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Preclinical study of a controlled release oral morphine system in rats[1]

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Abstract

The efficiency of a new controlled release oral system containing morphine hydrochloride elaborated by introducing the drug into the polymeric structure of Eudragit® L30D is evaluated. Its in vitro dissolution behaviour and its effectiveness using the tail flick test model of pain in rats are investigated. A significant reduction in the drug release rate from the morphine-Eudragit® L30D complex as well as a very high efficiency in the dissolution process have been found. The tested morphine complex has an analgesic effect in rats. This effect is very clear from 30 min to 8 h. At 12 h the effect remains clear but with a lower level of statistical significance. Furthermore, there are no significative differences between the different doses tested.

Keywords: Morphine hydrochloride; Chronic pain; Analgesia; Severe pain; Eudragit® L30D; Controlled release; MST Continus®; Tail flick test

Morphine given regularly by mouth is now recommended throughout the world for the management of severe pain in cancer, when less effective drugs are no longer adequate (World Health Organization, 1986). The most important controlled release system for the oral administration of morphine in the pharmaceutical market is the MST Continus®, which is a combination matrix consisting of a hydrophillic granular system inserted in a hydrophobic matrix. More investigations have been made in order to develop new controlled release systems of morphine (Hanks et al., 1995; Marín Bosca et al., 1995).

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In recent years, a complexation process between Eudragit® L and some basic drugs (morphine, carteolol, ephedrine, etc.) has been developed by us. The objective of these investigations was to prepare a controlled release system of morphine by introducing the drug into the polymeric structure of the acrylic resin. In previous papers (Alvarez-Fuentes, 1992; Caraballo et al., 1992; Alvarez-Fuentes et al., 1994; Fernández-Arévalo et al., 1994; Holgado et al., 1995), several experimental conditions of this complexation process between Eudragit® L and morphine were investigated; furthermore, thermal analysis, IR and NMR spectroscopy was performed in order to determine the nature of the interaction between the polymer and morphine. The morphine complex having the highest drug content (close to 42% w/w), corresponding to a 40% of neutralization degree of the polymer, was selected. The complexation process found between morphine and Eudragit® L was attributed to an interaction type of hydrogen bond (Fernández-Arévalo et al., 1994; Holgado et al., 1995).

The in vitro dissolution behaviours of morphine hydrochloride, 1:1 physical mixture morphine hydrochloride:Eudragit® L, morphine polymeric complex and MST Continus® tablets were investigated. The in vitro dissolution study was carried out at 37 ± 0.5°C in the USP XXIII basket apparatus (Turu Grau, mod. D-6) at a speed of 50 rpm. 700 ml of artificial gastric fluid without enzymes was employed as initial dissolution medium. A pH gradient technique was used. At predetermined time intervals, test solutions were assayed by a HPLC technique previously carried out by us (Alvarez-Fuentes et al., 1994).

The effectiveness of this new oral controlled release system of morphine was tested using the tail flick test model of pain in rats (D'Amour and Smith, 1941). Male Wistar rats weighing 225–250 g, supplied by the Reproduction Laboratory of the University of Cádiz (Spain), were used. The animals were housed in groups of five and maintained at a constant temperature (21 ± 1°C) with free access to food and water. 24 h before test animals were placed in an experimental room to allow adaptation to the test environment. All the experiments were carried out according to the Ethical Guidelines of the International Association for the Study of Pain (IAPS, 1980).

An automated tail flick analgesimeter (Socrel model) was used. It is basically a source of heat (100 watt bulb), that concentrates a light beam onto the sensitive surface of a photocell by means of a parabolic reflector. The tail of a restrained animal is placed on the sensitive surface of the photocell. When the animal flicks its tail away from the photocell, the light beam goes off. The time from placing the animal's tail on the sensitive surface of the photocell until it is flicked is considered as the tail flick latency.

Before starting the experiment, all the rats were weighed and tested on the tail flick test (basal response). After a test-baseline latency, all products were orally administered by means of a flexible canula. Then, the animals were treated with the corresponding dose of morphine polymeric complex or saline solution ($n = 11$), or Eudragit® L ($n = 10$):

(a) Control rats: 1 ml/kg of saline solutions (NaCl 0.9% w/v), or 1 ml/kg of aqueous suspension of Eudragit® L6D.

