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Pharmacokinetics of morphine 3-esters after oral administration in rabbits

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Pharmacokinetics of three morphine 3-esters-3-(2,2-dimethylvaleroyl) morphine (**A**), 3-(2-phenylbenzoyl) morphine (**B**), and 3-(2,2-diphenylpropionyl) morphine (**C**) was characterized after single oral administration in rabbits. Blood was sampled up to 24 h and cerebro-spinal fluid (CSF) was collected with the last blood sample. The concentration of the morphine 3-esters, morphine, morphine 3-glucuronide and morphine 6-glucuronide were determined in plasma and CSF using HPLC UV-detection. The morphine 3-esters were suggested to be a subject to marked presystemic elimination, since, in comparison to the administration of the un-esterified morphine, relatively low concentrations of morphine and morphine glucuronides were detected in plasma. The rate of disposition of morphine was dependent on the hydrolytic stability of the esters. The mean (± S.E.) plasma half-life of morphine was 0.9 ± 0.2 h, 2.5 ± 0.6 h and 3.5 ± 3.5 h after the administration of **A**, **B** and **C**, respectively, compared to 0.9 ± 0.2 h as estimated after the administration of non-esterified morphine. An analgesic effect will be achieved, since morphine was detected in CSF even 24 h after the application of the ester pro-drugs. It is concluded that esterification at the 3-position may be adapted to obtain sustained plasma levels of morphine.

1. Introduction

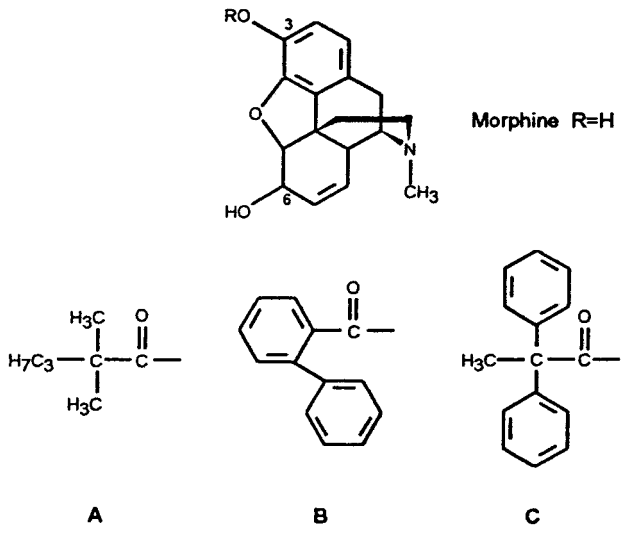
Morphine is the most convenient option for treatment of chronic pain. Several attempts have been made to retard the disposition of morphine in the systemic circulation and, thereby, overcome the drawbacks of the therapy, like too frequent dosing and side effects due to high plasma peaks.

Esterification of a parent compound has been described as a promising means not only to temporarily inactivate the drug, but also to control its release by varying the hydrolytic stability of the ester bond [1, 2]. Applying this concept to morphine, possible sites for esterification are at the 3- and 6-positions. The free phenolic hydroxyl group is important for both opioid receptor binding and analgesic activity [3–7]. Hence, the esterification at the 3-position may be adapted for a new type of slow-release formulations.

A series of morphine 3-esters was synthesized aiming to modify the release rate of morphine. The results of radioligand binding studies in guinea pig brain homogenates indicate a loss of receptor affinity of morphine by esterification at the 3-position [8].

The rate of hydrolytic cleavage of the esters in human plasma was determined *in vitro*. The half-life of plasma-catalysed hydrolysis for different esters was between 1 and more than 300 h, depending on the structure of the acid moiety [8].

Three morphine 3-esters-3-(2,2-dimethylvaleroyl) morphine (**A**), 3-(2-phenylbenzoyl) morphine (**B**), and 3-(2,2-diphenylpropionyl) morphine (**C**) – were chosen to study the pharmacokinetics of morphine 3-esters in rabbits after oral administration. In addition, the plasmakinetics of morphine and its glucuronides was determined.



