Cancer Chemother Pharmacol (1997) 39: 273–277

SHORT COMMUNICATION

Yuai Li · Greg A. Looney · Bruce F. Kimler
Aryeh Hurwitz

Opiate effects on 5-fluorouracil disposition in mice

Received: 22 December 1995 / Accepted: 25 May 1996

Abstract *Purpose*: This study was performed to investigate the effects of morphine on the disposition of 5-fluorouracil (5-FU). *Methods*: Mice were injected subcutaneously (s.c.) with saline or morphine, 20 mg/kg. 5-FU was administered intravenously (i.v.) 30 min later as a single bolus or by constant infusion. Blood samples were obtained by orbital sinus puncture. Urine samples were obtained from the bladder after ligation of the external urethra. 5-FU concentrations in plasma and urine were determined by HPLC. *Results*: Morphine markedly elevated plasma levels of 5-FU given at doses of 100 to 860 mg/kg. The plasma clearance rate of a bolus dose of 100 mg/kg 5-FU was significantly reduced from 54 to 28 ml/min per kg and the elimination half-life was increased from 6.9 to 12.2 min by prior administration of morphine. When 5-FU was infused at 0.5 mg/kg per min, morphine reduced its plasma clearance rate from 145 to 94 ml/min per kg. Mice made tolerant by prior morphine administration required higher doses of this opiate to raise 5-FU levels as well as to cause analgesia. The effects of morphine on 5-FU disposition were antagonized by naltrexone. Excretion of 5-FU in urine was not affected by morphine treatment. *Conclusions*: The plasma clearance rate of 5-FU in mice is significantly reduced by concomitant use of morphine. This effect of morphine is due to reduced hepatic elimination of 5-FU rather than to a decrease in its renal excretion.

This work is supported in part by the University of Kansas Cancer Center and U.S. Public Health Service Grant DA02477

Y. Li · G.A. Looney · A. Hurwitz (✉)
Division of Clinical Pharmacology, Department of Internal Medicine, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160–7320, USA
Tel. (913)–588–6060; Fax (913)–588–3995.

B. F. Kimler
Department of Radiation Oncology, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160–7321, USA

Key words 5-Fluorouracil · Morphine · Naltrexone · Pharmacokinetics · Mice

Introduction

5-Fluorouracil (5-FU) is a fluorinated pyrimidine antimetabolite which is widely used to treat adenocarcinomas of the gastrointestinal tract, breast and lung. Since cancer patients often are in severe pain, necessitating strong analgesics such as morphine, possible interaction between this narcotic and antineoplastic drugs may be clinically relevant. In animal experiments morphine has been shown to delay hepatobiliary elimination of other drugs [8, 10, 11] and model dyes [4, 15, 17]. Since 5-FU is rapidly eliminated by the liver, this study was planned to examine effects of morphine on the disposition of 5-FU in mice.

Materials and methods

Male and female ICR mice (Sasco Farms, Omaha, Neb.) weighing 20–35 g were used in this study. The animals were kept in approved facilities and the experiments were performed only after assessment and approval by the Institutional Animal Care and Use Committee.

5-FU USP injection was purchased from Solo Pak Laboratories, a division of Smith and Nephew, and morphine sulfate was from Eli Lilly and Co., Indianapolis, Ind. Crystalline 5-FU, naltrexone, 5-chlorouracil and 1-pentanesulfonic acid sodium salt (HPLC grade) were obtained from Sigma Chemical Co., St. Louis, Mo. A Perkin Elmer HPLC system, consisting of an ISS-100 autosampler, series 10 pump and LC-85B spectrophotometer detector, was attached to a Hewlett Packard 3390A integrator. The C18 column was from Waters Associates.

In this study, morphine and naltrexone were administered subcutaneously (s.c.) and 5-FU was injected intravenously (i.v.). Blood samples (100–500 μl) were obtained from the orbital sinus into heparinized microhematocrit tubes. Plasma was obtained by centrifugation. To collect urine from male mice, the external urethra was ligated [3] under ether anesthesia just before saline or morphine administration. The mice were sacrificed 30 min after 5-FU administration. The abdominal cavities were opened and bladder contents aspirated into a syringe.

