J. Pharm. Pharmacol. 1992, 44: 606–608
Communicated December 4, 1991

The effect of temperature and pH on the deacetylation of diamorphine in aqueous solution and in human plasma

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Abstract—The effect of temperature on the kinetics of the deacetylation of diamorphine and 6-monoacetylmorphine was studied in human plasma. Diamorphine was rapidly and quantitatively degraded to 6-monoacetylmorphine with initial half-lives of 354, 18 and 3 min at temperatures of 4, 25 and 37°C, respectively. Further deacetylation to morphine was not detected. In aqueous solution, diamorphine was quantitatively degraded to give 6-monoacetylmorphine as the major product and morphine as a minor product, the rate of deacetylation being dependent on temperature and pH. At pH 4·0 and 5·6 diamorphine had a half-life of greater than 14 days at all temperatures but at alkaline pH diamorphine was rapidly deacetylated. The rate of deacetylation of 6-monoacetylmorphine was consistently slower than that of diamorphine under identical conditions of pH and temperature. A method is described for the rapid stabilization and subsequent assay of diamorphine in plasma which will prevent errors in estimation of the drug due to unwanted hydrolysis.

Diamorphine (3,6-diacetylmorphine, heroin) is a synthetic opiate analgesic which is commonly used in pain therapy (Scott 1988). Previous studies have shown that the drug is rapidly deacetylated in aqueous solution at alkaline pH to form 6-monoacetylmorphine and further deacetylated to form morphine (Davey & Murray 1969; Nakamura et al 1975; Cooper et al 1981; Beaumont 1982). In biological fluids, diamorphine is subject to rapid hydrolysis by esterases and is completely deacetylated within 20 min in human or dog blood (Nakamura et al 1975; Garrett & Gurkan 1979; Lockridge et al 1980). The majority of the methods used for the assay of diamorphine require an alkali treatment of samples before extraction of the basic drug with organic solvent. It is, therefore, possible that the diamorphine will be rapidly degraded during the procedure before quantitation of the drug is achieved. The potential for degradation is even greater during the assay of diamorphine in biological fluids due to the presence of highly active tissue esterases.

There is a lack of kinetic data on the degradation of diamorphine and 6-monoacetylmorphine in human plasma and in aqueous solution at temperatures likely to be encountered in experimental work. Measuring the rate of deacetylation of diamorphine over a suitable range of pH values and temperatures will permit the prediction of conditions which will lead to unwanted hydrolysis.

Materials and methods

Chemicals. Diamorphine HCl and morphine HCl were obtained from May and Baker Ltd, Dagenham, UK. 6-Monoacetylmorphine HCl was obtained from Macfarlan Smith Ltd, Edinburgh, UK. Acetonitrile (far UV grade), diethylether (HPLC grade) and methanol (far UV grade) were purchased from May and Baker Ltd, Dagenham, UK; triethylamine (HPLC grade) from ROMIL Chemicals Ltd, Loughborough, UK; octanesulphonic acid from Fisons plc (Loughborough, UK) and potassium dihydrogen orthophosphate (HPLC grade) from BDH Chemicals Ltd, Poole, UK. All other chemicals were of reagent grade.

Equipment. The HPLC equipment used was as follows: Gilson 305 pump, Gilson 115 UV detector (wavelength 220 nm), Gilson 231/401 autoinjector, Gilson 712 system controller/integrator (Gilson Medical Electronics, Villers le Bel, France). A Grant Instruments (Cambridge, UK) thermostatic flow heater/cooler and a Grant block thermostat were used to maintain constant temperatures for the aqueous stability studies and plasma stability studies.