2. Investigations, results and discussion

In order to characterize the pharmacokinetics of morphine 3-esters **A**, **B**, and **C**, the concentrations of the esters, as well as of morphine, M6G and M3G, were measured up to 24 h in plasma and at 24 h in CSF after oral administration in rabbits.

The mean pharmacokinetic data are summarized in the Table, the plasma concentration-time curves of the quantified substances are shown in Fig. 1 and the comparison of the CSF and plasma concentrations of morphine and M3G is presented in Fig. 2.

The pharmacokinetic data obtained after single oral dosage of parent drug in rabbits served as control for the evaluation of the pharmacokinetics of morphine 3-esters. The experiments were performed with rabbits, as it allowed multiple blood sampling over 24 h. Additionally, the sampling of CSF in sufficiently large quantities for estimating the concentration of morphine esters, morphine, M3G and M6G.

The dose of the administered drugs was chosen taking into account, that the metabolic patterns for morphine in man and rabbit are not identical. In rabbits, only a minor part of morphine becomes glucuronidated at the 6-position [9] and, therefore, a relatively high dose (40 mg/kg b.w.) was administrated in order to obtain detectable concentrations of M6G in plasma.

The differences in plasmakinetics of morphine 3-esters could be explained by their differing susceptibility to hydrolytic cleavage. Under *in vitro* conditions, **A** was unstable with rapid hydrolysis into morphine. Consequently, its concentration in plasma was below the quantification limit already 30 min (time for first sample) after application. Differently, the plasma concentration of the more sta-

Table: Pharmacokinetic parameters of morphine 3-esters, morphine and morphine glucuronides after oral administration of morphine and morphine 3-esters in rabbits

	Morphine	A	B	C
Morphine 3-ester				
C_{max} (ng/ml)	–	n.m.	893 ± 346	762 ± 218
t_{max} (h)	–	n.m.	0.5 ± 0	1.2 ± 0.2
$t_{1/2}$ (h)	–	n.m.	1.8 ± 0.8	2.4 ± 0.5
AUC (ng/ml/h^{-1})	–	n.m.	317 ± 91	589 ± 117
Morphine				
C_{max} (ng/ml)	1157 ± 461	472 ± 37	149 ± 39	24 ± 6
t_{max} (h)	0.6 ± 0.1	1.3 ± 0.3	1.9 ± 0.3	2.0 ± 0.4
$t_{1/2}$ (h)	0.9 ± 0.2	0.9 ± 0.2	2.5 ± 0.6	3.1 ± 0.5
AUC (ng/ml/h^{-1})	2529 ± 866	1223 ± 163	594 ± 137	130 ± 35
Morphine 3-glucuronide				
C_{max} (ng/ml)	48765 ± 3773	56043 ± 4909	14060 ± 835	4275 ± 910
t_{max} (h)	1.3 ± 0.2	1.7 ± 0.2	1.4 ± 0.4	1.5 ± 0.3
$t_{1/2}$ (h)	1.1 ± 2.0	1.1 ± 0.1	2.6 ± 0.6	3.6 ± 0.5
AUC (ng/ml/h^{-1})	184710 ± 18371	208434 ± 21044	75316 ± 8364	28113 ± 4979
Morphine 6-glucuronide				
C_{max} (ng/ml)	866 ± 109	936 ± 70	219 ± 33	43 ± 6
t_{max} (h)	0.9 ± 0.1	1.8 ± 0.4	1.1 ± 0.3	2.0 ± 0
$t_{1/2}$ (h)	0.9 ± 0.2	1.0 ± 0.1	1.8 ± 0.4	3.4 ± 0.6
AUC (ng/ml/h^{-1})	2305 ± 240	3180 ± 434	843 ± 68	247 ± 30

