Oral morphine in cancer patients: *in vivo* kinetics and *in vitro* hepatic glucuronidation

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1 The kinetics of morphine and formation of the main metabolite, morphine-3-glucuronide (M3G) after single and intravenous doses of morphine were studied in six cancer patients and compared with the formation rate of M3G *in vitro* in microsomes isolated from liver biopsies obtained from the same patients at palliative laparotomy.

2 The results showed that high formation rates of M3G *in vitro* in microsomes isolated from liver biopsies were associated both with high apparent oral clearance values and high M3G/morphine AUC (area under the concentration vs time curve) ratios as measured *in vivo* in the same patients.

3 In accordance with previous results marked interindividual differences were seen in the kinetics of morphine; the oral bioavailability varied between 30 and 69% and the systemic plasma clearance between 18.6 and 34.0 ml min⁻¹ kg⁻¹. This variation correlated with the variation in morphine metabolism as assessed *in vitro*.

4 *In vivo*, a high M3G/morphine AUC ratio predicted a high oral clearance. Hepatic UDP-glucuronyl transferase activity is thus an important determinant of the *in vivo* kinetics of orally administered morphine.

Keywords morphine cancer pharmacokinetics glucuronidation

Introduction

In view of renewed interest in and increased use of high oral doses of morphine in the treatment of cancer pain (Twycross, 1978; Mount *et al.*, 1979; Agenäs *et al.*, 1982) it is important to gain further information about the kinetics and metabolism of morphine in cancer patients. Morphine metabolism in animals has been studied in detail whereas studies of the disposition of high oral doses of morphine in man are limited (Brunk & Delle, 1974; Yeh, 1975).

Morphine is subject to marked presystemic elimination (Brunk & Delle, 1974; Säwe *et al.*, 1981) predominantly by glucuronidation in the liver and the gut mucosa (Iwamoto & Klaassen, 1977; Bock *et al.*, 1978). In the clinical use of oral morphine this is compensated for by administra-

tion of higher oral than parenteral doses. The formation rates of morphine glucuronides in microsomes isolated from human liver specimens vary considerably between individuals (Säwe *et al.*, 1982). This is consistent with the large interindividual variation in oral bioavailability and steady-state drug concentrations during chronic therapy (Neumann *et al.*, 1982; Säwe *et al.*, 1983).

To evaluate to what extent the activity of the UDP-glucuronyl-transferase as measured *in vitro* in liver microsomes determines the *in vivo* metabolism of morphine, the kinetics of morphine in six cancer patients were compared with the enzyme activity in microsomes isolated from liver specimens from the same patients. Such com-

parative studies on drug metabolism *in vivo* and *in vitro* are difficult to perform and scarce in the literature.

Methods

Patients

Six male cancer patients aged 57 to 77 years were investigated at the initiation of chronic pain therapy with oral morphine. The clinical data are presented in Table 1. All patients had normal serum creatinine values and no history of kidney dysfunction. A wedge biopsy was taken from the liver during laparotomy which was performed for diagnosis or for palliation (Table 1). The study was approved by the Ethics committee of the hospital and required informed consent from the patients.

In vivo study

A single i.v. dose of 4 mg morphine hydrochloride was given about 1 week after surgery. Two days later a single oral dose of morphine hydrochloride was administered in an elixir containing morphine hydrochloride 20 or 25 mg, ethanol (95%) 2.5 g,

blackberry syrup 5 ml and purified water to a total volume of 20 ml. The patients were fasted overnight and remained in the supine position up to 4–5 h after the dose was administered. Venous blood samples were drawn over 24 h following the single i.v. and oral doses. The samples were collected in heparinized plastic tubes. Urine was collected over an interval of 0–12 h (or 0–6 h in patient 6) after the dose was administered.

In vitro study

Wedge biopsies, 1–3 g, obtained at surgery were immediately placed on dry ice and kept frozen at -80°C until the *in vitro* assay took place. The isolation of microsomes was performed as described previously (Pacifi *et al.*, 1982). Morphine glucuronidation was studied according to Bock *et al.* (1978) as modified by Pacifi *et al.* (1982). Incubates of 0.1 ml contained microsomes corresponding to 2 mg protein/ml, 15 mM uridine diphospho-glucuronic acid (UDPGA), and 40 μl of a solution containing equal parts of 50 mM MgCl_2 , 1 M Tris-HCl buffer, pH 7.4 and morphine (to give a final concentration of 0.85–17 mM in the incubate). Samples and blanks without UDPGA were incubated at 37°C for 20

