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Preclinical Study of an Oral Controlled Release Naltrexone Complex in Mice

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Abstract

Naltrexone, a long-acting, orally effective, opioid antagonist which blocks opioid effects as well as the development of physical dependence, would appear to be a drug ideally suited to addiction treatment. An optimal dosage regimen is critical for the treatment patient compliance in ambulatory opiate detoxification programs. The ideal dosage regimen would be an oral controlled-release system of naltrexone that allowed once-a-day administration providing stable plasma levels.

A naltrexone–Eudragit L complex was produced in aqueous medium from naltrexone hydrochloride solution and Eudragit L30D (30% w/v) previously diluted (6% w/v) and partially neutralized. The antagonistic activity of naltrexone–Eudragit L on morphine-induced thermal antinociception, in comparison with conventional naltrexone, was evaluated, using the mouse hot-plate model. Mice were administered 10 mg kg^{-1} morphine subcutaneously, 10 min before test, and the antagonist products, either naltrexone–Eudragit L or naltrexone hydrochloride, were administered orally at 0·5, 1, 2, 4, 6, 8, 10, 12, 14 and 16 h before the test.

The results showed that the antagonism induced by naltrexone–Eudragit L was effective until 12 h after drug administration, while that induced by naltrexone hydrochloride disappeared 10 h after its administration. A 23·47% increase of the area under curve was obtained when naltrexone–Eudragit L was administered, compared with that induced by conventional naltrexone. The time taken to decrease the inhibition of analgesic activity to 50% was delayed by 51·80%.

This complexation technique can be considered as a useful tool in the design of oral controlled-release systems capable of inducing a long-lasting effect in-vivo.

The therapeutic program of opioid dependence implies the detoxification of the patients addicted and the treatment of the psychological dependence. The use of an opioid antagonist, as an adjunct to the maintenance of the opioid-free state detoxified individuals, facilitates a successful detoxification (Crabtree 1984). Indeed, the oral administration implies a positive psychological factor in the treatment.

Naltrexone is a long-acting, orally effective, opioid antagonist which blocks opioid effects as well as the development of physical dependence. It would appear to be a drug ideally suited to addiction treatment. An optimal dosage regimen is critical for treatment patient compliance in ambulatory opiate detoxification programs. An oral controlled-release system of naltrexone that allowed once-a-day administration and provided stable plasma levels would be ideal.

Considering the optimal pharmacokinetic and physicochemical characteristics of naltrexone, it was introduced into the polymeric structure Eudragit L30D (anionic copolymer based on polymethacrylic acid and ethylacrylate). Alvarez-Fuentes et al (1997b) have evaluated the physicochemical properties and the in-vitro behaviour of naltrexone Eudragit L.

The aim of this study was to test in-vivo the antagonistic activity of naltrexone Eudragit L on morphine-induced thermal antinociception, in comparison with conventional naltrexone, in search

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for a compound with longer-lasting effects. The biopharmaceutical characterization of naltrexone–Eudragit L in comparison with naltrexone was carried out using the mouse hot-plate model.

Materials and Methods

Materials

Naltrexone hydrochloride was a gift from Laboratories Zambón S.A. (Barcelona, Spain). Eudragit L30D was obtained from Hüls Española S.A. (Barcelona, Spain), sodium chloride and sodium hydroxide from Panreac (Barcelona, Spain), morphine hydrochloride from Alcaliber, S.A. (Madrid, Spain) and sodium carboxymethylcellulose from Analema (Vizcaya, Spain).

Naltrexone–Eudragit L complex

Eudragit L30D (30% w/v) (Figure 1) is an anionic copolymer based on polymethacrylic acid and ethylacrylate (1 : 1), soluble above pH 5·5. A complexation technique successfully used with other drugs such as morphine (Alvarez-Fuentes et al 1994a, b) and carteolol (Holgado et al 1995) was used to produce the naltrexone–Eudragit L complex. The complex was produced in aqueous medium from naltrexone hydrochloride solution and Eudragit (30% w/v) L30D (6% w/v) previously diluted and partially neutralized (40%). This complexation technique has been patented with the number P9401748 (Alvarez-Fuentes et al 1994a, b, 1997b).

