Pharmacokinetics of intravenously administered haem arginate

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1 The pharmacokinetics of haem were investigated after intravenous administration of a therapeutic dose of haem arginate (3 mg haem kg⁻¹) to four healthy volunteers and four symptomless porphyric patients.

2 Plasma haem concentrations were measured also during a treatment course of four infusions in six patients with porphyria.

3 Plasma haem concentrations declined monoexponentially over 48 h in both healthy volunteers and porphyric patients, with a mean $\pm$ s.e. mean elimination half-life of 10.8 ± 0.6 h. Other kinetic parameters were also similar in the two groups, total plasma clearance was 3.7 ± 0.4 ml min⁻¹ and volume of distribution was 3.37 ± 0.34 l.

4 In the multiple dose study the elimination half-life increased significantly, from 11.3 ± 0.4 h to 18.1 ± 1.4 h over 4 consecutive days.

5 Plasma haemopexin values decreased with time after a single haem arginate dose.

6 The infusion of haem arginate did not cause thrombophlebitis.

Keywords pharmacokinetics haemin haem arginate haemopexin side effects

Introduction

The pharmacokinetics of haem (the term haem is used here to indicate an iron-protoporphyrin IX compound irrespective of the oxidation state of the iron) in healthy volunteers have not been described before. Owing to the poor solubility of haemin, haematin (haematin is a reaction product of haemin and sodium carbonate i.e. haemin hydroxide) is used for conventional haem infusions. The clinical use of haematin solutions prepared in hospitals for emergency cases of acute porphyrias has been associated with technical difficulties and complications. Because of the poor stability of haematin solutions (Mendenhall, 1984) they cannot be subjected to the quality control analyses (quantitative determination, sterility and pyrogen tests, etc.) normally performed on drug preparations before clinical use. Haematin infusions in porphyric patients are associated with a high risk of thrombophlebitis (McColl *et al.*, 1981) and coagulation

disturbances (Morris *et al.*, 1981, Glueck *et al.*, 1983), which are possibly caused by degradation products of haematin (Mustajoki, 1985). Transitory renal failure after a relatively large dose of haematin has been reported (Dhar *et al.*, 1978). At present haem is the treatment of choice in acute attacks of hepatic porphyrias (Pierach *et al.*, 1980), but according to Goldberg *et al.* (1984) more experience and fuller clinical assessment is still required.

Recently a new stable haem preparation, haem arginate (the term haem arginate refers to the reaction product of haemin and L-arginine in a solution mixture of propyleneglycol, ethanol and water), has been introduced. In preliminary studies (Tenhunen *et al.*, 1985) it was found to be safer than extempore prepared haematin solutions. We now report pharmacokinetic studies of this preparation with both healthy volunteers and porphyric patients.

Methods

Subjects

Eight subjects participated in a single dose study; four healthy volunteers (three women, mean age 34 years, range 26–50, mean weight 62.9 kg, range 45–71.5) and four porphyric patients (two women, mean age 38 years, range 30–45, mean weight 66.3 kg, range 47–83) with acute intermittent porphyria but without symptoms at the time of the study. Six patients (five men, mean age 42.5 years, range 31–64, mean weight 77.1 kg, range 43–97), of whom three suffered from acute intermittent porphyria, two from variegate porphyria and one from porphyria cutanea tarda, participated in a multiple dose study, in which biochemical effects of haem arginate treatment were also investigated.

Ethics

The research plans were approved by the Ethics Committee of the III Department of Internal Medicine, Helsinki University Central Hospital. The members of the research group were the first volunteers. Informed consent was obtained from each subject before the study.

Drugs and dosing

Haem arginate infusion concentrate (Normosang® 25 mg ml⁻¹, Huhtamäki Oy Pharmaceuticals (Medica), Helsinki, Finland) was diluted with 100 ml of physiological saline solution, and a dose equivalent to 3 mg haem kg⁻¹ was infused over 15 min into a forearm vein. The mean dose was 194 mg/subject in the single dose study. In the multiple dose study haem arginate infusion was given once a day on 4 consecutive days.

