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LACRIMAL AND PLASMATIC KINETICS OF MORPHINE AFTER AN OPHTHALMIC DELIVERY OF THREE DIFFERENT FORMULATIONS

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ABSTRACT

The aims of this work were to study the influence of a thermoreversible gel and an insert containing morphine on lacrimal and plasmatic kinetics in comparison with a simple solution. Lacrimal uptake was carried out with a Millipore filter which was characterized by a total recovery of morphine and an absence of interference on the CPG/SM procedure.

Contrary to the thermoreversible gel, the inserts prolonged the lacrimal and plasmatic kinetics. In the lacrimal fluid, Cmax was reduced from 21.9 to 4.8 mg/ml and Tmax delayed from 2.9 to 51.4 minutes. Likewise, in the plasma, Cmax was decreased from 116 to 37 ng/ml and Tmax prolonged from 17 to 135 minutes. This plasmatic prolongation was equivalent to the one reported with oral prolonged release devices containing morphine. Moreover, the insert areas under the curve (taking account of the delivered dose) in the lacrimal fluid

and in the plasma were multiplied by from 1.6 and 2.6, respectively, in comparison with the solution.

INTRODUCTION

The opportunity for systemic absorption of drugs from topically applied solution is well known and systemic passage after nasal or transdermic administration is abundantly described. Recently, some works carried out with animals have clearly demonstrated that systemic absorption of peptides or proteins after ophthalmic delivery could be equal or superior to the one obtained with nasal route (1, 2, 3, 4, 5, 6, 7, 8, 9). Ophthalmic route was considered with interest by these authors. Parenteral administration, though effective, is not suitable for the long term treatment. Because of the uniqueness in anatomical structure of the nose, the lack of reproductibility in the kinetic efficiencies is an inconvenience.

Chast et al. have delivered morphine acetate solution in the conjunctival cul-de-sac of New-Zealands rabbits (10, 11). Nevertheless, the kinetic profile was characterized by a very high peak magnitude but the decrease in concentrations was very rapid. The relative bioavailability was equal to 0.44. For these authors, ophthalmic route with intention to observe a systemic absorption could offer some advantages when classical routes are not possible according to the difficulties to swallow or to inject vehicles during the terminal phase of a cancer.

Although ophthalmic route interest and feasibility are not clearly demonstrated in the case of systemic passage, evaluation of prolonged release vehicle is important in order to improve kinetic profile and to understand absorption mechanisms. Two ophthalmic prolonged release vehicles were selected because of their satisfactory tolerance (i.e. a poloxamer 407 thermoreversible gel and a hydroxypropyl-cellulose insert) (12, 13, 14, 15, 16, 17). The aims of this work were to determine the morphine concentrations in lacrimal fluid and to compare them with the results obtained in plasma.

MATERIALS AND METHODS

Preparation of the formulations

*Ophthalmic solutions

The ophthalmic solution was prepared by dissolving 10% morphine acetate (MW=399.4, 1:2,5 soluble in water- Francopia, Paris-France), in distilled water at 5°C.

* Poloxamer 407 thermoreversible gel

The thermoreversible gel was manufactured with poloxamer 407 (20%- Lutrol F127- BASF- Allemagne). The dissolution was realized at 5°C, then morphine acetate was added and dissolved at the same temperature. In a previous work, gelification temperature was determined using rheological tests and was equal to about 23°C (18).

*Inserts

The polymer used was hydroxypropylcellulose (Klucel HF*, MW=1 115 000, Aqualon, Wilmington, U.S.A.). Morphine hydrochloride ($C_{17}H_{19}NO_3$-HCl.$3H_2O$, MW=375.8,1:24 soluble in water-Francopia, Paris, France). Morphine acetate could not be used according to its hygroscopy which was not compatible with direct compression and owing to its high solubility which may result in excessively rapid release from hydrophilic matrices. Inserts were obtained by direct compression. The sieving of the ground polymer, and morphine salt was performed with the use of a 0.05 mm mesh test sieve (Prolabo, Paris, France) , followed by the morphine salt being mixed with the polymer using a mixing drum (Turbula, Basel, Switzerland) over a period of one hour. Each insert was prepared by weighing out 15 mg of the mixture which was compressed on a single-punch press (K55 single-punch press, Milcott, Frogerais, Vitry sur Seine). Hardness was adjusted on the single-punch press to a value of 3.1-3.2 kPa, being monitored with a hardness tester (2E hardness tester, Schleuniger, Frogerais, Vitry sur Seine; n=6). The diameter and thickness were equal to 4 and 0.9 mm respectively.

