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Systemic absorption of morphine after ocular administration: evaluation of morphine salt insert in vitro and in vivo

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Summary

This work deals with the systemic absorption of morphine occurring after the administration of a morphine salt insert in the conjunctival cul de sac of New Zealand rabbits. The morphine salt inserts were manufactured using hydroxypropylcellulose (HPC) were prepared via direct compression. The release of drug was found to be mostly a diffusion-controlled process and was observed to increase in rate when HPC of lower molecular weight was employed. In vivo release was also due to diffusion of drug molecules through the matrix but was significantly retarded as compared to the in vitro rate (6.45 vs 4.25% $min^{-0.5}$). In contrast, hydration of the insert was observed to take place at a similar rate under both in vitro and in vivo conditions, and therefore does not appear to be the limiting factor in the release processes. The time course determined for the plasma concentrations resulting from the use of morphine hydrochloride-HPC inserts (25 : 75 wt%, 15 mg) was of longer duration than that in the case of a morphine acetate solution and the area beneath the curves was greater with the inserts.

Introduction

In previous work, plasma concentrations of morphine were determined after an ocular administration in the cul de sac of New Zealand rabbits (Chast et al., 1987, 1988). The vehicle used was a 10% aqueous solution of morphine acetate, 10 μl/kg (1 mg/kg) being delivered; these authors observed plasma concentrations of up to 101 ng/ml at the peak magnitude and the absolute bioavailability amounted to about 40%.

The ophthalmosystemic route corresponds to the delivery of a drug into the conjunctival cul de sac with the intention of observing systemic passage. Although the cul de sac has never been used for systemic administration, its possible employment has been considered as an interesting means of delivery which could possess some advantages such as hepatic first past absence, ease of administration, and the eventual withdrawal of vehicle is possible if an insert is used.

Nevertheless, the kinetic profile was characterized by a very high peak magnitude and the decrease in concentrations was very rapid (Chast et al., 1987, 1988). One possibility for avoiding such a profile involves the use of a controlled release vehicle, e.g. biosoluble inserts.

Ophthalmic inserts manufactured with alginate's salt (Loucas and Haddad, 1972; 1976), poly-

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vinylpyrrolidone (Urtti et al., 1985a), modified collagen (Rubin et al., 1973; Bloomfield et al., 1978; Vasantha et al., 1988), fibrin (Miyazaki et al., 1982), and hydroxypropylcellulose (Harwood and Schwartz, 1982; Urtti et al., 1985b; Attia et al., 1988) allow the prolonged release of drugs like pilocarpine, these matrix-associated polymers being biosoluble and not requiring removal from the eyes after use. A biosoluble ophthalmic insert (Lacrisert®, Merck Sharp&Dohme) composed of hydroxypropylcellulose (5 mg) was found to be effective in dry eye syndrome and tolerated well by most patients (Werblin et al., 1981; Lamotte et al., 1985; Lindhal et al., 1988).

The aims of this work were two-fold, namely, (i) the investigation of morphine release and insert hydration in vitro and in vivo with morphine salt-HPC inserts, and (ii) the determination of plasma concentrations using New Zealand rabbits, and comparison with those resulting from a simple morphine acetate solution.

Materials and Methods

Preparation of inserts

The polymers used were two cellulose derivatives: hydroxypropylcellulose (Klucel HF®, molecular weight, 1 115 000; Klucel GF®, molecular weight, 370 000; Aqualon, Wilmington, USA).

Two morphine salts were used: morphine sulphate ($(C_{17}H_{19}NO_3)_2 - H_2SO_4 \cdot 5H_2O$; molecular weight, 758.8; 1 : 21 soluble in water); and morphine hydrochloride ($C_{17}H_{19}NO_3 - HCl \cdot 3H_2O_3$; molecular weight, 375.8; 1 : 24 soluble in water); both salts were purchased from Francopia Laboratories (Paris). 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), followed by the morphine salt being mixed with the polymer using a mixing drum (Turbula, Basel) over a period of 1 h. Each insert was prepared by weighing out 15 mg of the mixture which was then 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.

Morphine salt release in vitro

This procedure had two different purposes, viz., (i) controlling the reproductivity of inserts and (ii) studying the mechanism of release of morphine.

The stirring basket dissolution apparatus (USP XXI, 1985) was used. The solution was an isotonic phosphate buffer (pH 7.3) at 33° C and stirring of the basket was adjusted to 50 rpm. One insert was placed into a stirring basket with 40 ml solution. Samples (3 ml) of the solution were withdrawn at appropriate intervals during a period of 3 h and were replaced by an equal amount of fresh buffer. Morphine hydrochloride release was determined spectrophotometrically at 285 nm. Each evaluation of release data was conducted with 6 inserts.