To induce morphine tolerance, mice were injected three times each day for 3 days with increasing doses of morphine sulfate [30]. On the first day three doses of 50 mg/kg were given s.c. On the second day two 50-mg/kg doses and one 100-mg/kg dose were given; three 100-mg/kg doses were administered on the third day. Control (naive) animals were given saline. To confirm tolerance to morphine, analgesic response was compared prior to 5-FU administration in naive and tolerant mice. Analgesia was ascertained by determining time not exceeding 30 s taken to flick the tail when immersed in water at 54 °C [22]. In addition to tolerance, another classical indicator of an opiate effect is its reversal by an opiate antagonist. Therefore naltrexone, an opiate antagonist, was administered to determine if this drug would reverse the effects of morphine on 5-FU disposition.

5-FU concentration was assayed in plasma and urine samples by an HPLC method [7]. To each 25–200 µl plasma sample (depending on anticipated 5-FU concentration) 1.25 µg 5-chlorouracil was added as an internal standard. The sample was then extracted twice with 1 ml ethyl acetate. After vortex mixing and 10-min centrifugation at 2500 rpm, the ethyl acetate supernatants were collected and pooled in a separate tube and evaporated to dryness at 45 °C under a gentle stream of nitrogen. The residue was reconstituted with 200 µl mobile phase (0.025 *M* 1-pentanesulfonic acid in HPLC-grade water) by placing the tube in an ultrasonic waterbath for 1 min. The reconstituted residue was then transferred to an autosampler microvial and a 10–40 µl sample injected onto a 30 cm × 3.9 mm reversed phase C18 column. The flow rate of the previously filtered, degassed mobile phase was 2.5 ml/min at a pressure of 10 MPa (approximately 1400 PSI). The UV detector was set at 270 nm, the absorption maximum of both 5-FU and 5-chlorouracil. With this method and equipment, the sensitivity of 5-FU assay is 100 ng/ml in plasma.

Data were analyzed for significant differences by Student's *t* test, ANOVA and Duncan's multiple range test. Analgesia data were analyzed nonparametrically by the Kruskal Wallis test. Pharmacokinetic analysis of plasma concentrations of 5-FU was carried out by a one-compartment model. For bolus doses of 5-FU, its clearance was calculated as dose/AUC, where AUC is the area under the plasma concentration-time curve extrapolated to infinity. Clearance of infused 5-FU was calculated as infusion rate/plasma concentration at steady state.

Results

Effect of morphine on disposition of various doses of 5-FU

Various doses of 5-FU were injected 30 min after saline or morphine, 20 mg/kg, was given s.c. to the mice. Blood samples were obtained 30 min after 5-FU administration and assayed for 5-FU concentration. Morphine elevated plasma 5-FU levels at all given doses (Fig. 1).

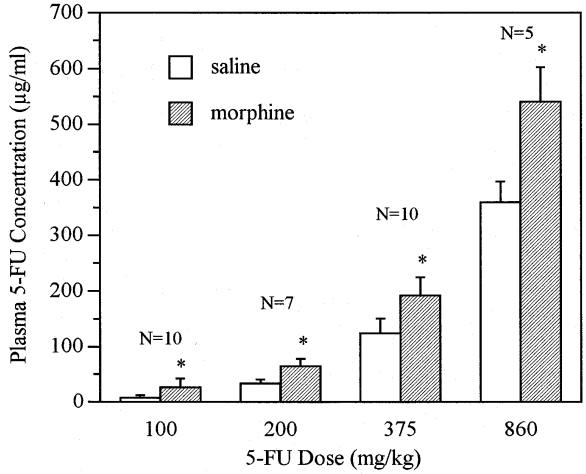

Fig. 1 Effects of morphine on disposition of varying doses of 5-FU. Saline or morphine, 20 mg/kg, was injected s.c., and 30 min later 5-FU was administered i.v. at doses indicated and blood samples obtained after another 30 min. Values are means ± SD; $*P < 0.01$ compared to saline group; *n* is the number of mice in each of the two treatment groups (saline and morphine at each dose)