Sampling

Timed venous blood samples for determination of plasma haem, haemopexin and haptoglobin were collected into heparinized tubes before the infusion and 1, 5, 15, 30, 45 min, 1, 2, 4, 6, 8, 12, 24, 36 and 48 h after it. In the multiple dose study samples for determination of plasma haem and haemopexin were obtained at 10 min and 24 h after every infusion.

Measurements

Haem concentrations were measured both spectrophotometrically (Dhar *et al.*, 1975) and as pyridine haemochromogens (Paul *et al.*, 1953). The coefficient of correlation between the two

methods was 0.985 ± 0.015 (mean $\pm$ s.e. mean). The specific method of pyridine haemochromogens was used to exclude possible interfering factors. In calculations we used values obtained with the spectrophotometric method. The coefficient of intra-assay variation of this method was 2.2% ($n = 16$) at $70 \mu\text{g ml}^{-1}$; the sensitivity of the method was $1 \mu\text{g ml}^{-1}$.

Haemopexin was measured by immunodiffusion (Nor-Partigen, Hoechst) and haptoglobin by immunoturbidimetry (Ojala *et al.*, 1981). Clinical chemical measurements relevant to drug safety (complete blood screen, platelets, serum aminotransferases, γ -glutamyltransferase, albumin and creatinine) were done on the first and last blood samples (0 h and 48 h). Routine methods of the University Hospital were used. Serum albumin was measured by immunonephelometry using Dacon antiserum and a Behringwerke Laser nephelometer.

Pharmacokinetic calculations

Curve fitting for each subject was done using a least squares computer program (Brown & Manno, 1978). Before calculation of the final parameters, a correction for the infusion time was made according to Loo & Riegelman (1970). Total plasma clearance (CL) was calculated from

$$\frac{\text{Dose}}{\text{AUC}},$$

where AUC is the total area under the plasma concentration–time curve calculated by the trapezoidal rule and with extrapolation to infinity. Volume of distribution was calculated from

$$\frac{\text{Dose}}{C_{(0)}},$$

where $C_{(0)}$ is the plasma haem concentration extrapolated to zero time after bolus injection.

Statistics

Student's *t*-test for paired data was used to compare the concentrations of haemopexin before and after haem arginate infusion and the elimination half-lives after each infusion.

Results

Plasma haem concentration–time curves for healthy volunteers and porphyric patients are shown in Figure 1. Since kinetic parameters for the two groups did not differ significantly, mean

Figure 1 Concentrations of haem in plasma following intravenous administration of haem arginate (3 mg haem kg⁻¹) to eight subjects in a single dose study. --- healthy volunteers, — porphyric patients.

Table 1 Pharmacokinetic parameters describing the disposition of haem following intravenous administration of haem arginate (3 mg haem kg⁻¹) to eight subjects (nos 1–4 are healthy volunteers and nos 5–8 porphyric patients)

Subject	Intercept with ordinate ($\mu\text{g ml}^{-1}$)	Exponential coefficient (h^{-1})	$t_{1/2}$ (h)	CL (ml min^{-1})	AUC ($\mu\text{g ml}^{-1} \text{h}$)	V (l)
1	97	0.059	11.7	2.1	1656	2.2
2	60	0.076	9.2	4.5	905	3.6
3	60	0.084	8.3	4.6	730	3.3
4	47	0.064	10.8	3.1	719	2.9
5	63	0.056	12.4	2.1	1181	2.3
6	43	0.055	12.7	3.5	947	3.8
7	49	0.058	11.9	5.0	848	5.1
8	63	0.072	9.6	4.6	918	3.8
Mean	60	0.065	10.8	3.7	998	3.4
± s.e. mean	± 6	± 0.004	± 0.6	± 0.4	± 108	± 0.3

values were calculated for all eight subjects. Individual data are shown in Table 1.

