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In vivo evaluation of a new sustained-release formulation of morphine

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The pharmacokinetics of a novel sustained-release oral formulation of morphine have been evaluated. The formulation consisted of tablets containing a morphine-Eudragit®L complex (MEC) which had shown good sustained-release properties in previous *in vitro* dissolution studies. MEC tablets were administered orally to beagle dogs and the morphine plasma levels and pharmacokinetic parameters obtained were compared with those obtained with MST Continus®, a commercially available sustained release form of morphine. Blood samples were withdrawn up to 12 h after dosing and plasma morphine concentrations were determined by HPLC with electrochemical detection. Both formulations presented a relatively rapid absorption of morphine with similar values of C_{max} (MST: 53 ng/ml; MEC: 50 ng/ml) and T_{max} (MST: 86 min; MEC: 88 min), and prolonged morphine plasma levels. Mean plasma morphine concentrations were higher for the MEC tablets than for MST tablets during the terminal phase of the corresponding curves and the mean $AUC_{0\text{-}12h}$ for MEC tablets was 138% of that obtained with MST tablets. Our findings indicate that MEC tablets can produce prolonged plasma levels of morphine and could be an alternative to commercially available morphine sustained-release forms.

1. Introduction

Morphine is the opioid analgesic of choice for the treatment of chronic moderate-to-severe pain associated with cancer and other pathologies (World Health Organisation 1986; Bonica and Ekstrom 1990), but its short half-life and duration of action necessitates a dosage schedule with frequent administration (4 to 6 doses per day) when this drug is administered orally to patients using conventional capsules, tablets or solutions (AHFS Drug Information 2001). Self-administered pain control using oral sustained-release dosage forms containing morphine is crucial, especially in home care or long-term care. Modified-release opioids clearly have benefits that extend beyond compliance. Several studies have demonstrated that modified-release opioids are effective and can be given safely in a variety of conditions, including cancer, back pain, diabetic neuropathy, laparoscopic surgery, arthritis, and other conditions (Bruera et al. 1998; Hale et al. 1999; Roth et al. 2000; Reuben et al. 2002; Warfield et al. 1998; Watson 2003). Some studies have also suggested that modified-release opioids may result in fewer side effects than are observed with short-acting opioids (Kaplan 1998; Caldwell 1999; Klepsted 2003). In fact, several sustained-release formulations of morphine have been developed, or are under development, in order to extend the dosage interval to 8 or 12 h or more (Bourke et al. 2000; Broomhead et al. 1997; Gourlay 1998; Sinatra et al. 2002; Nakamura et al. 2007; Imai et al. 2000). All commercial sustained-release formulations of morphine use morphine sulphate salt as an active ingredient. One of the first controlled release systems on the pharmaceutical market for the oral administration of morphine was MST Continus® tablets, which consist of a hydrophilic granular system held within a hydrophobic matrix. Another product, Avinza® capsules, contains both immediate release and extended release beads of morphine. Kadian® capsules is another product in which the morphine pellets are similar in general structure to the extended-release beads in Avinza®, but it does not contain the immediate-release component. Oramorph® sustained-release tablets differ from Avinza® and Kadian® capsules in that they contain the active ingredient in a simple matrix system instead of a polymer-coated reservoir.

A new approach to obtaining a sustained-release formulation of morphine has been developed, which involves preparing a complex of morphine with Eudragit® L, an acrylic polymer insoluble at low pH values but soluble above pH 5.5 (Alvarez-Fuentes et al. 1997). *In vitro* studies of morphine release from the complex showed a significant reduction in the release rate of drug as compared with morphine alone, and a release profile similar to that obtained with commercially available sustained-release tablets of morphine (MST Continus®) (Alvarez-Fuentes et al. 1996, 1997). However, when the complex was administered to dogs the plasma morphine concentration versus time curve was similar to that obtained after administration of morphine alone, indicating that the complex was not able to produce sustained–release of morphine *in vivo* (Araico et al. 2008). Additional *in vitro* studies with this formulation showed that the ionic strength of the surrounding medium has an important influence on the dissolution process, in addition to the effect of pH (Fernández-Arévalo et al. 2004). Therefore, the original formulation (morphine-Eudragit® complex) was modified to reduce the effect of ionic strength while maintaining the pH effect, and 38% of the total morphine