(n = 6, mean ± S.E.) n.m. = not measurable

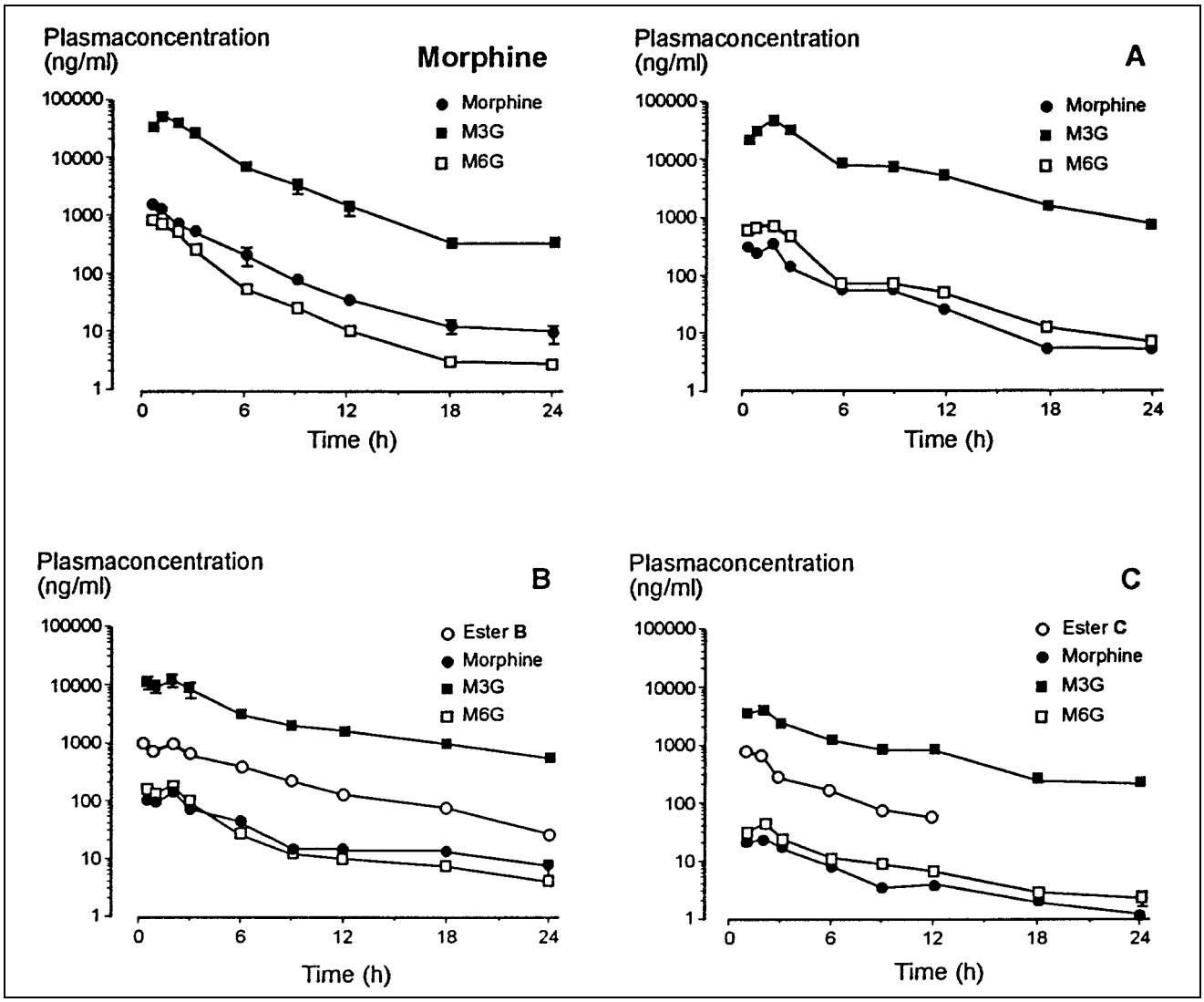


Fig. 1: Semilogarithmic plots of plasmaconcentrations of morphine 3-esters, morphine, morphine 3-glucuronide, and morphine 6-glucuronide after oral administration of morphine and morphine 3-esters **A**, **B**, and **C** in rabbits (n = 6, mean ± S.E.)