Effect of morphine on 5-FU pharmacokinetics

Saline or morphine, 20 mg/kg, was given s.c. to the mice, and 30 min later, 100 mg/kg of 5-FU was injected i.v. to all the mice. Plasma concentrations of 5-FU at 5, 20 and 45 min after 5-FU injection were assayed. 5-FU concentrations were higher in morphine-treated mice at all three time-points (Fig. 2). Pharmacokinetic analysis of these data showed halving of clearance after morphine, which was associated with a corresponding reduction in rate of elimination (Table 1). Similar results were obtained in another experiment in which blood samples were collected at 10, 30 and 60 min after 5-FU treatment.

When 5-FU was continuously infused i.v. at a rate of 0.5 mg/kg per min, plasma levels were higher after morphine administration than with saline. The plasma concentration of 5-FU had stabilized by 15 min after saline but was still rising in the morphine-treated animals (Fig. 3). The clearance rate of infused 5-FU was also significantly reduced by morphine treatment.

Effect of morphine on renal elimination of 5-FU

The external urethras of male mice were ligated under ether anesthesia. Saline or morphine, 20 mg/kg, was injected s.c.

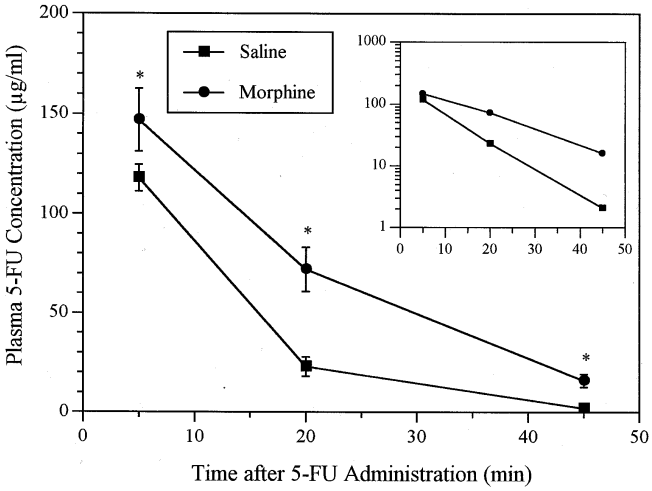

Fig. 2 Effect of morphine on pharmacokinetics of bolus injection of 5-FU. Saline or morphine, 20 mg/kg, was injected s.c., and 30 min later 5-FU, 100 mg/kg, was injected i.v. and blood samples were obtained after another 5, 20 and 45 min. The overlay graph shows a logarithmic *y*-axis transformation. Values are means ± SD; $n = 10$ in each group; $*P < 0.01$ compared to saline treatment

Table 1 Effect of morphine on 5-FU pharmacokinetics (Values are mean ± SD, n = 10)

	Saline	Morphine
k_e, elimination rate constant (min^{-1})	0.1017 ± 0.0101	0.0565 ± 0.0051*
$t_{1/2}$, elimination half-life (min)	6.88 ± 0.66	12.19 ± 1.20*
$AUC_{0-\infty}$, area under plasma drug concentration-time curve (μg/ml.min)	1860 ± 174	3639 ± 465*
Cl, total clearance of drug from plasma ($ml/min.kg^{-1}$)	54.2 ± 5.4	28.0 ± 4.1*
Vd, apparent volume of distribution (ml/kg)	535.1 ± 46.5	496.9 ± 74.9

* P < 0.01, Student's t-test

when the mice awoke, and 30 min later 5-FU, 100 mg/kg, was injected i.v. After another 30 min the mice were sacrificed, urine collected, and 5-FU assayed. Drug amounts eliminated in urine in 30 min were 949 ± 54 and 851 ± 54 μg (mean ± SEM, n = 8) in the control and morphine-treated groups, respectively. The difference between the two groups was not significant. Of the 5-FU dose, 24–28% was eliminated by the kidneys within 30 min.