Table 1 Clinical features of the patients

Patient	Age (years)	Weight (kg)	Cancer	Clinical findings/surgery	Concurrent medication and smoking habits
1	73	71	Oesophagus	Explorative laparotomy. Witzel fistula. Became icteric later.	None. Nonsmoker.
2	70	54	Gallbladder	Explorative laparotomy. Elevated bilirubin and liver transaminases in serum	Hydrochlorothiazide + amiloride, nitroglycerin. Smoker.
3	70	60	Carcinoma of the stomach	Palliative antecolic gastroenterostomosis. Postoperative gastric retention. Became icteric later.	Hydrochlorothiazide + potassium. Nonsmoker.
4	77	60	Carcinoma of the stomach	Explorative laparotomy. Witzel fistula.	Digoxin, dichlorotride, potassium, verapamil. Pre- and postoperative treatment with cytotoxic drugs. Nonsmoker.
5	68	58	Pancreas	Explorative laparotomy. Gastrostomy. Splenectomized.	Nystatin (1.5 m IU), phenoxymethylpenicillin. Nonsmoker.
6	57	61	Oesophagus	Explorative laparotomy. Witzel fistula. Splenectomized. Hepatomegaly.	None. Nonsmoker.

min and the reaction was stopped by freezing on dry ice in acetone.

Analytical method

Morphine, morphine-3-glucuronide (M3G) and 'putative', morphine-6-glucuronide (M6G) in incubates, plasma and urine were measured by reversed phase ion-pair high pressure liquid chromatography (Svensson *et al.*, 1982). An increased sensitivity was achieved by the following modifications: 1 ml plasma was mixed with 1.5 ml of 0.5 M ammonium sulphate adjusted to pH 9.3 with ammonia, then passed through a Chromprep® PRP-1 320 µl cartridge (Hamilton Bonaduz AG, Bonaduz, Switzerland). The cartridge was washed with (1): 7.5 ml 10 mM ammonium sulphate adjusted to pH 9.3 with ammonia, (2) 0.7 ml 10 mM phosphate buffer, pH 2.1 and (3) 0.3 ml 5% acetonitrile in phosphate buffer pH 2.1. Morphine and its metabolites were eluted with 0.8 ml 5% acetonitrile in phosphate buffer pH 2.1. All of the eluate was injected on the column. The lower limit of detection of morphine M3G and M6G in plasma with this modification was 4 nmol/l (1.1 ng/ml). The coefficient of variation was 3.8% for morphine (at 17 nmol/l; 4.9 ng/ml) and 4.0% for M3G (at 100 nmol/l; 46.1 ng/ml), $n = 5$). The standard curves were linear for both morphine and M3G. Interfering peaks did not allow the quantitation of M6G in plasma from two patients. M6G was not accurately measurable in plasma after intravenous administration of morphine.

Calculations

The terminal half-life ($t_{1/2,z}$) was calculated from the least squares regression line fitted to a semi-log plot of the plasma drug concentration vs time. The area under the plasma drug concentration vs time curve (AUC) was calculated by the trapezoidal rule with extrapolation to infinity.

The apparent oral clearance (CL_{po}) and the systemic clearance after intravenous dosing (CL) were determined as dose divided by AUC, and the volume of distribution (V) as CL divided by the elimination rate constant. The oral bioavailability (F) was calculated as:

$$F = \frac{AUC_{po}}{D_{po}} \cdot \frac{D_{iv}}{AUC_{iv}}$$

The Michaelis-Menten constant (K_m) and the maximum velocity (V_{max}) of the formation of M3G and M6G *in vitro* were calculated from Lineweaver-Burke plots (substrate concentration⁻¹ vs reaction velocity⁻¹).

Results

In vivo data

Higher plasma concentrations of M3G than morphine were observed both after i.v. and oral dosing (Figure 1). The pharmacokinetic data are given in Table 2. CL varied between 18.6 and 34 ml min⁻¹ kg⁻¹. The average value of V was 4.0 l/kg and of $t_{1/2,z}$ was 1.7 h. F varied between 29.9 and 69.3% (mean 47.1%).

Figure 1 Plasma concentrations of morphine and its glucuronides after single i.v. (4 mg; upper panel) and oral (20 mg; lower panel) doses in patient no. 2.

Consistent with the high M3G concentrations the average M3G/morphine AUC ratios (an 'index' of glucuronidation rate under certain assumptions; see below under discussion) after i.v. and oral dosing were 11.1 and 24.4, respectively (Table 2). The corresponding value for the M6G/morphine AUC ratios after oral dosing was 2.5 in four of the patients in whom M6G was measurable in plasma. The average AUC_{po} for M3G was 8.9 times larger than the average AUC_{po} for M6G in these patients.