Four types of morphine salt - HPC inserts were studied with the intention of evaluating the influence of the molecular weight of HPC, morphine salt/HPC ratio and type of morphine salt used. Their respective compositions were as follows: morphine hydrochloride (25%)/high molecular weight HPC (75%) (reference insert); morphine hydrochloride (50%)/high molecular weight HPC (50%); morphine hydrochloride (25%)/low molecular weight HPC (75%); and morphine sulphate (25%)/high molecular weight HPC (75%).

Release data were fitted using a method similar to that of Urtti et al. (1985a,b). Release data were analysed up to an extent of 70% of the total release for 4 types of inserts: the slope of the logarithm of the quantity released vs logarithm of time was determined. When this slope is equal to 0.5, drug release is only governed by the diffusional process (Urtti et al., 1985a,b).

Release data were fitted to the dissolutional cube root (Hixson-Crowell, 1931; Eqn. 1), first-order (Wagner et al., 1974; Eqn. 2) and diffusional

square root of time dependence equations (Touitou and Donbrow, 1982; Eqn. 3):

$$\sqrt[3]{100} - \sqrt[3]{m} = bt + a \quad (1)$$

$$\ln(m) = -bt + a \quad (2)$$

$$100 - m = b\sqrt{t} + a \quad (3)$$

where m designates the percentage of undissolved morphine salt at time t, a denotes the intercept with the y-axis and b is the slope. Statistical differences between the 4 release rates were analysed with the Dunnett test which was conducted using as a reference the release data obtained from the reference inserts (degree of freedom = 20, $p = 3$).

Hydration of inserts in vitro

Hydration was evaluated with reference inserts. Each insert was put into a stirring basket with 40 ml solution for a period of 3 h under the same conditions as above. At fixed time points, each insert was removed and weighed. Each determination, for every time point, was made with 3 different inserts. The water content in the hydrated matrix was evaluated taking into account the weight increment and the morphine released; preliminary determination indicated that HPC dissolved no more than 5% until the third hour, therefore polymer dissolution was considered negligible in comparison to the weight of absorbed water.

Morphine salt release and insert hydration in vivo

In vitro drug release is generally used in order to evaluate vehicle efficiency and the rate of drug dissolution from ocular matrices (Harwood and Schwartz, 1982; Miyazaki et al., 1982; Saettone et al., 1984; Urtti et al., 1985a; Habib and Attia, 1986). Nevertheless, in vitro conditions are very different from those pertaining to the physiological characteristics of the tear film and the anatomy of the conjunctival cul de sac. Morphine release and insert hydration were studied in the cul de sac with the aim of specifying in vivo processes and comparing them with the in vitro results.

Reference inserts were placed in the lower cul de sac of 6 New Zealand rabbits, removed at the appropriate time point, and then weighed. Residual morphine salt content was calculated according to a spectrophotometric method at 285 nm after dissolution in distilled water. In vivo hydration was evaluated with the weight increment and quantity of residual morphine being taken into account.

Morphine concentrations in plasma

Six New Zealand rabbits received a reference insert in the cul de sac. Blood samples were collected from the ear artery at fixed intervals during a period of 12 h, then centrifuged and frozen at $-25\,^{\circ}$C. Concentrations of morphine base vs time were determined via a radioimmunoassay procedure, using highly selective antibodies and [^{3}H]morphine, as described elsewhere (Chast et al., 1987). Various pharmacokinetic parameters such as the peak magnitude (C_{max}), peak delay (T_{max}) and area under the curve (using the trapezoidal rule) were calculated and compared with the results obtained by Chast et al. for a simple morphine acetate solution.

Results and Discussion

Morphine salt release in vitro

The percentage of morphine released vs the square root of time is depicted for the 4 types of insert in Figs. 1–3, the principal release parameters being summarized in Table 1. Release was found to reach completion within 4 h.

The K values were close to 0.5, therefore Fickian diffusion appeared to represent the principal mechanism of release. Nevertheless, a slight deviation from 0.5 was observed; this deviation has also been reported with pilocarpine-HPC inserts and is due to polymer relaxation (Urtti et al., 1985a,b) (Table 1).