Antagonism by naltrexone of morphine effect on 5-FU disposition

Saline, naltrexone 5 mg/kg, morphine 20 mg/kg or the combination of naltrexone and morphine were injected s.c. 30 min before i.v. 5-FU, 100 mg/kg. After another 30 min blood samples were collected for determination of 5-FU levels in plasma. Elevation of plasma 5-FU by morphine was reversed completely by naltrexone (Table 2).

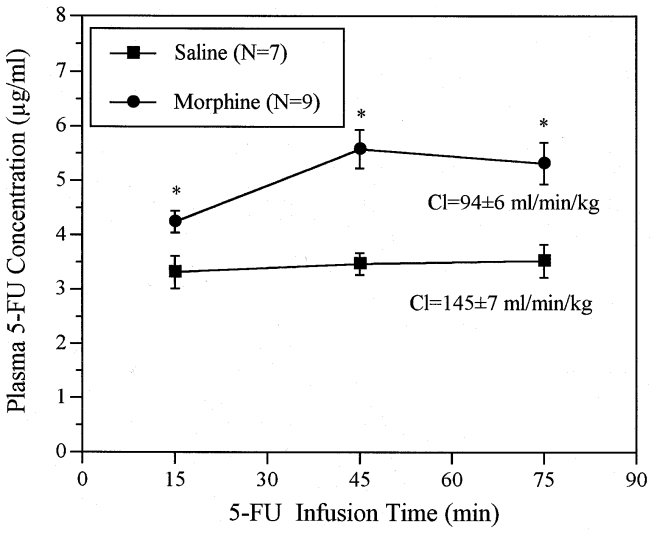

Fig. 3 Effect of morphine on pharmacokinetics of infused 5-FU. A continuous i.v. infusion of 5-FU, 0.5 mg/kg per min, was started 30 min after s.c. injection of saline or morphine, 20 mg/kg. At 15, 45 and 75 min blood samples were obtained. Values are means ± SD; *P < 0.01 compared to saline at corresponding time-point

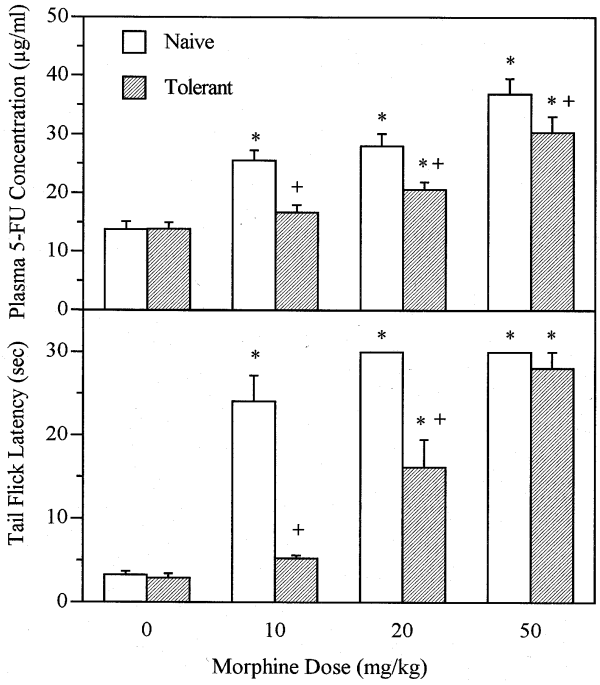

Fig. 4 Effects of morphine on 5-FU disposition (*upper panel*) and analgesia (*lower panel*) in morphine-naive and -tolerant mice. Saline or increasing doses of morphine (to 100 mg/kg) were injected s.c. three times daily for 3 days. One day later, 30 min after a single s.c. injection of saline or of morphine at the dose indicated, warm-water tail-flick was determined and 5-FU, 100 mg/kg, was administered i.v. After another 30 min blood samples were obtained for 5-FU assay. Values are means ± SD; n = 10 in each group; *P < 0.05 of morphine response compared to corresponding acute saline treatment; ^{+}P < 0.05 of tolerant mice compared to corresponding naive group