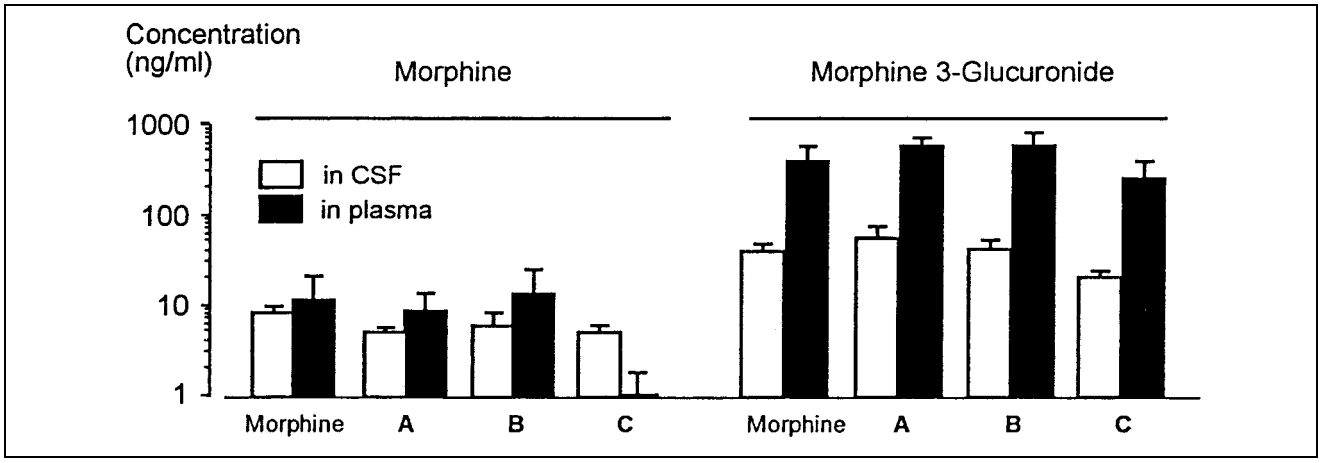


Fig. 2: Logarithmic plots of the concentrations of morphine and morphine 3-glucuronide in CSF and plasma 24 h after oral administration of morphine and morphine 3-esters **A**, **B**, and **C** in rabbits (n = 4–6, mean ± S.E.)

bile esters **B** and **C** could be measured and $t_{1/2}$ was determined (Table). It is suggested that the morphine 3-esters are subject to incomplete absorption or marked presystemic elimination when given orally, since the degradation of esters **B** and **C** *in vitro* was significantly slower.

Morphine 3-esters were shown to be a reliable source of morphine after oral administration in rabbits, since morphine was present within 30 min with all three esters. The plasma concentration of morphine after the administration of **A**, **B** and **C** increased slowly and the respective C_{max} values were 2.5-, 7.8-, and 48-fold lower than with non-esterified morphine. The $t_{1/2}$ of morphine after administration of **A** and the parent drug were similar. In comparison, the $t_{1/2}$ of morphine in plasma was prolonged after administration of **B** and **C** (Table).

Morphine is glucuronidated at the 3 and 6 position. M6G has been shown to contribute to the analgesic effect [10]. M3G, although analgesically inactive, is considered a possible source of morphine in the terminal phase, since it is present in higher concentrations, enters into enterohepatic circulation and may be unconjugated in the intestine [11–13].

M3G and M6G were present in plasma after administration of the parent compound and the ester-prodrugs. During the whole observation period the mean plasma levels of M3G exceeded that of morphine. After administration of morphine, as well as esters **A** and **B**, the concentration of M6G was lower, after the administration of **C** marginally higher than that of morphine. The elimination of morphine glucuronides ran in parallel with the parent drug. In all study groups the mean apparent half-lives of the morphine glucuronides did not differ significantly from each other or from the half-life of morphine.

The major analgesic effect of morphine is caused by interaction with receptors in the CNS, thus requiring the penetration of the drug into the brain and spinal cord. Morphine was detected in the CSF even 24 h after application of the studied ester pro-drugs, suggesting that an analgesic effect may be obtained after the oral administration of morphine 3-esters.