The best fit to the release data was obtained with the diffusional equation (Eqn. 3; $0.996 < r < 0.998$) in comparison with the Hixson-Crowell equation (Eqn. 1; $0.991 < r < 0.997$) or Wagner

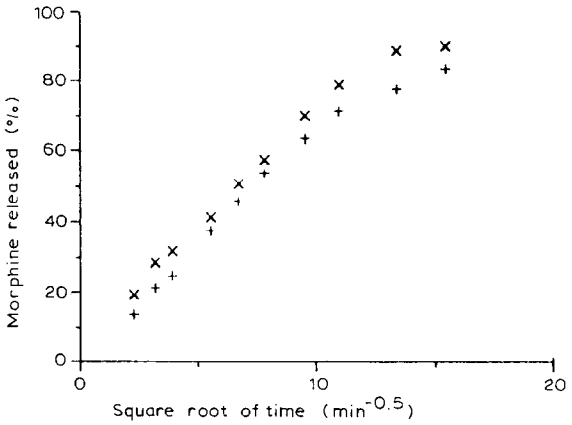

Fig. 1. Morphine released (%) vs square root of time (min$^{-0.5}$) obtained with the reference inserts (+) and inserts made with 50% morphine hydrochloride and 50% high molecular weight HPC (×). For the sake of clarity error bars have been omitted.

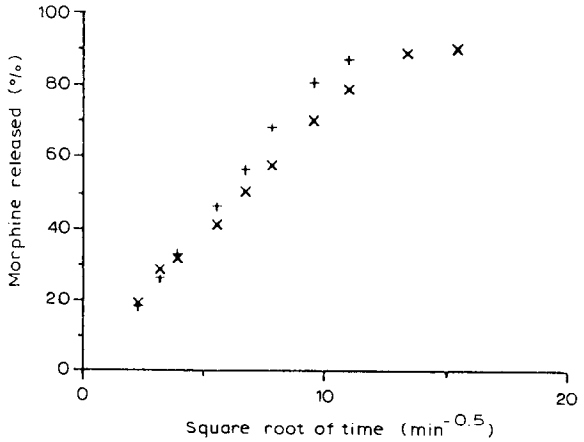

Fig. 2. Morphine released (%) vs square root of time (min$^{-0.5}$) obtained with the reference inserts (×) and inserts made with 25% morphine hydrochloride and 75% low molecular weight HPC (+). For the sake of clarity error bars have been omitted.

equation (Eqn. 2; $-0.998 < r < -0.991$) (Table 1). A certain degree of dependence on the square root of time was consistent with the results obtained by many authors, with inserts of pilocarpine-cellulose derivative (Harwood and Schwartz, 1982; Saettone et al., 1984; Urtti et al., 1985b; Habib and Attia, 1986).

On using HPC of lower molecular weight, the release rate was significantly increased ($p < 0.01$), as observed previously (Harwood and Schwartz, 1982) with pilocarpine-HPC films. Replacing hydrochloride by sulphate had little effect on the results, being a consequence of the similar solubilities of the two salts (Fig. 2). The increment of the morphine hydrochloride/HPC ratio (from 25 : 75 to 50 : 50 wt%) did not change the release rate, this finding being indicative of a diffusional process (Fig. 3).

Therefore, release of morphine salt was mainly governed by a diffusional mechanism and was very similar to that of pilocarpine salt. This similarity arose from the hydrophilic nature of both molecules. In vitro release was influenced by the molecular weight of HPC and, probably, by the solubility of morphine salt.

TABLE 1

Releaase parameters obtained with the 4 types of inserts

	Morphine salt (%)/molecular weight (low/high) of HPC			
	HCl (25%)/low	HCl (25%)/high	HCl (50%)/high	H_2SO_4 (25%)/high
K (±S.D.)	0.51 ± 0.01	0.42 ± 0.04	0.51 ± 0.02	0.45 ± 0.03
Diffusional equation (Eqn. 3)				
r	0.998	0.998	0.998	0.996
b (slope, ±S.D.) (% min$^{-0.5}$)	8.9 ± 0.4 *	6.45 ± 0.3	6.44 ± 0.3	7.6 ± 0.2 *
Hixson-Crowell equation (Eqn. 1)				
r	0.997	0.991	0.992	0.991
Wagner equation (Eqn. 2)				
r	−0.998	−0.995	−0.997	−0.991

* $p < 0.01$.