(b) Treated animals: the tested morphine polymeric complex (SLCM) suspended in carboxymethylcellulose (1% w/v). Amounts of complexes to provide 10, 20, 40 and 60 mg of morphine/kg were used (SLCM 10, 20, 40 and 60, respectively).

All the animals were subjected to subsequent tail flick test at 30 min, 1, 2, 4, 8, 12 and 16 h. Tail flick latencies were measured in three different areas of the rat tail at intervals of 1 min. The mean of three consecutive measures was retained as the latency. A 30 s cut off was predetermined to avoid tissue damage to the animals.

The results were expressed as the mean ± S.E. value of the latencies (seconds) of each group. For statistical analysis, individual group comparisons were made using a two-way ANOVA with repeated measures. The factors of variation were treatment effects (between subjects) and time (within subjects). Individual treatment effects (differences between groups) were analyzed using a Student-Newman-Keuls test following significant main effects of treatment by ANOVA. Time

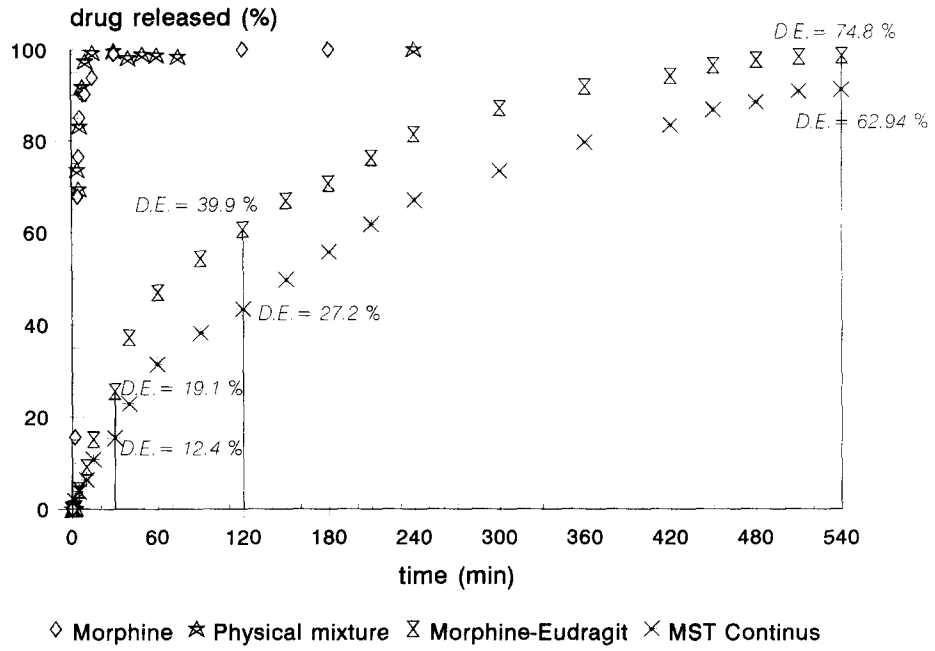

Fig. 1. In vitro release profiles of the indicated products (D.E. = dissolution efficiency at the indicated times).

course effects (differences within groups) were analyzed using a Dunnett test following significant main effects of treatment by ANOVA. A P value ≤ 0.05 was considered as significant.

In respect to the main results of this paper, the in vitro dissolution study of morphine hydrochloride, 1:1 physical mixture morphine hydrochloride-Eudragit® L, morphine polymeric complex and a MST Continus® 60 mg tablet were considered for comparison purposes.

A significant reduction in the release rate of drug from the two controlled release systems assayed has been found in comparison to those corresponding to morphine hydrochloride and the physical mixture morphine hydrochloride-Eudragit® L (Fig. 1). On the other hand, the complex used as a morphine controlled release system has allowed a very high efficiency in the dissolution process. In order to compare the release profiles of the complex and the MST Continus® system the dissolution efficiency values found for the two controlled release systems at several time intervals are indicated. So, the dissolution efficiency values of the morphine complex was higher than that showed by the MST Continus® system and a higher initial release rate is observed for the morphine complex. These results are more significant considering that the morphine content in the complex (42 mg) was lower than the charge of the MST Continus® tablet (60 mg).

In relation to the in vivo study, the results obtained from the tail flick latency curves did not indicate a significant time-dependent variation in latencies corresponding both to saline solution or Eudragit® L treated animals (Fig. 2). However, an increase was observed after 30 min in rats treated with different doses of morphine polymeric complex compared to control rats. After this time-point, the analgesia was maintained at 8 h and 12 h at different signification (Table 1). At 16 h, no significant differences in the pain thresholds were observed in any group.