HPLC analysis. The diamorphine stability samples were analysed by HPLC under the following conditions: the column was a Spherisorb (Phase Separations, Queensferry, UK) ODS 1 (15 cm × 4·6 mm int. diam.) with 3 μm particle size; for the mobile phase aqueous potassium phosphate buffer (0·05 M, 65%), acetonitrile (35%) and triethylamine (0·1%) adjusted to pH 3·0 with orthophosphoric acid was used. The flow rate was 1·0 mL min^{-1} and the injection volume 50 μL. The 6-acetylmorphine stability studies were carried out under the following conditions: the column was a Spherisorb ODS 2 (15 cm × 4·6 int. diam.) with 5 μm particle size; the mobile phase comprised water (65%), methanol (35%), triethylamine (0·1%) and octanesulphonic acid (1 g L^{-1}) adjusted to pH 2·1 with orthophosphoric acid. The flow rate was 1·5 mL min^{-1} and the injection volume was 50 μL.

Assessment of diamorphine stability in plasma. Diamorphine stability in plasma was determined at a concentration of 100 μg mL^{-1} at 4, 25 and 37°C. Diamorphine was added to plasma maintained at the appropriate temperature by a heating block. Samples (0·5 mL) were removed at timed intervals into 10 mL glass screw-top tubes and frozen immediately in liquid nitrogen for 30 s. The frozen samples were stored and analysed later in batches. To each frozen 0·5 mL sample was added diethylether (3 mL) and pH 9·0 aqueous borate buffer (0·1 M, 0·5 mL) and the samples were vortex-mixed for 10 min. The ether layer (upper) was separated from the aqueous layer by centrifugation (3000 *g*, 10 min) and removed to a clean test tube. The sample was extracted with a further volume of diethylether (3·0 mL) and the two ether extracts combined and evaporated to dryness under nitrogen at 45°C. The residue was redissolved in HPLC mobile phase (0·5 mL) and 50 μL was injected for HPLC analysis.

Assessment of diamorphine and 6-monoacetylmorphine stability in aqueous solution. Diamorphine and 6-monoacetylmorphine stabilities were assessed at concentrations of 100 μg mL^{-1}. A freshly made up solution of the compound in aqueous acetate buffer (0·1 M; pH 4·0 and 5·6), aqueous phosphate buffer (0·1 M; pH 7·0 and 7·4) or borate buffer (0·1 M; pH 8·0–11·0) as appropriate, was transferred to a jacketed glass vessel or test tube maintained at 4, 25 or 37°C. Samples (50 μL) were removed from the vessel at appropriate time intervals and injected for analysis by HPLC using a programmed autosampler/injector. The HPLC system was calibrated at the start, during, and at the end of the stability study and the concentrations of diamorphine, 6-monoacetylmorphine and morphine were determined by interpolation from the averaged calibration lines.

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Calculation of kinetic parameters. The time-concentration data from the stability studies were analysed using a curve-fitting program (Minim; R. D. Purves, Department of Pharmacology, University of Otago, New Zealand) to assess whether the rate of degradation followed a monoexponential or biexponential decline. The half-life was calculated by dividing 0·693 by the rate constant. The following equations were used in these calculations:

$$C_t = A \cdot e^{-kt} \text{ (monoexponential)}$$
$$C_t = A \cdot e^{-k_1 t} + B \cdot e^{-k_2 t} \text{ (biexponential)}$$

where C_t is the concentration of reactant at time t; A, B are the intercepts on the concentration axis; and k, k_1 and k_2 are the rate constants.

Results and discussion

Chromatographic separation and validation of assay. The retention times for diamorphine, 6-monoacetylmorphine and morphine were 3·6, 2·6 and 2·2 min, respectively, for both the aqueous solution assay and the plasma assay. The within-day and between-day reproducibility (expressed as a coefficient of variation) for the plasma assay was less than 6% (n = 6) and that of the aqueous solution assay was less than 4% (n = 6) for each compound at concentrations of 1·0, 5·0, 25, 50 and 100 $\mu g\ mL^{-1}$. The recoveries of diamorphine, 6-monoacetylmorphine and morphine in the extraction procedure, determined by HPLC analysis, were 82·0 ± 3·4% (n = 6), 78·0 ± 3·2% (n = 6) and 70·0 ± 2·2% (n = 6), respectively. Both assays had a limit of detection of 50 ng mL^{-1} and were linear over the range of concentrations between 1·0 and 100 $\mu g\ mL^{-1}$. A typical chromatogram showing the separation of diamorphine, 6-monoacetylmorphine and morphine extracted from plasma is shown in Fig. 1.