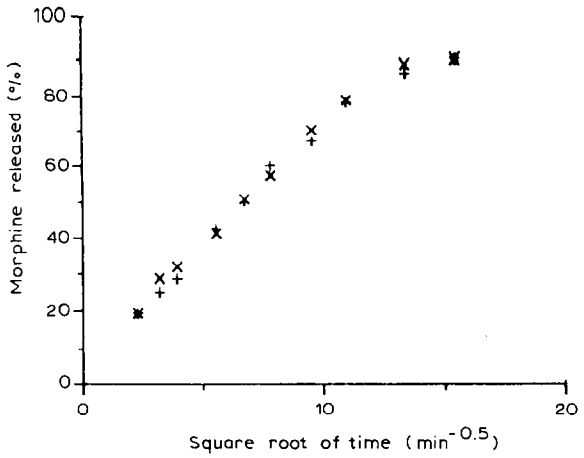

Fig. 3. Morphine released (%) vs square root of time ($min^{-0.5}$) obtained with the reference inserts (+) and inserts made with 25% morphine sulphate and 75% high molecular weight HPC (×). For the sake of clarity error bars have been omitted.

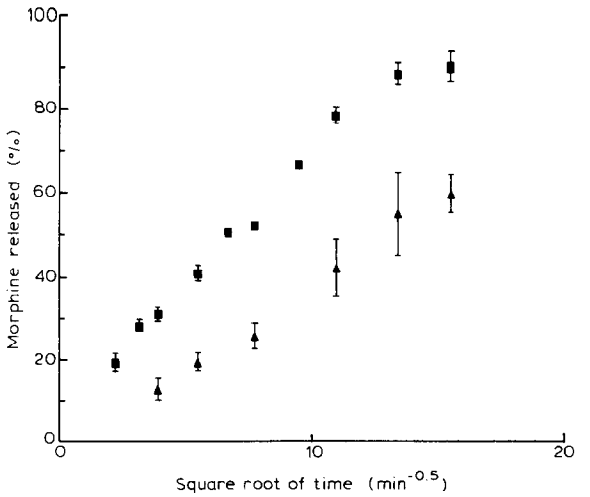

Fig. 4. Morphine released (%) vs square root of time ($min^{-0.5}$) in vitro (■) and in vivo (▲) (±S.D.).

In vivo morphine release

The best fit of the release data was obtained with the expression for the diffusional square root of time dependence ($r = 0.996$) as compared with the Hixson-Crowell equation ($r = 0.991$) or Wagner equation ($r = -0.994$). K was equal to 0.57. Hence, as for the in vitro assay, in vivo morphine release was essentially due to diffusion of the molecules.

Nevertheless, release differed significantly at all time points on the basis of the Mann-Whitney U-test ($p < 0.01$). The rate of release decreased from 6.45 (in vitro) to 4.25% $min^{-0.5}$ (in vivo). The $T_{50\%}$ (time required to release 50% of the total quantity) was lengthened from 45 min (in vitro) to 165 min (in vivo) (Fig. 4).

This decrease probably resulted from differences in experimental procedures. The omission of stirring, or a small volume of the tear film may disturb the release mechanism. Moreover, diffusion is linearly related to the area of surface exchange between the vehicle and solution (Urtti et al., 1985a,b); in vivo, the area of surface exchange between the insert and the lachrymal film was affected by contact with the conjunctival mucosa; consequently, this could involve a marked decrease in release rate. To our knowledge, however, studies of drug release from an insert in the conjunctival cul de sac are sparse in number. This investigation has pointed out the great differences between in vitro and in vivo procedures. In vitro studies should be restricted to the control of inserts; in contrast, our method of in vivo evaluation allows us to define the availability of the drug in the cul de sac before systemic absorption. Nevertheless, in vivo study is not always possible, in particular when inserts are rapidly dissolved in the cul de sac (for example, with sodium alginate (Loucas and Haddad, 1972)). Consequently, it would be interesting to devise an in vitro system that more closely approaches in vivo conditions.

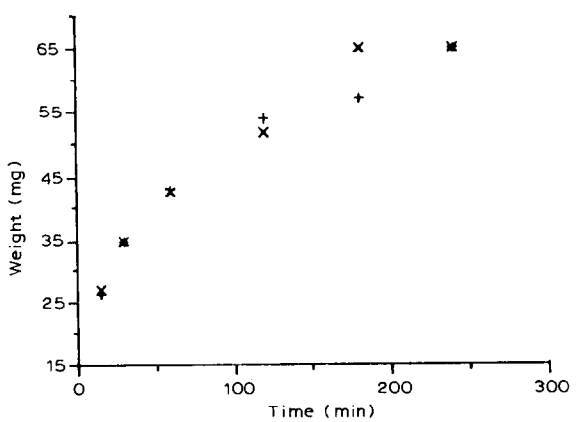

Fig. 5. Weight increment obtained with reference inserts in vitro (+) and in vitro (×). For the sake of clarity error bars have been omitted.