Lacrimal uptake

The basic problem of levels determination in lacrimal fluid results from the collection methods. Two procedures are classically reported. Direct method using capillary tubes requires stimulation of tears secretion. The error in concentrations determination is difficultly controlled and the values are drastically reduced (19, 20, 21). On the contrary, indirect method is preferable (20). This one is carried out with absorbent materials like blotting paper strips. Their principal advantages are the following : small uptake, satisfactory tolerance, easy use and an no preliminary stimulation. Nevertheless, previous tests are necessary to verify the total recovery of the molecule in the solution before the analytical procedure. Several materials were tested in order to study the morphine recovery and the eventual interference on the analytical procedure :

-Schirmer strips (Faure, Annonay, France)

-Whatman paper U 92000 455000 (Waters, Saint Quentin en Yvelines, France)

-Millipore filter AP 20 (Waters)

Simulations tests were performed using strips (5mm/30mm) impregnated with 3 µl of morphine acetate solution (0.5, 4.5, 14.5 mg/ml). Then, the strips were disposed in glass tubes with 2 ml methanol and submitted to supersonic waves during 2 minutes.

Morphine determination in lacrimal fluid

Morphine levels were determined by capillary gaz chromatography coupled with mass spectrometry. Codeine (Francopia) was the internal standard and a previous silylation was necessary and was obtained with BSTFA/TMCS (99/1, Bis-trimethylsilyl-2,2,2-trifluoroacetamide / Trimethylchlorosilan, Pierce Chemical Company, Mallet S.A., Choisy-Charles de Gaulle, France). The apparatuses used were a Hewlett-Packard 5988 A chromatograph with a 0V1 fused silica capillary column (12 m, 0.2 mm i.d.) and a 5899 A Hewlett-Packard mass spectrometry (Les Ulis, France). The head of the column was held at 100°C for 30 seconds and then the temperature programmed to 215°C with 60°C/minute. The chromatography was started at 215°C and programmed

to 230°C with 4°C/minute. The column was connected to the electron impact source of the mass spectrometer. The efficiencies of the determination procedure were characterized by an interday reproductibility of 2,6%, an innerday reproductibility of 7,6% and a sensibility of 0,1 ng/ml.

Morphine determination in plasma

They were performed via radioimmunoassay procedure using highly selective antibodies and ^{3}H morphine described elsewhere (10). This method was choosen by Chast et al. for the morphine determination in plasma after morphine acetate solution administration in conjunctival cul-de-sac.

In vivo study

*Lacrimal study

Morphine acetate solution (0.76 mg morphine base/kg), thermoreversible gel (0.76 mg morphine base/kg) and morphine hydrochloride morphine insert (3 mg morphine base) were delivered in the conjunctival cul-de-sac of New-Zealands rabbits (n=7, for each formulation). Lacrimal samples were collected with Millipore filter strips (5 mm / 30 mm) and submitted to the recovery and dosage procedure.

*Plasmatic procedure

Thermoreversible gel (0.76 mg morphine base/kg) and insert (3 mg morphine base) were disposed in the conjunctival cul-de-sac using 5 and 6 New-Zealands rabbits, respectively. The blood samples were collected, then centrifuged and frozen at 25°C.

RESULTS AND DISCUSSION

Millipore filter has been selected for the following reasons : total recovery, no interference on the analytical determination and easy use. Simulation tests are essential in order to obtain interpretable and reproducible results.