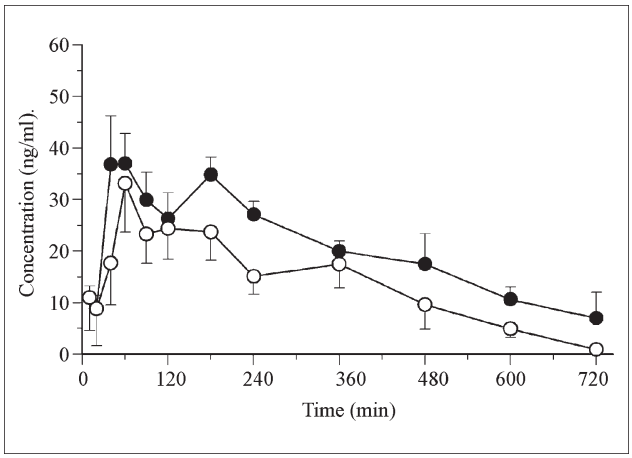

Fig.: Plasma morphine concentrations (mean ± SEM), obtained in fasted dogs (n = 6) after oral administration of MST Continus® (◯) and MEC tablets (●)

in the formulation was incorporated as free morphine in order to obtain early plasma levels of the drug (Alvarez-Fuentes et al. 1994).

In the present study we evaluated this new morphine-Eudragit® complex formulation *in vivo* using beagle dogs, and compared its pharmacokinetic profile with that obtained with MST Continus®, a commercially available morphine sulfate sustained-release formulation.

2. Investigation, results and discussion

Mean plasma morphine concentrations obtained after administration of MEC and MST tablets to dogs are shown in the Fig., and the values of pharmacokinetic parameters are presented in Table 1.

As can be seen in the Fig., both formulations of morphine gave rise to relatively rapid absorption of morphine, with similar values of C_{max} and T_{max}. Furthermore, both curves displayed prolonged morphine plasma levels. In our previous study we showed that the administration of free morphine hydrochloride to fasted dogs give rise to a rapid elimination phase, with no detectable plasma concentrations of morphine 8 h after dosing (21), whereas, in the present study, the mean plasma level 12 h after dosing was still about 7 ng/ml when morphine was administered as MEC tablets.

Most dogs showed secondary peaks in the elimination phase of the plasma morphine concentration versus time curve, which made it difficult to estimate the value of the elimination rate constant. Nevertheless, it can be observed in the Fig. that mean plasma levels showed a similar terminal slope for MEC and MST tablets. On the other hand, mean plasma morphine concentrations were higher for the MEC tablets than MST tablets during the terminal phase of the respective curves.

The mean $AUC_{0\text{-}12h}$ for MEC was significantly higher than that obtained with MST, which suggests that MEC tablets gave rise to a higher relative bioavailability of morphine (138%) based on the $AUC_{0\text{-}12h}$ of MST (Table 1).

In a previous study (17), Nakamura et al. applied a different approach to produce a 24-h sustained-release formulation of morphine hydrochloride. They obtained core granules containing the drug and then coated them with a mixture of pH-dependent swelling polymer, and water insoluble and water soluble polymers (SPILA granules). The pharmacokinetic profile of this formulation was also tested in beagle dogs by oral administration of 60 mg, when the values of the pharmacokinetic parameters obtained were 336 min and 20.7 ng/ml, for T_{max} and C_{max}, respectively. However, the administration of MEC tablets gave faster absorption of morphine (T_{max} = 88 min) and a higher C_{max} (50 ng/ml). On the other hand, the plasma morphine concentration 12 h after dosing was similar for both formulations, approximately 6 ng/ml (SPILA granules) and 7 ng/ml (MEC tablets).

In conclusion, MEC tablets offer a novel mechanism for a controlled-release dosage form of morphine. Our findings indicate that MEC tablets can produce prolonged plasma levels *in vivo* and could be an alternative to commercially available sustained-release forms of morphine.

Table 1: Pharmacokinetic parameters of morphine (mean ± SD) obtained in fasted dogs (n = 6) after oral administration of MST Continus® tablets (MST) or morphine-Eudragit® complex tablets (MEC)

Parameter	MST	MEC	Statistical significance[a]
C_{max} (ng/ml)[b]	53 ± 25	50 ± 20	n.s.
T_{max} (min)[c]	86 ± 29	88 ± 37	n.s.
$AUC_{0\text{-}12h}$ (ng.min/ml)[d]	9332 ± 2382	12884 ± 1956	$P = 0.03$

[a] Anova test; n.s.: not significant ($p > 0.05$)
[b] Maximum plasma morphine concentration
[c] Time to maximum plasma morphine concentration
[d] Area under the plasma morphine concentration versus time curve from time zero to last sampling time

3. Experimental

3.1. Drug and chemicals

The morphine-Eudragit®L complex (MEC) was prepared as previously described (Alvarez-Fuentes et al. 1994). Tablets consisting of 62.5% (m/m) of MEC, 15% (m/m) of morphine hydrochloride trihydrate, and 22.5% (m/m) of Eudragit®RS were prepared as previously described (Holgado et al. 1994). The total amount of morphine in the tablets, expressed as morphine hydrochloride trihydrate, was 60 mg. A commercially available sustained-release formulation of morphine (MST Continus® 60 mg, Asta Medica, Madrid, Spain) was also administered. To avoid vomiting after morphine administration, dogs were given with 5 mg of chlorpromazine (Largactil®, Rhône-Poulenc Rorer, Madrid, Spain). All other chemicals were of reagent or HPLC grade.

