{-1} morphine. Results are means ± s.d., n = 4.

Ratios of M3G/morphine and M6G/morphine are based on the AUC values. Student's paired *t*-test was used to check whether the trough levels of morphine in the plasma of animals receiving chronic morphine dosage were significantly different between day 1 and day 10.

Data are given as mean ± s.d.

Results

Study A

The plasma concentration-time curves for morphine and glucuronidated metabolites are shown in Fig. 1. Mean pharmacokinetic parameters are presented in Table 1. The plasma concentration of morphine after subcutaneous administration rapidly rose to a maximum plasma concentration (C_{max}) of 3130 ± 950 ng mL^{-1}. The corresponding value for time to peak concentration (t_{max}) was 55 ± 27 min. The plasma levels of morphine then declined with a $t_{\frac{1}{2}}$ of 107 ± 26 min. The CL was calculated to be 50·7 ± 15·3 mL min^{-1} kg^{-1} and the Vd was 8·1 ± 3·5 L kg^{-1}.

The degradation of morphine to its glucuronides proceeded rapidly; detectable quantities of M3G and M6G were found in the plasma 5 min after application. From this time the mean plasma levels of M3G, but not of M6G, exceeded that of morphine throughout the remaining observation period. The plasma AUC ratio of M3G/morphine was 11·1 ± 2·4 compared with 0·14 ± 0·03 for M6G/morphine.

Both M3G and M6G achieved their peak plasma values at 75 ± 17 min. The values for C_{max} of M3G and M6G were 24 900 ± 6300 and 340 ± 100 ng mL^{-1}, respec-

Table 1. Pharmacokinetic parameters of morphine, morphine-3-glucuronide, and morphine-6-glucuronide after subcutaneous administration of morphine. Data are means ± s.d., n = 4.

	$t_{\frac{1}{2}}$ (min)	Vd (L kg^{-1})	CL (mL min^{-1} kg^{-1})	C_{max} (ng mL^{-1})	t_{max} (min)	Metabolite : morphine plasma AUC ratio
Morphine	107 ± 26	8·1 ± 3·5	50·7 ± 15·3	3130 ± 950	55 ± 27	—
Morphine-3-glucuronide	122 ± 22	—	—	24 900 ± 6300	75 ± 17	11·1 ± 2·4
Morphine-6-glucuronide	105 ± 19	—	—	340 ± 100	75 ± 17	0·14 ± 0·03

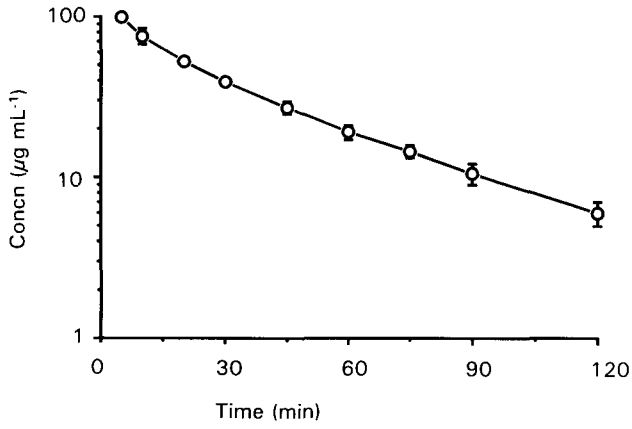

FIG. 2. Plasma concentrations of morphine-3-glucuronide in rabbits after intravenous administration of 25 mg kg^{-1}. Results are means ± s.d., n = 4. $t_{\frac{1}{2}}$ = 33 ± 3 min.

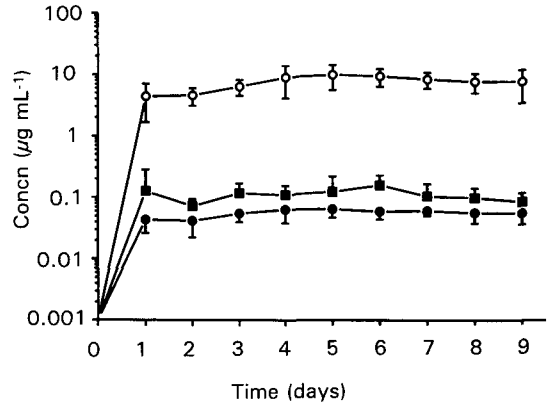

FIG. 3. Trough levels of plasma concentrations of ■ morphine, ○ morphine-3-glucuronide, and ● morphine-6-glucuronide in rabbits receiving repeated morphine dosage (6 × 10 mg kg^{-1} day^{-1}) for nine days. Results are means ± s.d., n = 6.

tively. The elimination half-lives for M3G and M6G were 122 ± 22 and 105 ± 19 min, respectively.

The parallel decline of the plasma levels of morphine and its glucuronides suggest the glucuronidation to be the rate limiting step. This conclusion is supported by the elimination half-life of only 33 min for M3G when M3G is applied intravenously (Fig. 2). Due to the limited supply of M6G similar experiments were not performed with this metabolite.

Study B

The trough levels of morphine, M3G, and M6G in the plasma of animals receiving chronic morphine dosage showed no significant differences between day 1 and day 10, i.e. steady state was reached within the first day of treatment (Fig. 3).

The concentration of morphine and glucuronides in plasma and CSF after single morphine dosage and after the last dose following long-term administration is shown in Table 2. Both morphine and M3G were present in the CSF regardless of the form of treatment. M6G could not be detected in CSF 4 h after administration of a single dose of morphine; upon chronic administration its concentration was just at the detection level 4 h after the last injection.