Effect of morphine on analgesia and 5-FU disposition in morphine-naive and -tolerant mice

Saline or increasing doses of morphine were injected s.c. three times daily for 3 days. One day later a single injection of saline or morphine was administered s.c. at the dose indicated in Fig. 2. A tail-flick response time 30 min later

Table 2 Morphine effect on 5-FU disposition; reversal by naltrexone. Saline, naltrexone 5 mg/kg, morphine 20 mg/kg or the indicated combination was injected s.c. 30 min before i.v. 5-FU, 100 mg/kg. After another 30 min blood samples were collected for determination of levels of 5-FU in plasma. Numbers in parentheses indicate number of animals in each group. Values are means ± SD

	5-FU plasma concentration (μg/ml)
Saline-saline	4.94 ± 1.99 (5)
Naltrexone-saline	4.63 ± 0.91 (6)
Saline-morphine	13.47 ± 3.80 (6)*
Naltrexone-morphine	4.17 ± 0.76 (4)**

* P < 0.01 compared to saline–saline group; ** P < 0.01 compared to saline–morphine group

was determined, and immediately thereafter 5-FU, 100 mg/kg, was administered i.v.. After another 30 min blood samples were obtained for assay of 5-FU concentration. After 3 days of morphine administration mice became tolerant to its analgesic effects, requiring higher doses for acute suppression of the tail-flick response. Tolerance also developed to morphine-induced elevation of plasma concentrations of 5-FU. Acute doses of morphine elevated plasma levels of 5-FU more in naive than in tolerant mice (Fig. 4).

Discussion

The present study showed that hepatic disposition of 5-FU can be changed by concomitantly administered morphine. 5-FU plasma half-life was prolonged, elimination rate decreased and plasma concentration was elevated markedly. Earlier studies have shown that opiates slow hepatobiliary [4, 8, 10, 11, 15, 17] and renal [9, 14, 18] elimination of other drugs and model dyes in mice and rats. These interactions with opiates have been found to enhance toxicity of some of these drugs, including the antineoplastic agent, doxorubicin [21]. 5-FU is primarily eliminated by the liver in rodents and humans by rapid enzymatic reduction [12, 27], though one study in mice suggests that up to 30% of a dose of 5-FU is excreted unmetabolized in the urine within 30 min [5]. In the present study, 24–28% of a 100-mg/kg dose of 5-FU was excreted into the urine by 30 min. Urinary excretion of 5-FU was not affected by morphine, suggesting that its elevated levels in plasma were due to inhibition of hepatic elimination. In the present animal study, the observations were made at a 5-FU dose of 100 μg/kg i.v., which corresponds to 300 mg/m^2 body surface area [23], a dose well within the range used to treat cancer patients. This 5-FU dose has been reported to cause regression of susceptible mouse colon tumors [28] and to achieve plasma levels needed to suppress transplanted human tumors in nude mice [20]. When higher doses of 5-FU were administered, levels in plasma rose disproportionately, as expected with saturable, nonlinear elimination. At each of these doses, treatment with morphine further elevated the levels of 5-FU (Fig. 1).

To avoid unpredictable plasma levels which result from its erratic gastrointestinal absorption and to reduce the bone marrow toxicity associated with bolus injection, 5-FU is often given by continuous i.v. infusion [12]. Other investigators have reported that plasma clearance of 5-FU is higher when infused than when administered as a single bolus [1]. While this finding was confirmed in the present study, administration of morphine markedly reduced clearance of infused 5-FU by 35%. This is consistent with the longer half-life of 5-FU when given with morphine (Table 1).