In this paper, the in vivo study was not carried out with the MST Continus® system. This system is a matrix: its oral administration by means of a flexible canula is impossible without breaking it. This possibility would not be viable because the matrix would be damaged and destroyed. On the

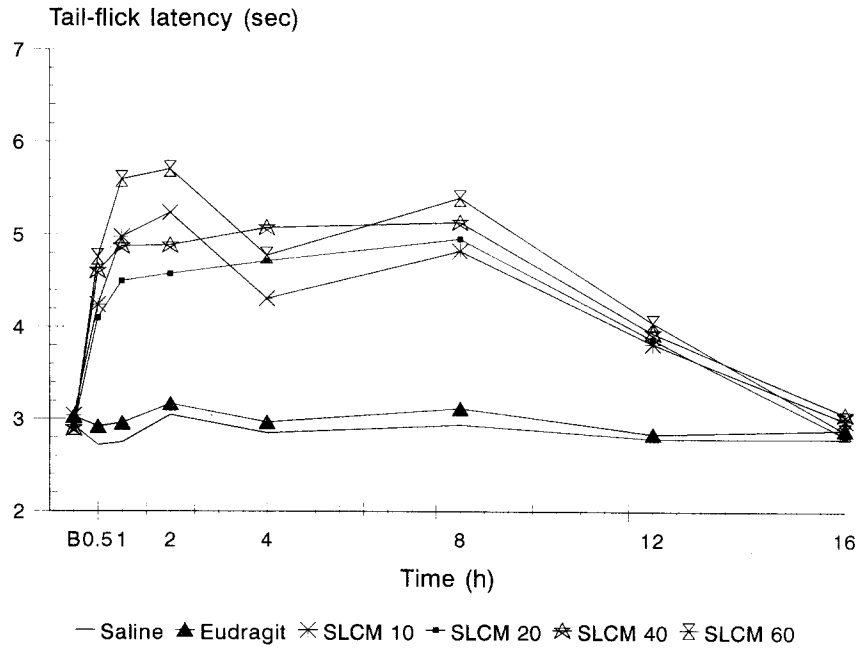

Fig. 2. Tail flick latency curves.

other hand, a bibliographical review of the study of the in vivo behaviour of MST Continus® in rats has been performed.

Analysis of the treatment effects at the different time points. Rats treated with morphine complex showed significantly increased latencies from the first time-point tested. Thus, one factor ANOVA (factor of variance = treatment) performed on the different time points, showed that from 30 min to 12 h there was a significant increase in nociceptive threshold (Table 1). Post hoc comparison showed that this significant increase in nociceptive threshold ($P \leq 0.005$) could be attributed to any of the tested doses. This effect declined at 16 h.

Analysis of the time course effect. One factor ANOVA (factor of variance = time) performed on the different treated groups showed a significant increase in nociceptive threshold in all the treated groups (Table 2). Post hoc comparison showed that this significant increase in nociceptive threshold ($P \leq 0.001$) was attributed to time points from 30 min to 8 h. This effect was decreasing at 12 h and reached basal values at 16 h.

As a conclusion of the in vivo assays, the results obtained demonstrate that the tested morphine polymeric complex has an analgesic effect in rats. This effect is very clear from 30 min to 8

Table 1
Analysis of the treatment effects at the different time points

Time	d.f.	F	P
30 min	57	7.409	<0.0001
1 h	57	11.364	<0.0001
2 h	57	10.479	<0.0001
4 h	57	7.269	<0.0001
8 h	57	6.178	<0.0001
12 h	57	4.275	0.0025
16 h	57	0.440	0.8186

Table 2
Analysis of the time course effects in the different groups

Dose (mg/kg)	n	d.f.	F	P
10	10	72	7.161	<0.0001
20	10	72	5.835	<0.0001
40	11	80	6.014	<0.0001
60	11	80	8.409	<0.0001

h. At 12 h the effect remains clear but with a lower level of statistical significance. Furthermore, there are no significative differences between the different doses tested. So, by means of using this test on rats and with this experimental protocol, it can be concluded that the analgesia elicited by the test morphine polymeric complex seems to be effective after 30 min and for more than 12 h.

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