An HPLC method was used to quantify the naltrexone hydrochloride (pump Kontron Instruments, type 420, injector Rheodyne type 7125, detector Kontron Instruments, type 432, integrator Konik Instruments, type DataJet 4600, column Merck, Aluspher 100 RP-select B (5 μm particle size, 12·5 cm × 4 mm i.d.). The flow rate was set at 1 mL min^{-1} and the variable wavelength detector was set at 283 nm. The selected mobile phase was methanol : purified water : diammonium phosphate 70 : 30 : 0·1 v/v/w. The calibration curve obtained ($y = (0{\cdot}1222 \pm 1{\cdot}80\times10^{-4})x + (4{\cdot}35\times10^{-5} \pm 2{\cdot}34\times10^{-4})$) was linear from 6·25 to 1000 μg mL^{-1}, giving r = 0·9999 as correlation coefficient (n = 27) and F = 459596 as Snedecor ratio ($P < 0{\cdot}0001$). The accuracy (−8·4%) and the intra- and inter-assay precision (CV < 1%) were adequate (Alvarez-Fuentes et al 1997b).

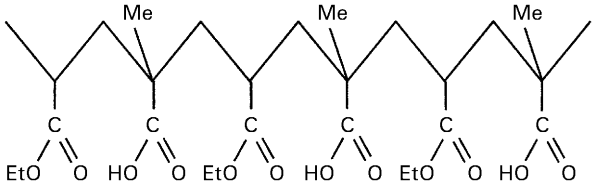

Figure 1. Structure of Eudragit L30D.

The obtained naltrexone-complex was characterized using an ^{1}H NMR and a ^{13}C NMR spectroscopic study (Bruker 200-AC type spectrometer, DMSO-d6 (ICN Biomedical Inc., Cambridge, UK) as solvent). The ^{1}H NMR spectra of the complex naltrexone–Eudragit L with those of the separate components suggested that naltrexone was present in the polymer as free base and not in its ammonium salt form. The analysis of ^{13}C chemical shifts showed that the spectroscopic behaviour of naltrexone carbons in the complex was closer to naltrexone base ($\Delta\delta_i = 0{-}1{\cdot}7$ ppm) than to naltrexone hydrochloride ($\Delta\delta_i = 0{-}3{\cdot}5$ ppm). It can be deduced that naltrexone was present in the complex as free base and not as ammonium salt; so, naltrexone interacted with the polar groups of the polymer by means of hydrogen bonds (Alvarez-Fuentes et al 1997b).

Release profile of naltrexone–Eudragit L

An in-vitro dissolution study (USP XXIII basket apparatus, Turu Grau, mod. D-6) using a pH gradient technique showed that the release of half of the drug charge was assured between the first 2 h, and the rest of the drug charge was gradually released during the following 6 h. This release profile was adequate for our objectives: to obtain an immediate and effective antagonism of opiate effects and to maintain this action over a long period of time (Alvarez-Fuentes et al 1997b).

Animals

Albino male OF1 mice (25–30 g; Central Animal Service, University of Cádiz) were housed in the laboratory under controlled experimental conditions. The temperature was maintained at 21 ± 1°C with a 12-h light–dark cycle. The air was removed at 15 changes h^{-1} (relative humidity 55 ± 10%). Free access to food and water was allowed (rodent pellets, Panlab, Barcelona, Spain) until 1 h before the drug administration. The mice were randomly assigned to groups of 10. These groups were housed in plexiglass cages (25 × 50 × 15 cm) and placed in the test room the day before testing to allow adaptation to the testing environment.

The experimental protocol was approved by the Ethical Committee for Experiments on Animals (CEEA) of the Faculty of Medicine of the University of Cádiz (licence number 079604) and

carried out according to the Ethical Standards of the International Association for the Pain of Study (1980).

Nociceptive test

The dosages of morphine and naltrexone are expressed as the salt form. Morphine was diluted in 0·9% NaCl and injected subcutaneously. Naltrexone hydrochloride and naltrexone–Eudragit L complex were suspended in carboxymethylcellulose (1% w/v) and administered orally by means of a flexible cannula. All the drugs were administered in a volume of $10\,mL\,kg^{-1}$.

Mice were placed on a hot plate (Socrel, model DS 37 Digital) thermostatically maintained at $55{\cdot}0 \pm 0{\cdot}2^{\circ}C$ (Woolfe & Macdonald 1944). A plastic cylinder was used to confine the mouse to the heat surface of the hot plate. The time elapsed until the first response of the animal (either forepaw or hindpaw licking or jumping) was measured as the pain latency.

To avoid tissue injury, animals not responding within 45 s (cut-off time) were removed from the hot plate and a maximal analgesic effect was recorded.

Experimental procedure

In a previous work (Alverez-Fuentes et al 1997a), the time interval between morphine administration and the test, as well as the administration route that induced an adequate analgesic effect were evaluated. Thus, the subcutaneous administration of $10\,mg\,kg^{-1}$ morphine, 10 min before test, was chosen. Alvarez-Fuenteset al (1997a) verified that the polymer Eudragit L had neither analgesic nor proalgesic activity.