Whereas the plasma concentrations of morphine and M6G were similar, in CSF the concentration of M6G was much lower than that of morphine (Table 2).

To estimate penetration of morphine, M3G, and M6G, the ratio of CSF and plasma trough levels was calculated (Table 3). The ratios of the concentrations in CSF and plasma of morphine and its glucuronides were 0·4–0·5 and ⩾0·1, respectively.

Discussion

The analgesic activity of M6G, one of the polar metabolites of morphine, has been documented in animal experiments (Paul et al 1989; Frances et al 1992) and in patients suffering from pain (e.g. Osborne et al 1992). Despite increasing evidence for additional sites of morphine action outside the CNS (Junien & Wettstein 1992; Stein 1993), it is still widely accepted that the major analgesic effect is caused by interaction with receptors in the CNS, thus requiring the penetration of the drug into the brain or spinal cord. With respect to M6G, the question arose as to whether the high polarity of the glucuronide moiety would allow the molecule to penetrate the blood-brain barrier. We, therefore, studied the distribution of morphine and its glucuronides in rabbits. This species was chosen as it allows the sampling of liquor cerebrospinalis in sufficiently large quantities to estimate the concentration of morphine, M3G and M6G.

The metabolic pattern for morphine in man and rabbits is not identical, since in rabbits only a minor part of morphine becomes glucuronidated at the 6-position (Yoshimura et al 1969). To obtain detectable concentrations of M6G, the animals had to be treated with high doses of morphine. Apart from initial sedation the doses of 10–20 mg kg^{-1} were well tolerated.

Morphine elimination $t_{\frac{1}{2}}$ = 107 min) in rabbits proceeds at similar rates in other species e.g. rat, monkey or man (Rane et al 1984; Osborne et al 1990; Bhargava et al 1991).

Table 2. Trough levels of plasma and cerebrospinal fluid (CSF) concentrations of morphine, morphine-3-glucuronide and morphine-6-glucuronide in rabbits.

		Morphine (ng mL^{-1})	Morphine-3-glucuronide (ng mL^{-1})	Morphine-6-glucuronide (ng mL^{-1})
A. Single dose 20 mg kg^{-1} morphine	Plasma	440 ± 170	11 000 ± 3900	94 ± 39
	CSF	170 ± 80	430 ± 210	—
B. Repeated morphine dose of 10 mg kg^{-1} morphine 6 times daily	Plasma	85 ± 32	7600 ± 4200	54 ± 19
	CSF	38 ± 33	460 ± 250	4 ± 3

Results are means ± s.d., n = 6–8.

Table 3. Distribution of morphine, morphine-3-glucuronide, and morphine-6-glucuronide in plasma and cerebrospinal fluid (CSF) of rabbits after single and repeated administration of morphine. Data are means ± s.d., n = 6–8.

	CSF/plasma concentration ratios after morphine treatment	
	Single	Repeated
Morphine	0·40 ± 0·10	0·48 ± 0·33
Morphine-3-glucuronide	0·06 ± 0·05	0·08 ± 0·04
Morphine-6-glucuronide	—	0·10 ± 0·09

Morphine-6-glucuronide was not detectable in CSF after a single morphine treatment.

Elimination of the glucuronides runs in parallel with that of the parent drug, suggesting glucuronidation as the common rate-limiting process. Since the elimination of glucuronides proceeds faster than their formation, the rate of glucuronidation will also be reflected in the rate of elimination of M3G and M6G. This is demonstrated by the observation that M3G is eliminated four times faster when applied intravenously.

The concentration of both glucuronides in CSF achieved only about one-tenth that in plasma. Since the penetration of M6G into CNS was comparable with that of other glucuronides e.g. 7-hydroxycoumarin (Ritschel & Hardt 1983) or zidovudine (Cretton et al 1991), the particular lipophilicity of M6G—as estimated by an RP-HPLC method (Carrupt et al 1991)—does not seem to be of significance under physiological conditions. Despite poor penetration of M3G across the blood-brain barrier, the concentration of this metabolite in CSF exceeded that of morphine; in the rabbit M3G is predominantly formed and its plasma concentration is more than tenfold the concentration of morphine. Also, in man glucuronidation at the 3-position is the main metabolic step, but glucuronidation of the hydroxyl group in the 6-position seems to be more prevalent than in rabbits, since the plasma concentration of M6G exceeds that of morphine by a factor of 2–5 (Peterson et al 1990; Breda et al 1991). Assuming a similar distribution ratio between plasma and CNS in rabbits and man, concentrations of morphine and M6G in CNS of man may attain comparable levels. This may explain the analgesic effect of M6G without assuming a higher receptor affinity than that of morphine, high penetration across blood-brain barrier, or an accumulation of the drug in CNS.

Our experiments do not provide any evidence for an accumulation of M6G in CNS during chronic treatment (as suggested by Hanks et al (1987)) and there are no indications of changes in the metabolic pattern, such as an increased formation of M6G as suggested by Lehmann & Zech (1993).

Finally the question should be addressed, whether glucuronides penetrate the blood-brain barrier or are found within the CNS. Despite the known ability of glucuronidation in brain homogenates, glucuronidation in CNS seems to be of minor importance in-vivo, since only very small concentrations of M6G were found in CSF of patients, treated with chronic epidural morphine (Samuelsson et al 1993). Penetration of M6G may be carrier-mediated or by diffusion (e.g. via leaks in the barrier or simply by partition). There is no necessity to postulate an uptake of M6G by an anion transport system since the ratio of CSF/plasma concentration of less than 0·1 is also found with other polar hydrophilic substances.

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