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Ocular distribution of new Hydrogel based on Xanthan Gum vs Eye drops containing Netilmicin and Dexamethasone

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PURPOSE

The aim of this work was to compare the *in vivo* ocular distribution of two preservative free formulations, the new xanthan gum hydrogel vs the traditional eye drops (reference group), both containing 0.1% dexamethasone (DEX) and 0.3% netilmicin (NET).

MATERIAL AND METHODS

Animals:

A total of 120 New Zealand White rabbits (60 males and 60 females) were supplied by Francucci Enzo, Rieti, Italy. Animals weighed approximately 1.89-2.61kg (males) and 2.01-2.53 kg (females) were 7 to 11 weeks old. Female animals were nulliparous and non-pregnant. An acclimatisation period of 25-27 days was allowed before treatment, during this period the health status of the animals was assessed by through observations. On the day of allocation all animals were weighed and allocated to the 2 groups by computerised stratified randomisation to give approximately equal initial group mean body weights.

Treatment and sampling

The two groups of treatment, (the new xanthan hydrogel vs the reference item eye drops) consisted of 28 male and 28 female rabbits. The test item (the new xanthan hydrogel) or reference item (eye drops) formulations were administered to the animals of the relevant group by direct introduction into the right eye. A volume of 50 µl (approximately 1 drop) of the indicated items was introduced into the lower conjunctival sac with a graduated pipette (Gilson), previously calibrated for the gel. The eye was held closed for a few seconds to prevent loss of the item and to aid distribution over the surfaces of the eye and conjunctival membranes. The left eye of all animals remained untreated and acted as a control.
Sampling was performed approximately at 15, 30, 60 minutes and 2, 4, 6 and 12 hours following the administration of the items, relevant animals being killed at each time point. The 7 time points corresponded to the 7 sub-groups (groups 1 and 2). Immediately on killing the animal the external surfaces of the eye (including the cornea and conjunctivae) and surrounding skin/fur were washed to remove residual substance. The aqueous humour was collected from treated eyes by use of a syringe and hypodermic needle. This structure was washed with isotonic saline to remove adherent aqueous humour and placed into labelled tubes. The cornea and conjunctivae were then separated, washed with isotonic saline to remove adherent aqueous humour and placed into labelled tubes. The vitreous was removed and placed into labelled tubes. The retina was removed and placed into labelled tubes. Blood samples, of 2 ml each, were taken from the animals and placed into heparin anticoagulant tube.
A number of untreated eyes, from stock animals, previously maintained in dry ice during collection, were sampled as described above to provide sufficient material to establish baseline analytical data.
All samples were stored frozen at approximately -21°C pending analysis.

Chemical analysis

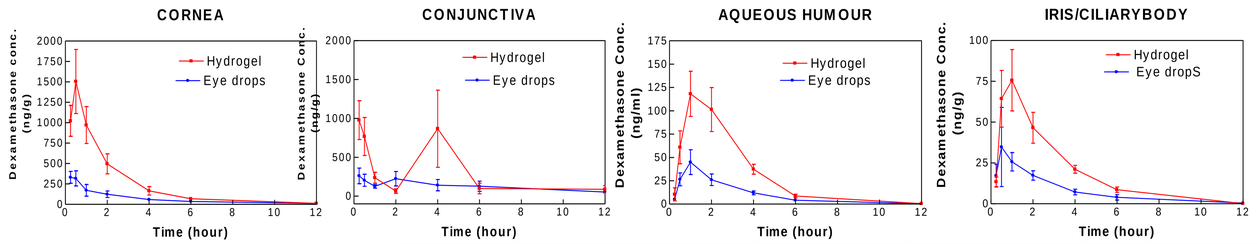
Netilmicin: HPLC/UV methods suitable to determine the antibiotic in aqueous humour, conjunctivae and cornea from rabbits were established. No analysis of netilmicin was performed in plasma samples because no levels of this active ingredient were detected in a previous study.
Dexamethasone:Suitable LC/MS-MS methods in order to determine the content of dexamethasone alcohol in aqueous humour, conjunctivae, cornea, iris/ciliary body and plasma from rabbits, were established.