A drastic elimination of lacrimal morphine levels was observed with the solution (Table 1). The constant of elimination (0,285 min^{-1}) was similar to the

Table 1

Comparison between pharmacokinetic parameters in lacrimal fluid obtained with morphine acetate solution, thermoreversible gel and morphine hydrochoride inserts

	Solution	Thermoreversible gel	Insert
Dose(mg/kg)	0.76	0.76	0.84
Cmax(mg/ml) (± S.E)	21.9±2.5	17.3±1.6	4.8±0.3
Tmax(min)(± S.E)	2.9±0,5	5	51.7±8.6
AUC(mg/ml.min)	333	289	570

one described with drugs like pilocarpine or fluoresceine (22, 23) and radioactive tracers like 99mTc (24). And these results confirmed that morphine concentration was assumed to decrease exponentially with time.

Morphine lacrimal concentration resulting from the solution could be evaluated just after the delivery, taking account of the volume administered and the physiological lacrimal film volume (7,5 µl). This concentration was equal to 62,5mg/ml and was very superior to the one noted at the time two minutes. Several phenomenona induced such a drastic"elimination". Bioavailability of topically applied drugs is mainly defined by spillage, drainage via the lacrimal passage (canaculi, lacrimal sac, nasolacrimal duct) and systemic absorption through conjunctival mucosa (23, 25, 26). Corneal penetration is generally limited. Nevertheless, a large part occurs drainage so far as the normal resident tear volume is significantly inferior to the volume delivered. Chrai and al. have fitted the lacrimal fluid evolution in function of the time (t) (23):

$$V_t = V_i \exp^{-kt} + 7.5$$

where V_i is the instilled volume, 7.5 µl the resident volume and k equal to 0.25+0.0113Vi.(23). In our study, V_t decreased from 42 µl just after the instillation to 17 µl at the second minute and 7.5 µl at the tenth minute. Taking account of these local phenomenona, most of the morphine was drained and absorbed through the nasal mucosa. The lacrimal and plasmatic kinetics (tables 1

Table 2

Comparison between pharmacokinetic parameters in plasma obtained with morphine acetate solution, thermoreversible gel and morphine hydrochoride inserts.

	Solution	Thermoreversible gel	Insert
Dose(mg/kg)	1	0.76	0.90
Cmax(ng/ml)(± S.E)	116±28	51.3±13.2	37.0±4.7
Tmax(min.)(± S.E)	17.0±4	11.0±2.5	135±26.5
AUC(ng/ml.min)	5320	4915	12680

and 2, Figures 1 and 2) were very similar but the obtention of the lacrimal peak magnitude was delayed from about 16 minutes in comparison with the plasmatic one. This delay resulted from the drainage to the nasal mucosa and from the morphine distribution in the organism. The lacrimal and plasmatic kinetics observed with the thermoreversible gel were not different from the solution. Nevertheless, the lacrimal and plasmatic Cmax were slightly reduced, which was interesting in order to minimize the local and systemic toxicities. Moreover, the plasmatic Tmax appeared sooner which could induce a faster pharmacological action of morphine but the kinetics differences between the thermoreversible gel and the solution were not significant and the areas under the curve, taking account to the administered dose, were equivalent.

On the contrary, inserts induced a significant prolongation in lacrimal film and plasma. Drastically in the both fluids, the Cmax were reduced and the Tmax delayed. The plasma elimination half times T1/2 were equal with the solution and the inserts to 70 and 260 minutes, respectively.

Kinetic characteristics (Tmax: 135 min., T1/2 : 259 min.) observed with the inserts were similar to those reported with oral controlled release containing sulphate morphine (Tmax: 150min., T1/2: 156-246 min.) (27). These findings signified that ophthalmic route allowed a kinetic prolongation equivalent to oral administration. Local and relative bioavailabilities were widely increased with the