The antagonistic activity of naltrexone–Eudragit L on morphine-induced thermal antinociception, in comparison with conventional naltrexone, was evaluated at different time intervals. Each animal group was tested at one specific time and was not re-used thereafter. Each experiment involved three groups of 10 mice. All the three groups received morphine ($10\,mg\,kg^{-1}$) subcutaneously. Group 1 received saline orally and was used as the control group. Group 2 received naltrexone–Eudragit L orally ($16\,mg\,kg^{-1}$, expressed as naltrexone). Group 3 received naltrexone hydrochloride orally ($16\,mg\,kg^{-1}$). Naltrexone–Eudragit L or naltrexone hydrochloride was administered at 0·5, 1, 2, 4, 6, 8, 10, 12, 14 and 16 h before the test. The duration of action of either antagonist was determined by their capability to reverse the analgesic effect of morphine.

Statistical analyses

The data are expressed as a percentage of the response of the control group (group 1).

Statistical analyses were performed on raw data. The differences between groups were analysed using a Student-Newman-Keuls test following significant main effects of treatment by analysis of variance. Statistical significance was accepted at the 5% level ($P < 0{\cdot}05$).

Furthermore, other parameters such as the percentage of inhibition of analgesic activity (IAA) were studied to compare the effect of naltrexone hydrochloride and naltrexone–Eudragit L complex during the assayed time.

$$IAA\,(\%) = ((latency_{control} - latency_{antagonist/morphine})/latency_{control}) \times 100 \quad (1)$$

where $latency_{control}$ is the result from the control group (saline + morphine) and $latency_{antagonist/morphine}$ is the result from group 2 or 3 (antagonist + morphine).

Results

The morphine-induced antinociceptive effect was significantly antagonized by the administration of either antagonist ($F_{(2,27)} = 48{\cdot}9852$, $P < 0{\cdot}01$), 30 min before the hot-plate test. This antagonism was maintained until 10 h after drug administration ($F_{(2,27)} = 29{\cdot}5542$, $P < 0{\cdot}01$) (Figure 2). At this time, significant differences between the effect induced by naltrexone–Eudragit L and conventional naltrexone were found ($P < 0{\cdot}05$). A significant antagonism of morphine antinociception was induced by naltrexone–Eudragit L 12 h after drug administration, but not by naltrexone hydrochloride ($F_{(2,27)} = 3{\cdot}4051$, $P < 0{\cdot}05$). No significant

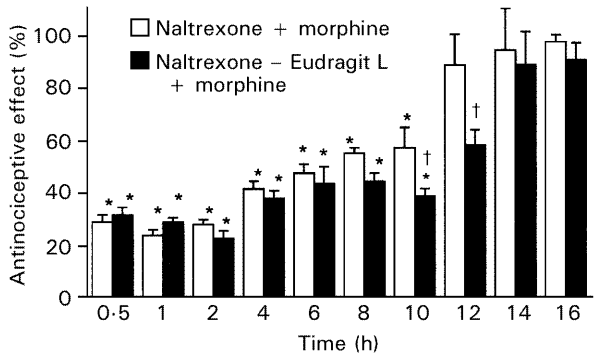

Figure 2. Antinociceptive effect of the groups expressed as percentages of response of the control group (group 1, administered morphine subcutaneously plus saline orally). *$P < 0{\cdot}05$ compared with saline only; †$P < 0{\cdot}05$ compared with group 3, administered morphine subcutaneously plus naltrexone orally.

Table 1. Parameters obtained from the statistical analyses of the data.

Time	Analysis of variance		Post hoc		
	$F_{(2,27)}$	P	Group 3 vs group 1	Group 2 vs group 1	Group 2 vs group 3
30 min	48·9852	< 0·01	$P < 0·05$	$P < 0·05$	–
1 h	64·1276	< 0·01	$P < 0·05$	$P < 0·05$	–
2 h	148·4395	< 0·01	$P < 0·05$	$P < 0·05$	–
4 h	32·6131	< 0·01	$P < 0·05$	$P < 0·05$	–
6 h	15·2160	< 0·01	$P < 0·05$	$P < 0·05$	–
8 h	28·7302	< 0·01	$P < 0·05$	$P < 0·05$	–
10 h	29·5542	< 0·01	$P < 0·05$	$P < 0·05$	$P < 0·05$
12 h	3·4051	0·0480	–	$P < 0·05$	$P < 0·05$
14 h	0·4725	0·6285	–	–	–
16h	0·1242	0·8837	–	–	–

All the three groups received morphine subcutaneously. Group 1 received saline orally and was used as the control group. Group 2 received naltrexone–Eudragit L orally (16 mg kg^{-1}, expressed as naltrexone). Group 3 received naltrexone hydrochloride orally (16 mg kg^{-1}).