The coefficient of correlation for the linear regression between CL_{po} and the M3G/morphine AUC_(oral) plasma ratio was 0.88 ($P < 0.05$) (Figure 2). As a corollary this ratio was inversely related to F ($r = -0.85$; $P < 0.05$, Figure 2). However, the CL correlated poorly with F ($r = 0.32$, NS; Figure 2).

Table 2 *In vivo* pharmacokinetic data for morphine after single intravenous and oral doses

Patient	Dose (mg)	$t_{1/2,z}$ (h)	V (l/kg)	CL ($\text{ml min}^{-1} \text{ kg}^{-1}$)	AUC plasma ratio		Urinary excretion ratio		F (%)
					M3G morphine	M6G morphine	M3G M6G	M3G morphine	
1	i.v. 4	3.0	8.6	33.3	15.9	—	—	10.2	51.5
	oral 25	3.2	—	65.2	29.6	—	—	23.7	
2	i.v. 4	1.6	3.9	29.0	16.2	—	—	6.9	29.9
	oral 20	5.0	—	96.8	34.9	—	—	16.0	
3	i.v. 4	2.2	3.5	18.6	6.3	—	—	4.2	48.2
	oral 20	4.9	—	38.3	18.5	1.8	10.2	7.9	
4	i.v. 4	0.9	2.1	27.7	9.5	—	—	4.3	33.9
	oral 20	2.9	—	81.7	29.9	4.3	6.9	21.4	
5	i.v. 4	1.1	3.1	34.0	9.3	—	—	4.7	69.3
	oral 20	1.9	—	49.2	13.0	1.4	9.3	10.2	
6	i.v. 4	1.3	3.0	25.6	9.2	—	—	6.0	49.9
	oral 20	1.9	—	51.2	21.0	2.3	9.1	11.5	
Mean	i.v.	1.7	4.0	28.0	11.1	—	—	6.1	47.1
	oral	3.3	—	63.7	24.4	2.5	8.9	15.1	

Figure 2 Relationships between different *in vivo* kinetic parameters in the six patients.

M3G was the major excretion product both after i.v. (79.9%) and oral dosing (81.5%). The average urinary recovery of morphine, M3G and M6G was 55.3% after i.v. (42.8–74.9%; $n = 4$) and 56.4% after oral dosing (21.1–78.5%; $n = 5$). In patient 6 the recovery in urine collected during 6 h was 54.7 and 60.2%, after i.v. and oral dosing, respectively. The urinary recovery increased to about 85% after i.v. and 79% after oral dosing when the sampling period was extended to 24 h (two patients).

The ratio between the amounts of M3G and morphine excreted was linearly related to the M3G/morphine AUC plasma ratio ($r = 0.90$; Figure 3).

In vitro data

The rate of formation of M3G and M6G in liver microsomes as a function of morphine concentrations is shown in Figure 4. The enzyme kinetic

data obtained from the liver biopsy experiments are given in Table 3. The $V_{\max}$ for the formation of M3G was 7.7 times higher than the $V_{\max}$ for M6G.

Comparison of in vivo and in vitro data in the individual patient

The r value for the relationship between the $V_{\max}$ for M3G in liver microsomes and the M3G/morphine AUC ratio was 0.80 ($P < 0.1$) after oral and 0.89 ($P < 0.05$) after i.v. dosing (Figure 5). The corresponding r values between $V_{\max}$ and the M3G/morphine urinary excretion ratios were 0.72 ($P < 0.1$) and 0.87 ($P < 0.05$), respectively. The liver microsomal $V_{\max}$ value did not correlate significantly with the CL_{po} ($r = 0.64$, NS) or the CL ($r = 0.41$, NS). Replacement of $V_{\max}$ with $V_{\max}/K_m$ ratios did not yield any better correlations.

Figure 3 Relationship between the M3G/morphine AUC ratio in plasma and the M3G/morphine urinary excretion ratio in six cancer patients (i.v. ♦ and oral ●).

Figure 4 Rates of appearance of M3G in liver microsomes at different substrate concentrations and the Lineweaver-Burke plots for the same reactions.