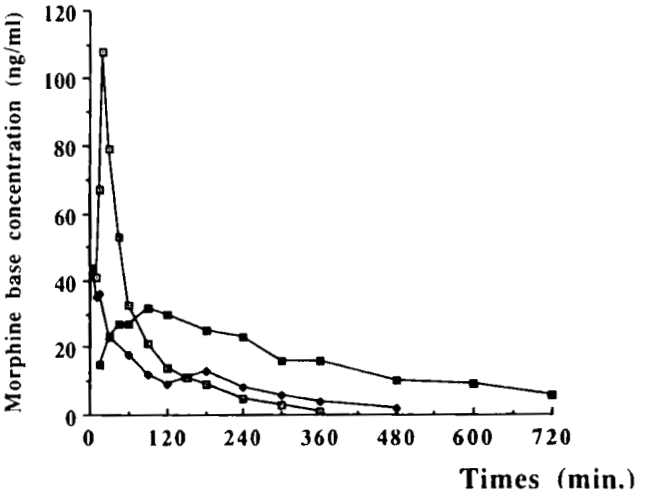

Figure1

Comparison between lacrimal profiles obtained with morphine acetate solution (▫) thermoreversible gel (◆) and morphine hydrochloride insert (■).For the sake of clarity error bars have been omitted.

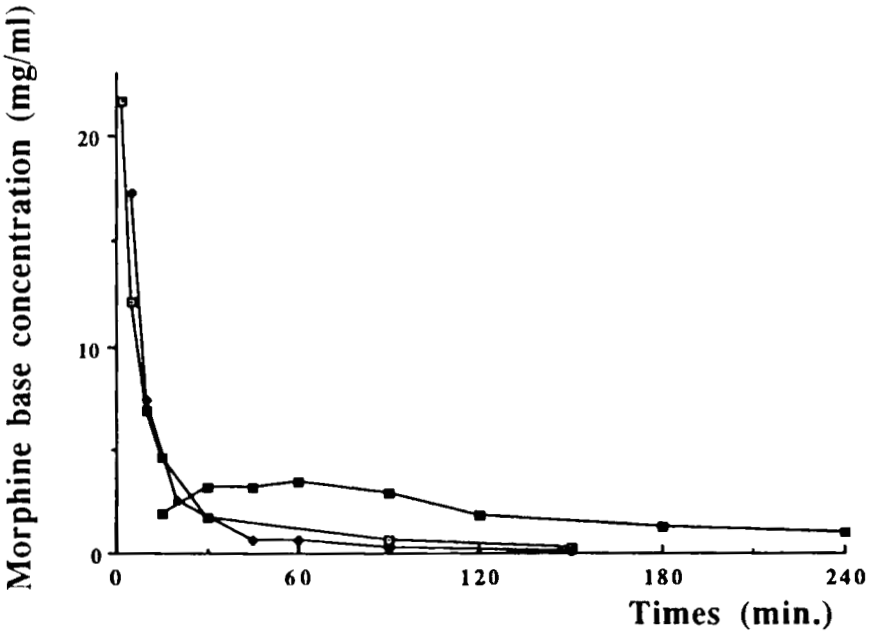

Figure 2

Comparison between plasmatic profiles obtained with the morphine acetate solution (▫) thermoreversible gel (◆) and morphine hydrochloride insert (■). For the sake of clarity error bars have been omitted.

inserts: lacrimal and plasmatic areas under the curve were multiplied by from 1.6 and 2.6, respectively (taking account of the delivered dose). Prolonged release vehicles are assumed to restrict lacrimal drainage in comparison with solution instillation, and conjunctival absorption is supposed to become predominant (28). Our experiments demonstrated the great power of conjunctival absorption in the case of morphine. An other experiment performed by Chast (55), confirmed this observation. This author has blocked lacrimal passage by disposing collodion in the canaliculi and this blocage did not influence the areas under the curve.

CONCLUSION

Mechanisms contributing to systemic passage are influenced by lacrimal drainage. Ophthalmic inserts, by reducing this drainage, have modified the lacrimal and plasmatic kinetics and this vehicle, contrary to the gel, corresponded to an alternative in order to resolve the three main inconvenients of the solution (i.e. high peak magnitude, rapid elimination, and limited bioavailability). Its use could reduce the rythm of administration and general toxicity of morphine just like oral prolonged release device. But, whereas oral vehicles did not improve the relative bioavailability, the inserts have increased strongly this parameter. Taking account of the very weak quantity which could be delivered in the conjunctival cul-de-sac, this last point seemed particularly encourageous.

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