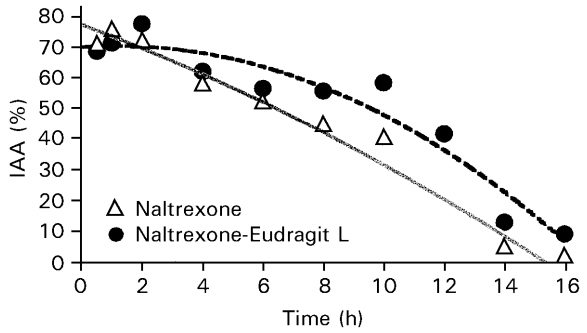

Figure 3. Percentage of inhibition of analgesic activity (IAA; %) of naltrexone and naltrexone–Eudragit L complex.

differences in the pain responses were observed in any group at the 14-h time-point. Table 1 shows the results of the statistical analyses.

The plot of inhibition of analgesic activity (IAA %) vs time shows the comparison of the potency of either drug in antagonizing the morphine-induced analgesia (Figure 3). A 23·47% increase of the area under curve (AUC) was obtained when naltrexone–Eudragit L was administered, compared with that induced by conventional naltrexone.

The time taken to decrease IAA to 50% (t50) was calculated by semilogarithmic regression. The value of this parameter was 4·30 h when naltrexone was administered; however, the pretreatment with naltrexone–Eudragit L provided a value of 6·54 h.

Discussion

Alvarez-Fuentes et al (1997a) showed that naltrexone–Eudragit L complex displayed an effective antagonism of the analgesic action induced by subcutaneous administration of morphine. This effect was observed when the complex was administered at least 2 h before the beginning of the experimental test. Those in-vivo results were in agreement with the in-vitro release profiles of the naltrexone–Eudragit L assayed. However, the capability of naltrexone complexes to induce a long-lasting antagonism remained to be determined.

The mouse hot-plate model is usually employed as a method for studying opiate antagonists, using morphine (positive control) as the model analgesic (Heilman et al 1976; Carrara et al 1990; Rosland & Hole 1990; Fuentes et al 1994). The test was carried out as a method for determining the oral effectiveness and the duration of action of a standard narcotic antagonist, naltrexone, and a new product, the naltrexone–Eudragit L complex.

Thus, the effect of naltrexone-Eudragit L on morphine-induced thermal antinociception was evaluated at different time intervals, and then compared with that induced by conventional naltrexone. Naltrexone hydrochloride inhibited significantly the analgesic effect of morphine from 30 min to 10 h after its administration. However, naltrexone–Eudragit L induced an effective antagonism from 30 min to 12 h after treatment. Moreover the antagonism induced by naltrexone–Eudragit L 10 h after administration, was significantly higher than that induced by naltrexone hydrochloride at the same time interval. Furthermore, the plot of the inhibition of analgesic activity vs time shows that the naltrexone–Eudragit L complex provided an enhancement of the AUC by 23·47% and a delay of 51·80% in the t50 parameter, compared with those induced by naltrexone hydrochloride.

Thus, naltrexone–Eudragit L showed a longer-lasting effect than conventional naltrexone using

the hot-plate test model of pain in mice. Similar results were obtained with a controlled-release system of morphine based on the same complexation technique, and using the tail-flick test as the pain model in rats (Alvarez-Fuentes et al 1996). In this study, the morphine–Eudragit L-induced analgesia was effective from 30 min to 12 h after administration. Both results sustained the viability of the proposed complexation technique with Eudragit L30D as a tool in the design of oral controlled-release systems capable of inducing long-lasting effects in-vivo.

The capability of this complex to induce an effective and long-lasting antagonism of opiate effects appears to be ideally suited to treat opiate addiction. Once the patients have been detoxified and opiate free, an opioid antagonist is usually employed as an adjunct to the maintenance of the opioid-free state (Crabtree 1984). Under naltrexone treatment, the possibility of relapse decreases rapidly, as opiate agonists produce few or no effects. However, an optimal dosage regimen is critical for the treatment-patient compliance (Verebey 1981) and stable plasma levels are needed to block the effects of agonist if resumption of opiate use occurs.

In conclusion, the optimal results obtained in this study with the naltrexone–Eudragit L complex advance the development of controlled-release systems of naltrexone. The complexation technique used may provide controlled-release systems, which could allow once-a-day administration and may account for treatment compliance as well as provide stable plasma levels. However, chronic administration is needed to establish a more accurate dosage-regimen.

These complexes can be formulated as several dosage forms: suspensions, capsules, tablets, inert matrices, etc. This will allow a dosage form to be designed that can obtain the required plasma levels. Further investigations are necessary to evaluate the effectiveness of these complexes after its formulation as inert matrices, by means of the corresponding preclinical, clinical and pharmacokinetic studies.

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