Table 3 *In vitro* parameters describing the glucuronidation of morphine by human hepatic microsomes

Patient	V_{max} ($\text{nmol min}^{-1} \text{kg}^{-1}$)		K_m (mM)	
	M3G	M6G	M3G	M6G
1	1.32	0.20	2.05	4.28
2	1.21	0.23	0.84	1.77
3	0.59	0.07	0.63	0.57
4	0.88	0.12	0.50	0.52
5	0.62	0.06	1.00	1.86
6	1.02	0.12	0.47	0.94

Discussion

The high doses of morphine that are required for oral administration are a result of the low oral bioavailability (F) of morphine. The low average value of F is a result of extensive presystemic elimination rather than incomplete absorption since the total recovery in urine after parenteral and oral administration of morphine is similar (present data, Brunk & Delle, 1974; Yeh, 1975). The present study confirms our previous results (Säwe *et al.*, 1981) showing pronounced inter-individual differences in the disposition of oral morphine in cancer patients. This has clinical consequences, especially at the start of oral therapy since F is unknown and hence both under- and overtreatment may occur.

The major metabolic pathway of morphine includes formation of M3G (Boerner *et al.*, 1975). This metabolite accounts for about 80% of the excreted products identified with our analytical method. The M3G concentration in plasma rose rapidly after oral dosing and 25-fold higher concentrations (AUC) of M3G than morphine were found.

Our results indicate that high M3G/morphine ratios as measured *in vivo* in plasma and urine after single i.v. and oral doses of morphine were associated with high conversion rates of morphine to M3G *in vitro* in the liver. The M3G/morphine ratio, as measured in blood or urine is thus a gross indicator of the hepatic metabolism of morphine similar to the 'metabolic ratio' of debrisoquine which identifies extensive and poor metabolizers of this drug (Sloan *et al.*, 1983). Parallel studies of the *in vivo* and *in vitro* metabolism of oxidized drugs have been described for debrisoquine (von Bahr *et al.*, 1983) and anti-pyrene (Boobis *et al.*, 1981).

Further studies are needed to clarify the usefulness of the M3G/morphine ratio in urine or plasma as a predictor of the individual capacity to glucuronidate morphine. For the urinary ratio

Figure 5 The relation between *in vivo* kinetic parameters and the maximum formation ($V_{\max}$) of M3G *in vitro* in microsomes isolated from liver specimens from the same patients.

to be used in this manner it is necessary that kidney function is normal assuming that the rate of formation of M3G is the rate limiting step for excretion and that the rate of formation of the metabolites is proportional to the substrate concentration, i.e. first-order kinetics apply. These two conditions were shown to be true in cancer patients (Säwe *et al.*, 1983). In addition the $V_{\max}$ for the formation of M3G may affect the M3G/morphine ratio in plasma.

Considering the many factors which might affect the comparison of *in vitro* and *in vivo* data, it is interesting that the r value was higher between $V_{\max}$ and CL_{po} ($r = 0.64$) than between $V_{\max}$ and the CL ($r = 0.41$), although none of these relations was significant. This is in accordance with the concept that the apparent CL_{po} of a drug that is subject to extensive elimination and is completely absorbed, such as morphine, is mainly determined by the intrinsic clearance of the presystemic organs (gut and liver), i.e. the maximum enzyme activity, whereas the CL is primarily determined by the hepatic blood flow (Nies *et al.*, 1976).

Among several factors which may influence the estimation of drug metabolism *in vivo* and *in vitro*, such as the obviously different conditions under which the enzymes operate one must consider the possible formation of unmeasured unknown metabolites as well as extrahepatic metabolism. Studies in animals have shown that metabolism in the gut wall contributes 46% to the overall presystemic extraction of morphine (Dahlström & Paalzow, 1978). It is possible that the human intestine also metabolizes morphine in a similar manner to that established for other drugs. Intestinal drug metabolism in man has been observed for ethinyloestradiol (Back *et al.*, 1982) and flurazepam (Mahon *et al.*, 1977).

In the present study there was a marked similarity in the relative *in vivo* and *in vitro* formation of both M6G and M3G; the AUC_{po} of M3G in plasma was 8.9 times higher than the AUC_{po} of M6G and the $V_{\max}$ for M3G was eight-fold higher than $V_{\max}$ for M6G in the liver. This indicates that any extrahepatic metabolism of morphine has a similar pattern to that observed in the liver.

In conclusion, our results indicate that hepatic UDP-glucuronyl-transferase activity is an important determinant of the *in vivo* kinetics of oral morphine. They show that the *in vivo* indices of drug elimination, i.e. CL_{po} and metabolite/parent drug ratios in urine or plasma reflect the enzyme activity in the liver. This raises the question of the usefulness of such ratios as indices of initial dose requirements for oral morphine in cancer patients, since the inter-subject differences in oral bioavailability are mainly due to differences in enzyme activity in the drug metabolizing organs rather than incomplete and varying gastrointestinal absorption. The present investigation also supports the use of *in vitro* preparations of liver microsomes as a tool for studying various factors which might influence drug metabolism *in vivo*.

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