The mechanism whereby morphine slows 5-FU elimination in liver was not addressed in the present study. In other experiments, using mice and rats, opiates have been shown to raise plasma levels of other compounds which, like 5-FU, are rapidly eliminated by the liver. This hepatic effect of morphine, though centrally mediated [19], is independent of other opiate actions, including hypothermia, biliary spasm and the hypoxia, hypercarbia and acidosis associated with respiratory depression [15–17]. Unpublished data (Li, Looney and Hurwitz) suggest that opiates slow hepatobiliary elimination of other compounds by markedly reducing portal blood flow, which is associated with the reduction in splanchnic blood flow caused by morphine [24, 25]. Although cimetidine [13] and misonidazole [26] have been claimed to slow 5-FU elimination by inhibiting its hepatic metabolism, most evidence suggests that hepatic blood flow is rate-limiting in 5-FU elimination [1, 31]. Thus, elevation of plasma 5-FU concentrations by morphine, a drug which reduces hepatic blood flow, is not unexpected. Reversal of impairment of morphine-induced drug clearance by the antagonist, naltrexone, eliminates the possibility of interference of morphine with the 5-FU assay and suggests involvement of opioid receptors rather than competition for rate-limiting elimination between the narcotic and 5-FU. Tolerance to its impairment of 5-FU elimination after repeated administration of morphine is also characteristic of many opioid effects.

The lowest morphine dose shown to raise 5-FU levels in the present study (10 mg/kg) is in the same range as is needed to slow elimination of other drugs [8, 10, 15, 17]. This dose is about one-fiftieth the LD_{50} in the mouse [2] and barely produces any noticeable behavioral effects in this species. When calculated according to surface area, this dose would convert to about 1 mg/kg in humans [23]. Although even this dose would be considered quite high, it is well within the range which is needed to control pain in cancer patients [29].

In conclusion, morphine slows the elimination of 5-FU in mice. Since 5-FU is only minimally bound to plasma proteins [1] and is equally distributed between plasma and red blood cells [1], and its disposition is qualitatively similar in several rodent species and in humans [6], opiates may affect 5-FU disposition similarly in humans. Under some circumstances 5-FU dose adjustment may be necessary to avoid possible toxicity.

Acknowledgement We wish to acknowledge the World Health Organization for their support of Dr. Yuai Li.

References

1. Au JL-S, Walker JS, Rustum Y (1983) Pharmacokinetic studies of 5-fluorouracil and 5′-deoxy-5-fluorouridine in rats. J Pharmacol Exp Ther 227(1):174
2. Barnes CD, Eltherington LG (1973) Drug dosage in laboratory animals, revised edition. University of California Press, Berkeley
3. Becker BA, Gibson JE (1967) A simple method for the production of anuria in mice. Proc Soc Exp Biol Med 124:296
4. Ben-Zvi Z, Hurwitz A (1986) Effects of morphine and clonidine on sulfobromophthalein disposition in mice. J Pharm Pharmacol 38:481
5. Chadwick M, Rogers WI (1972) The physiological disposition of 5-fluorouracil in mice bearing solid L1210 lymphocytic leukemia. Cancer Res 32:1045