3.2. *In vivo* study

3.2.1. Animals and experimental protocols

Six purpose-bred beagles (2 males and 4 females), approximately 1 year of age were used in the experiments, which were performed with the approval of our university, and in accordance with its rules for the use of animals for research purposes. The average weight of the dogs was 9.5 kg (range 8.2–11.6 kg). Dogs were housed in pairs in floor pens containing soft wood bedding. Rooms had automatically timed lighting providing 12 h of light and 12 h of dark per day. Dogs were fed in quantities sufficient to maintain a stable body weight (Harland Interfauna Ibérica, S.A., Barcelona, Spain) and water was available *ad libitum*.

The absorption of morphine from MEC and MST tablets was compared in fasted dogs. Dogs were fasted overnight and MEC and MST tablets were administered at 9:00 a.m. using a two-period crossover design. The washout period between the two administrations was one week and the sequence of administration was assigned randomly according to the schedule shown in Table 2.

Table 2: Study design

Group	Dogs	Treatment	
		Period I	Period II
I	1, 2, 4	MST	MEC
II	3, 5, 6	MEC	MST

MST: MST Continus® tablets
MEC: Morphine-Eudragit® complex tablets

3.2.2. Drug administration and blood sampling

Prior to the administration, a catheter was placed in the cephalic vein and secured using aseptic techniques to facilitate blood sampling. Tablets were administered with 30 ml of tap water and blood samples (2.0 ml) were withdrawn through the catheter at the following times: 0, 10, 20, 40, 60, 90, 120, 180, 240, 360, 480, 600 and 720 min. After each blood sampling heparinized normal saline was flushed through the catheter. Blood samples were placed in heparinized tubes and immediately centrifuged (2000 g for 5 min) at 8 °C, and the supernatant plasma was stored at −20 °C until analysis. Since in preliminary studies it was observed that morphine caused vomiting in dogs after dosing, it was decided to administer 5 mg of chlorpromazine (1 ml of Largactil®) through the intravenous catheter immediately before the administration of any morphine preparation.

3.3. Analytical method

The equipment used to determine morphine concentration in the plasma samples was a Waters (Milford, MA, USA) high-performance liquid chromatograph, comprising the following modules: a Waters 515 HPLC Pump, a 7725i Rheodyne injector, a Waters Concorde electrochemical detector containing a VT-03 flowcell with an *in situ* Ag/AgCl reference electrode, and a Waters 743 Data Module. The potential was set at +0.70 V, the oven temperature was 35 °C and the sensitivity was set at 2 nA full scale.
The eluent consisted of a mixture of acetate buffer (100 mM, pH 4.5)-methanol (75:25 v/v) containing sodium 1-octanesulfonate (500 mg/L), EDTA (100 mg/L) and KCl (150 mg/L). The mixture was delivered at 1 ml/min through a Nova Pak C_{18} cartridge of 15 cm × 3.9 mm I.D. (Waters). Plasma samples were processed as follows: 0.1 ml of plasma, 0.1 ml borax buffer (0.1 M borax containing 1 mg/ml of disodium ethylenediaminetetraacetate and 1 mg/ml of sodium bisulfite) and 1.0 ml extraction solvent (chloroform-isopropanol, 95:5 v/v) were placed in a 1.5 ml Eppendorf tube. The mixture was shaken for 10 min in a type DSG-301 Heidolph shaker and centrifuged for 5 min (2000 g). The upper aqueous phase was removed and 0.9 ml of the organic phase was transferred to a new tube containing 0.1 ml of 0.2 M acetate buffer (pH 3.5). After shaking and centrifuging again, 25 µl of the supernatant was injected into the HPLC system.
The coefficient of variation of the analytical method was less than 5%, and the detection limit was approximately 1 ng/ml.

3.4. Pharmacokinetic and statistical methods

The area under the plasma morphine concentration *versus* time curve from time zero to the last sampling time ($AUC_{0\text{-}12h}$) was estimated by means of a combination of the regular trapezoidal and the logarithmic trapezoidal rules (Wagner 1983). The maximum plasma morphine concentration (C_{max}) and the time of its occurrence (T_{max}) were observed values. Most dogs showed secondary peaks in the elimination phase of the plasma morphine concentration versus time curve, which made it difficult to estimate the value of the elimination rate constant. Since this value is necessary to estimate the elimination half-life and the total area under the plasma morphine concentration curve, these parameters have not been calculated.
Pharmacokinetic parameters obtained after the administration of MEC and MST tablets to fasted dogs (Table 1) were compared by means of a 3-way ANOVA test (factors: treatment, dog and period).
For all statistical tests, a p value of < 0.05 was considered to be statistically significant.

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