Probably due to relatively low plasma concentration, morphine 3-esters could not be quantified in CSF 24 h after oral administration.

Glucuronides are hydrophilic substances and therefore considered to penetrate poorly across the blood-brain barrier. Nevertheless, both M3G and M6G have been detected in CSF after chronic administration of morphine in rabbits [9]. In the present study only M3G could be quantified in concentrations exceeding that of morphine, whereas the concentration of M6G was below the quantification limit in CSF.

In summary, the pharmacokinetics of three morphine 3-esters was characterized after single oral administration in rabbits. Morphine became bioavailable and penetrated the blood-brain barrier, suggesting that an analgesic effect could be achieved after the administration of the morphine 3-esters. The ester pro-drugs are suggested to be a subject to extensive presystemic elimination when given orally. Still, the retarded disposition of morphine maintains the plasma concentrations with increased half-lives of elimination dependending on the hydrolytic stability of the esters. Taking into account the prolonged plasma half-life of morphine after the administration of some morphine 3-esters, it is concluded that esterification at the 3-position may provide a promising approach to obtain sustained plasma levels of morphine.

3. Experimental

3.1. Chemicals

3-(2,2-Dimethylvaleroyl) morphine, 3-(2-phenylbenzoyl) morphine, and 3-(2,2-diphenylpropionyl) morphine were synthesized as described recently [8, 15]. Morphine hydrochloride was provided by Mundipharma GmbH (Limburg, Germany). All other analytical or HPLC grade chemicals were obtained from Merck (Darmstadt, Germany) or Aldrich (Steinheim, Germany).

3.2. Animals

The pharmacokinetics of morphine 3-esters was measured in male Chinchilla-Bastard rabbits (Charles River, Sulzfeld, Germany) weighing 3.5–3.8 kg. Rabbits were maintained in a controlled environment (22 ± 2 °C; 65% ± 15% relative humidity, 12 h light-dark cycle) for at least 1 week before use in the studies. Standard rabbit food and water were available ad libitum.

3.3. In vivo pharmacokinetics

The animals received using gavage a single dose of morphine (40 mg/kg b.w.) or morphine 3-ester (dose equivalent to 40 mg/kg b.w. morphine). Blood samples were taken before the injection and 0.5, 1, 2, 3, 6, 9, 12, 18, and 24 h thereafter. CSF was collected together with the last blood sample. The study protocol of the pharmacokinetic studies *in vivo* was approved by the animal research committee of the University of Kiel. 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 physiological solution. The plasma was separated by centrifugation and frozen at −20 °C until analysed.

To obtain cerebro-spinal fluid (CSF) the *cisterna magna* was punctured after the animals were fully anaesthesized with ketamine (5 mg/kg). CSF samples were collected into plastic tubes and were frozen at −20 °C until analysed.

3.4. Assay procedures and pharmacokinetic analysis

Quantification of the concentrations of morphine 3-esters, morphine, morphine 3-glucuronide (M3G) and morphine 6-glucuronide (M6G) in plasma was performed after reversed-phase solid-phase extraction by HPLC-UV detection using methods published elsewhere [14, 15].

Quantitative determinations were obtained by relating peak areas of the studied substance and an internal standard. Calibration curves were constructed and used to determine the concentration of unknown samples. The quantification limits were 20, 10, and 10 ng/ml for **A**, **B,** and **C**, respectively. The respective quantification limits for morphine, M3G, and M6G were 5, 10, and 5 ng/ml. The pharmacokinetic parameters were calculated using computer software InPlot (version 4.03, GraphPad Inc., San Diego, USA).

The highest plasma concentration measured was C_{max}, and the time at which the peak plasma concentration occurred was t_{max}. The apparent plasma elimination rate constant (k_e) was estimated using linear regression of the ln(concentration)-time curve. The area under the concentration-time curve (AUC) in plasma from 0 to 24 h was estimated using a linear trapezoidal method. The $t_{1/2}$ was calculated by $0{,}693/k_e$.

The data are given as mean ± S.E.

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