PK analysis

The following pharmacokinetic parameters were calculated according to standard non compartmental analysis:
C_{max}: maximum observed concentration
T_{max}: time to C_{max}
$AUC_{0-\infty}$: area under the concentration-time curve calculating the extrapolation to infinity by the logarithm trapezoidal rule

RESULTS

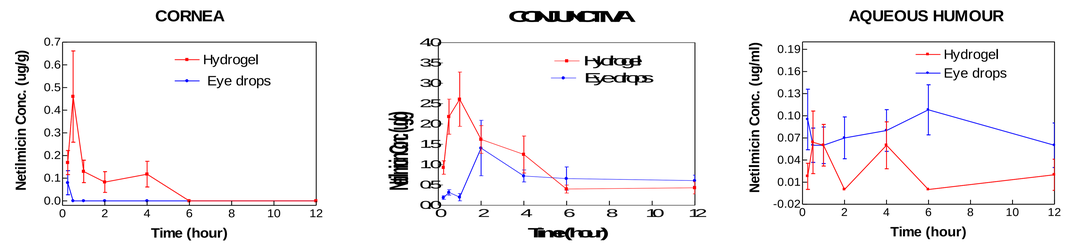
Dexamethasone PK profile



Treatment	Tissues	T_{max} (h)	C_{max} (ng/g-ml)	AUC_{0-12h} ± SEM (ngxhour/g-ml)
HYDROGEL	cornea	0.5	1505.8 ± 392.4*	2658.1 ± 123.9*
	conjunctivae	0.25	977.2 ±249.2*	2778.7 ± 522.6*
	aqueous humour	1	118.3 ± 24.2*	348.5 ± 14.6*
	iris/ciliary body	1	75.7 ±18.8*	209.0 ± 7.6*
	plasma	2	6.3 ± 1.5*	28.6 ± 1.6*
EYEDROPS	cornea	0.25	331.1 ± 72.5	702.6 ± 38.9
	conjunctivae	0.25	260.7 ± 100.5	1138.8 ± 83.8
	aqueous humor	1	45.0 ± 13.2	116.8 ± 5.2
	iris/ciliary body	0.5	52.3 ± 33.6	92.4 ± 5.4
	plasma	2	12.6 ± 1.8	56.55 ± 3.0

*Student's t test ($p<0.05$), n=8

Netilmicin PK profile



Treatment	Tissues	T_{max} (h)	C_{max} (ng/g-ml)	AUC_{0-12h} ± SEM (ngxhour/g-ml)
HYDROGEL	cornea	0.5	0.46 ±0.2*	0.45 ± 0.07*
	conjunctivae	1	2.61 ± 0.7*	10.50± 0.70
	aqueous humour	NA	NA	0.51 ± 0.07
EYEDROPS	cornea	NA	<LOQ	NA
	conjunctivae	2	1.41 ± 0.7	8.24 ± 0.63
	aqueous humor	6	0.11 ± 0.03	0.95 ± 0.10

NA: Not Applicable $LOQ_{cornea:}$ 0.205 mg/g *Student's t test ($p<0.05$), n=8

CONCLUSIONS

Briefly, analysis of the data indicates a statistically significant increase of DEX concentrations after treatment with the hydrogel formulation in cornea, conjunctivae, aqueous humour and iris/ciliary body when compared to that of the eye drops. Consequently, a statistically significant decrement of DEX was detected in plasma in animals treated with the hydrogel respect to the reference group. Analysis of NET content revealed a statistically significant increase in the cornea and conjunctivae of animals treated with the hydrogel up to 4 and 1 hour respectively.

In conclusion, we observed that Xanthan hydrogel formulation shows an increment in the absorption of DEX and NET in all ocular tissues with respect to the eye drops. Moreover, a significant reduction of systemic uptake of dexamethasone following a single ocular administration of the hydrogel was observed. Furthermore, analysis of NET showed a statistically significant increment in cornea and conjunctivae of hydrogel treatment group with respect to the eye drops group. This effect is due to the Xanthan gum mucoadesivity (ref.1) properties that allow a better adherence of the gel to the ocular surface. Therefore, it can be explored a possible reduction of the dose when using xanthan hydrogel that will be tested further in clinical studies.

Reference:
1. C. Civiale et al; IOVS, 2006; Vol.47 Issue 13