TABLE 2

In vitro and in vivo hydration

Time (min)	Hydration (mg)	
	In vitro	In vivo
15	12.7	13.2
30	21.5	19.7
60	30.2	29.2
90	33.6	29.7
120	41.9	37.1
180	51.3	49
240	53.3	55

This implies a less significant solution volume and a reduction in the area of surface exchange.

Insert hydration in vitro and in vivo

Any significant differences in the plots of the weight increment in vitro and in vivo vs time, were indicated by the Mann-Whitney U-test (Fig. 5). Likewise, hydration in vitro and in vivo was very similar (Table 2). In view of these results, hydration does not represent the limiting factor in morphine release in the cul de sac.

Determination of plasma drug concentrations

The kinetic profiles resulting from delivery of reference inserts were strongly modified in comparison with morphine acetate solution (Chast et al., 1988) (Fig. 6).

T_{max} was lengthened from 16.8 to 135 min and C_{max} was reduced from 105.5 to 36.6 ng/l (Table 3). The area beneath the curve increased by 52%, although the quantity of morphine administered was only 16% higher. Thus, the absolute bioavailability seemed to improve with inserts. Likewise, Urtti et al. (1985b) reported prolongation of the kinetics and a decrease in peak magnitude when pilocarpine-HPC inserts were used instead of the solution.

Drug absorption after ophthalmosystemic delivery is due to differences between the sites, particularly the conjunctival mucosa. Nasolachrymal and gastrointestinal absorption is considered to be minor and is due to drainage from the conjunctival fornix via the canaliculi to the lachrymal sac and to the nasolachrymal duct (Sieg and Robinson, 1976). Few studies have dealt with the influence of the ophthalmic vehicle on systemic absorption. Vehicles may modify the availability of drug to absorption sites. In particular, nasolachrymal drainage may be altered, but further studies are needed to clarify the mechanisms of absorption after delivery to the conjunctival cul de sac with regard to the vehicle used. The main difference between solution and insert delivery concerned the availability of the drug to administration sites.

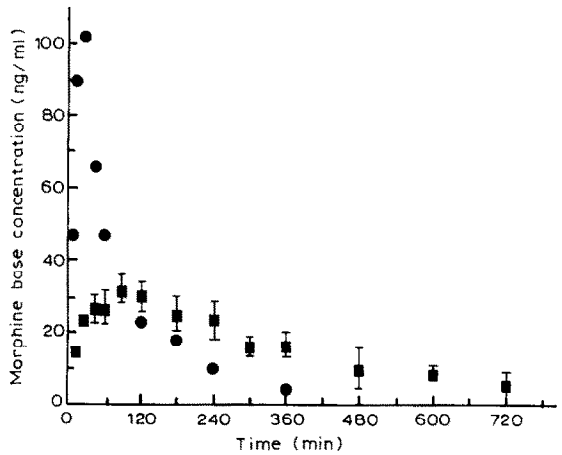

Fig. 6. Morphine concentration vs time resulting from morphine acetate solution (●) and insert (■) (±S.E.).

With the solution, absorption results from the conjunctival mucosa and other absorption sites. A

TABLE 3

Comparison between pharmacokinetic parameters obtained with reference inserts and morphine acetate solution

	Morphine acetate solution	Reference inserts
Number of rabbits (n)	11	6
Morphine administered (mg/kg) (in morphine base equivalent)	0.76	0.88
T_{max} (min) (±S.E.)	16.8±8.8	135±26
C_{max} (ng/ml) (±S.E.)	101.1±28.4	36.6±4.8
Area under curve (ng min ml^{-1}) (±S.E.)	8289±955	12617±1470

large quantity of drug being administered implies the rapid saturation of conjunctival sites, therefore, a significant proportion of drug is susceptible to drainage via the nasolachrymal duct to other absorption sites. Moreover, the loss of drug is not negligible, and is a consequence of overflow problems and eyelid-blinking and tear reflexes.

In contrast, with controlled release vehicles, the loss of drug is diminished and drug availability in the lachrymal fluid is more regular; absorption from the conjunctival mucosa is probably increased and, as observed in our sudy, absolute bioavailability is improved.

Conclusion

In view of this work, HPC could be considered as a useful polymer in order to prolong in vitro and in vivo release. Work is now in progress with the aim of clarifying the morphine absorption sites after cul de sac administration and the influence of the vehicle.

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