Stabilization of diamorphine in plasma samples. The rapid stabilization of diamorphine in plasma was successfully achieved using the method of freezing the sample in liquid nitrogen followed by liquid-liquid extraction using reagents cooled to 4°C. No detectable breakdown of diamorphine was observed during the stabilization and extraction procedures nor during the storage of plasma samples containing diamorphine at −20°C. The method was found to be simple and convenient to use. Previously reported techniques for the stabilization of diamorphine use esterase inhibitors (Garrett & Gurkan 1979) or involve sample freezing with solid carbon dioxide (Umans et al 1982). Both of these techniques require complex and time consuming sample preparation and result in only partial stabilization of diamorphine.

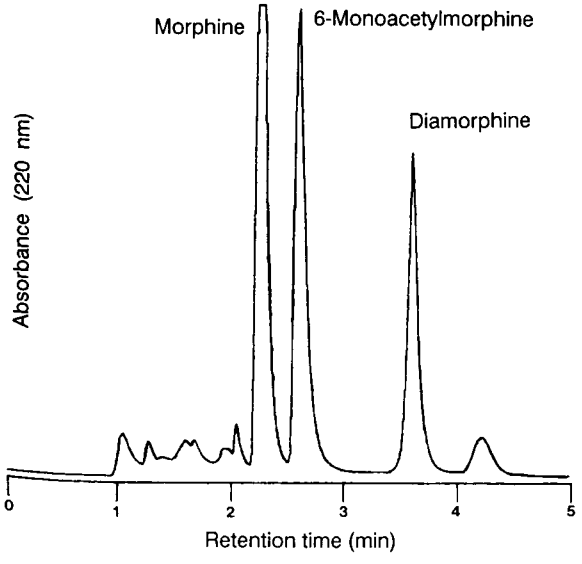

FIG. 1. HPLC chromatogram of diamorphine, 6-monoacetylmorphine and morphine extracted from human plasma.

Plasma stability studies. The rate of deacetylation of diamorphine in human plasma increased with temperature (Table 1). The kinetics of deacetylation were best described by a monoexponential function at 4°C and a biexponential function at 25 and 37°C. 6-Monoacetylmorphine was the final decomposition product in plasma over the 2 h duration of the experiment. Morphine was not detected.

The initial and terminal half-lives of 3·2 and 15 min, respectively, at 37°C in this study compares with reported half-lives (at 37°C) of 9 and 15 min in human blood (Nakamura et al 1975; Inturrisi et al 1984), and 22 min in human serum (Nakamura et al 1975) and 3 min in-vivo after intravenous administration to man (Inturrisi 1986). The data on the stability of diamorphine in plasma at 25 and 4°C have not been reported previously and indicate that diamorphine is degraded rapidly at 25°C and is hydrolysed at a significant rate even at the reduced temperature of 4°C. The observation that 6-monoacetylmorphine is not further metabolized to morphine in plasma confirms previous studies (Nakamura et al 1975; Smith & Cole 1976; Barrett et al 1991).

Aqueous stability studies. The rate of deacetylation of diamorphine in aqueous solution is dependent on pH and temperature (Table 2). First order kinetics were apparent in all cases. The rate of degradation was substantially increased at higher pH, with 6-monoacetylmorphine being the major degradation product and morphine a minor product. In all cases, the rate of appearance of 6-monoacetylmorphine mirrored the disappear-

Table 1. Kinetics of diamorphine degradation in plasma at 4, 25 and 37°C.

	Half-life (min)	
Temp (°C)	Initial	Terminal
4	354*	354*
25	18	19·2
37	3·2	15

Mean of 3 determinations.
* Mean of duplicate determinations.

Table 2. Effect of temperature and pH on the stability of diamorphine in aqueous solution.