6. Collins JM (1985) Pharmacokinetics of 5-fluorouracil infusions in the rat: comparison with man and other species. Cancer Chemother Pharmacol 14:108
7. El-Yazigi A, Al-Humaidan A (1987) Rapid analysis of 5-fluorouracil in plasma or formulations by high-performance liquid chromatography. J Pharm Biomed Anal 5(7):747
8. Garty M, Hurwitz A (1985) Morphine effects on ampicillin disposition in mice. Antimicrob Agents Chemother 28:489
9. Garty M, Hurwitz A (1986) Tolerance to morphine effects on renal disposition of xenobiotics in mice. J Pharmacol Exp Ther 239:346
10. Garty M, Ben-Zvi Z, Hurwitz A (1985) Opioid effects on lidocaine disposition and toxicity in mice. J Pharmacol Exp Ther 234:391
11. Garty M, Ben-Zvi Z, Hurwitz A (1989) Interaction of clonidine and morphine with lidocaine in mice and rats. Toxicol Appl Pharmacol 101:255
12. Grem JL (1990) Fluorinated pyrimidines. In: Chabner BA, Collins JM (eds) Cancer chemotherapy. J.B. Lippincott Co., Philadelphia, pp 180–224
13. Harvey VJ, Slevin JL, Dilloway MR (1984) The influence of cimetidine on the pharmacokinetics of 5-fluorouracil. Br J Clin Pharmacol 18:421
14. Hurwitz A (1981) Narcotic effects on phenol red disposition in mice. J Pharmacol Exp Ther 216:90
15. Hurwitz A (1983) Narcotic effects on sulfobromophthalein disposition in rats. J Pharmacol Exp Ther 227:68
16. Hurwitz A, Fischer HR (1984) Effects of morphine and respiratory depression on sulfobromophthalein disposition in rats. Anesthesiology 60:537
17. Hurwitz A, Fischer HR, Innis JD, Ronsse S, Ben-Zvi Z (1985) Opioid effects on hepatic disposition of dyes in mice. J Pharmacol Exp Ther 232:617
18. Hurwitz A, Garty M, Ben-Zvi Z (1988) Morphine effects on gentamicin disposition and toxicity in mice. Toxicol Appl Pharmacol 93:413
19. Hurwitz A, Looney G, Sullins M, Ben-Zvi Z (1990) Hepatobiliary effects of morphine are mediated in the brain. Hepatology 12:1406
20. Inaba M, Kobayashi T, Tashiro T, Sakurai Y (1988) Pharmacokinetic approach to rational therapeutic doses for human tumor-bearing nude mice. Jpn J Cancer Res 79:509
21. Innis JD, Meyer M, Hurwitz A (1987) A novel acute toxicity resulting from the administration of morphine and adriamycin to mice. Toxicol Appl Pharmacol 90:445
22. Janssen PAJ, Neimegeers CJE, Dony JGH (1963) The inhibitory effect of fentanyl and other morphine-like analgesics on the warm water-induced tail-withdrawal reflex in rats. Arzneimittelforschung 13:502
23. Klaassen CD, Doull J (1980) Evaluation of safety: toxicologic evaluation. In: Doull J, Klaassen CD, Amdur MO (eds) The basic science of poisons, 2nd edn. Macmillan, New York, pp 21–22
24. Leaman DM, Levenson L, Zelis R, Shiroff R (1978) Effect of morphine on splanchnic blood flow. B Heart J 40:569
25. Mailman D (1980) Effects of morphine on canine intestinal absorption and blood flow. B J Pharmacol 68:617
26. McDermott BJ, van der Berg HW, Martin WMC, Murphy RF (1983) Pharmacokinetic rationale for the interaction of 5-fluorouracil and misonidazole in humans. Br J Cancer 48:705
27. Milano G, Etienne M-C (1994) Dihydropyrimidine dehydrogenase (DPD) and clinical pharmacology of 5-fluorouracil (review). Anticancer Res 14:2295
28. Peters GJ, van Dijk J, Laurensse E, van Groegingen CJ, Lankelma J, Leyva A, Nadal JC, Pinedo HM (1988) In vitro biochemical and in vivo biological studies of the uridine “rescue” of 5-fluorouracil. Br J Cancer 57:259
29. Twycross RG (1988) Opioid analgesics in cancer pain: current practice and controversies. Cancer Surveys 7:29
30. VonVoigtlander PF, Lahti RA, Ludens JH (1983) U-50,488: a selective and structurally novel non-mu (kappa) opioid agonist. J Pharmacol Exp Ther 224:7
31. Warren BS, LaCreta FP, Kornhauser DM, Williams WM (1987) Dose and flow dependence of 5-fluorouracil elimination by the isolated perfused rat liver. Cancer Res 47:5261