The decline in haem concentrations was mono-exponential and a good fit was obtained between the observed and estimated values (r^2 values ranged from 0.84 to 0.99; median 0.97). The mean ± s.e. mean elimination half-life was 10.8 ± 0.6 h. The volume of distribution of haem was small having a mean value of 3.4 l.

Plasma haemopexin concentrations decreased in parallel with those of haem (Figure 2).

Plasma haptoglobin values did not change after the haem arginate infusion. The individual mean values, 14 measurements per subject (with ranges from time 0 to 48 h) were: No. 1: 2.7 mg ml⁻¹ (2.4–2.9), No. 2: 1.0 (1.0–1.1), No. 3: 1.0 (0.5–1.2), No. 4: 1.8 (1.4–1.9), No. 5: 0.4 (0.3–0.5), No. 6: 0.5 (0.4–0.6), No. 7: 0.3 (0.2–0.3) and No. 8: 0.9 (0.8–1.0).

In the multiple dose study the apparent elimination half-life of haem in plasma increased significantly ($P < 0.01$ – 0.001) during therapy.

Figure 2 Concentrations of haemopexin in plasma (mean $\pm$ s.e. mean) before and after haem arginate infusion (3 mg haem kg⁻¹) to eight subjects in a single dose study. $\square$ all subjects $n = 8$, ▨ healthy volunteers $n = 4$, ▤ porphyria patients $n = 4$.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; significantly different from pretreatment values.

Thus, the value was 11.3 ± 0.4 h (mean $\pm$ s.e. mean) after the first dose, then 13.9 ± 0.5 h, 16.5 ± 0.4 h and 18.1 ± 1.4 h after the second, third and fourth infusion, respectively. Ten minutes after the first infusion plasma haemopexin was 0.28 ± 0.06 g l⁻¹. It was only detectable in one subject after subsequent infusions. In this patient the concentration was 0.11 g l⁻¹ 10 min after the second infusion. During treatment with haem arginate high urinary excretion of porphyrin precursors in acute intermittent porphyria and high values of faecal porphyrins in variegate porphyria fell to normal (data not shown here, see Mustajoki *et al.*, 1985).

No objective side-effects were observed either in healthy volunteers or in the porphyric patients. No abnormalities were found in clinical chemical laboratory values relevant to drug safety before and 48 h after the haem arginate infusion.

Discussion

Of the proteins measured, haemopexin is a high affinity binding protein for free haem. When the binding capacity of haemopexin is saturated haem binds to other serum proteins, especially albumin (Muller-Eberhard & Vincent, 1985). Haemopexin may also transfer the haem from liver back to plasma (Schmid, 1983) thus prolonging the terminal half-life. Haptoglobin is a

binding and transfer protein for haemoglobin and the reason why it was measured was that if the haem arginate infusion had caused haemolysis (which also depletes haemopexin) then the concentration of haptoglobin should decrease.

The elimination half-life of haem obtained in this study in both healthy volunteers and porphyric patients after a single infusion of haem arginate is in agreement with the rapid phase half-life in porphyric patients reported by Petryka *et al.* (1976). They also suggested that the elimination of haematin in porphyric patients after repeated intravenous administration can be described by a biexponential equation where the terminal slow elimination is determined by the rate of formation of haemopexin to replace that which has been removed.

The slower, terminal elimination half-life of haem could not be determined in our study after a single dose. However, when we treated porphyric patients with repeated daily haem arginate infusions, cumulation occurred even after the infusion given on the third day. This supports the observation that elimination slows down as the haemopexin level decreases and the binding of haem thus declines. Only one infusion of 3 mg kg⁻¹ was sufficient to deplete plasma haemopexin.

Haem arginate infusions were not irritant to veins nor did they cause thrombophlebitis or any other objective side-effects.

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