	Half-life (h)		
pH	37°C	25°C	4°C
4·0	1018	1129	—
5·6	321	592	—
7·0	49·4	222	—
7·4	32·9	96·8	773
8·0	8·3	60·8	—
9·0	—	5·9	—

Mean of duplicate determinations.

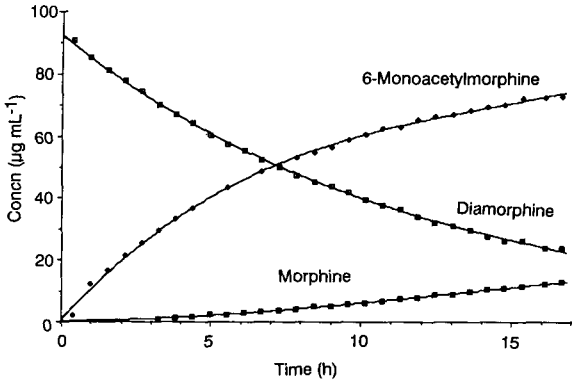

FIG. 2. Diamorphine deacetylation in aqueous solution at pH 8·0 and 37°C.

Table 3. Effect of pH on the stability of 6-monoacetylmorphine in aqueous solution at 25°C.

pH	Half-life (h)
4·0	*
5·6	*
7·4	*
8·5	129
9·0	45·9
10·0	7·9
10·35	4·7
10·7	2·5
11·3	0·44

Mean of duplicate determinations.
* No detectable degradation over 14 days.

ance of diamorphine (Fig. 2). The mean half-life of diamorphine was 32·9 ± 2·9 h (n = 3) at 37°C, 96·8 ± 1·3 h (n = 3) at 25°C and 774 h (n = 2) at 4°C.

From an Arrhenius plot (temp^{-1} vs log k) of the aqueous degradation of diamorphine at pH 7·4 at 4, 25 and 37°C the activation energy of the aqueous chemical hydrolysis was calculated to be 69·2 kJ mol^{-1}. This calculated value of activation energy is similar to that of 65·7 kJ mol^{-1} calculated by Davey & Murray (1969) at pH 4·9 in aqueous solution. The plasma degradation of diamorphine showed non-Arrhenius behaviour and the data could not be used to calculate an activation energy for the enzyme-catalysed deacetylation.

The effect of pH on the stability of 6-monoacetylmorphine in aqueous solution at 25°C is shown in Table 3. The ester bond of 6-monoacetylmorphine is known to be labile under alkaline conditions (Smith & Cole 1976; Barrett et al 1991) but there is no information available on the kinetics of this deacetylation reaction. In this study the degradation from 6-monoacetylmorphine to morphine was found to proceed at a consistently slower rate than that from diamorphine to 6-monoacetylmorphine at all pH values measured, thus supporting the view that the 3-acetyl group of diamorphine is more labile than the 6-acetyl group. This is in accordance with the known increased reactivity of a phenolic ester (3-acetyl) compared with an alcoholic ester (6-acetyl).

The instability of diamorphine in aqueous solution at neutral pH means that care must be taken, when using the compound in in-vitro experiments, to ensure that the conditions of temperature and pH are chosen to avoid degradation of diamorphine. The data in this study supports the observations that significant degradation of diamorphine will occur over the prolonged periods of intravenous infusion from a reservoir or when the drug is made up in solution and stored at room temperature before use (Jones et al 1987; Omar et al 1989; Kleinberg et al 1990; Northcott et al 1991).

In conclusion, we have shown that the rates of deacetylation of diamorphine in human plasma and aqueous solution at temperatures of 4, 25 and 37°C are sufficiently rapid to permit significant breakdown of the drug in aqueous solutions and in biological fluids over the course of an in-vitro or in-vivo experiment. We have described a method for the rapid stabilization and subsequent assay of diamorphine in plasma which will prevent errors in estimation of the drug due to hydrolysis.

The authors wish to thank Ms Å. Carlsson and Mr B. G. Davis for technical assistance.

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