

IN VIVO AND OCULAR SAFETY STUDY OF DORSOLAMIDE HCL OCULAR INSERT

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Summary

Dorsolamide HCl is a carbonic anhydrase inhibitor widely used in the treatment of glaucoma available in the form of conventional eye drop form. This conventional dosage form is facing certain drawbacks like poor bioavailability, tear turnover, lacrymal drainage and conjunctival absorption. Optimized formulation was evaluated for ocular safety study, *In vivo* release study, *In vivo* intra ocular pressure lowering activity. The results revealed that the optimized ocular insert was safe for ocular administration and showed better *in vivo* intra ocular pressure lowering activity compare to eye drop and control.

Key Words: Dorsolamide HCl, Glaucoma, Ocular

Introduction

Glaucoma is the second cause of vision loss in the world. They are now an estimated 12 million people affected by glaucoma in India [1] and 60.5 million in the world and by 2020 this is expected to be 16 million in India and 79.6 million in the world [2]. Improved methods of screening and drug delivery systems are the urgent need to address this issue. Dorsolamide HCl is a topically active carbonic anhydrase inhibitor developed to circumvent the side effects of acetazolamide [3]. Its ocular formulation has been marketed in USA since 1995 [3]. Dorsolamide HCl available in conventional eye drop and widely used in treatment of glaucoma. But this conventional dosage form is suffering from inherent drawbacks like patient has to take several times [4], allergic reaction [5], limited bio availability [6], tear dilution, solution drainage [7], tear turnover and conjunctival absorption [8]. Here an attempt was made to circumvent the drawbacks associated with conventional dosage form.

Materials and Methods

Dorsolamide HCl was received as gift sample from INTAS pharmaceutical, Ahmedabad, Dialysis membrane was procured from Himedia laboratories, schioetz tonometer. Other chemicals and solvents used in this study were of analytical grade.

OCULAR SAFETY STUDY

To carry out animal study permission was taken from institutional animal ethical committee of Sumandeep Vidyapeeth (947/ac/06/CPCSEA). The sterile optimized formulation will be placed in one eye of each rabbit by gently pulling the lower eyelid. The eyelids were then being gently held together for one second and animal will be released. The other eye remaining untreated was served as the control. The eye of each rabbit will be examined 24, 48 and 72 hrs. After treatment for irritation, inflammation by necked eye or by means of pen torch. At the time of examination period rabbits will be scored for ocular reaction as per standards mentioned in Table 1, 2 and 3. Total three animals taken in this group [9].

IN VIVO RELEASE STUDY

To carry out animal study permission was taken from institutional animal ethical committee of Sumandeep Vidyapeeth (947/ac/06/CPCSEA). Total seven Albino rabbit of either sex was used in the experiment weighing 2.5 to 3.5 Kg. Inserts were sterilized by using UV radiation for one to two minutes before the study. Albino rabbits of either sex were selected for experiment. The animals were housed in individual cages and customized to laboratory condition for a day and received free access to food and water. On the day of experiment the sterilized inserts were placed into the lower *cul-de-sac* of rabbits. The inserts were inserted into one eye of seven rabbits. After 1, 2, 4, 6, 10, 22 and 24 hrs, the inserts were carefully removed and analyzed for remaining drug content by UV Spectrophotometer. The amount of drug release was calculated by subtracting the remaining amount of drug from initial amount of drug. Observations for any fall out of insert were also recorded throughout the experiment[10].

IN VIVO INTRA OCULAR PRESSURE LOWERING ACTIVITY

To carry out animal study permission was taken from institutional animal ethical committee of Sumandeep Vidyapeeth (947/ac/06/CPCSEA). *In vivo* intra ocular pressure lowering activity of selected Ocusert preparation of Dorsolamide HCl was studied in normotensive albino rabbits of either sex weighing 2 to 3.5 Kg. The animals were housed under well controlled conditions of temperature (22 ± 2 °C), humidity ($55 \pm 5\%$) and 12/12 – h, light-dark cycle, was given access to food and water. To induce acute glaucoma, 5% dextrose solution (15 ml/kg) was intravenously infused through marginal ear vein. The basal intraocular pressure was measured by schiøtz tonometer. The drug formulations were placed at *cul-de-sac* to rabbits. Total 12 rabbits were divided into three groups each contains 4 rabbits. In first group marketed preparation of Dorsolamide HCl eye drop equivalent to 0.45 mg was administered. In second group placebo film was inserted which acted as control. In third group medicated film of Dorsolamide HCl was inserted into lower *cul de sac* of rabbits. In all cases the preparation was administered after 15 minutes of dextrose injection. The intraocular pressure (IOP) changes were recorded every 30 min till the pressure difference between the control eye and treated eye is zero. Intraocular pressure (IOP) was measured by tonometry method with the help of schiøtz tonometer and mean was taken at three times fixed interval. All IOP measurements were carried out by the same operator, using same schiøtz tonometer. Each rabbit was given washout period of three days after every treatment. The ocular hypotensive activity was expressed as the average difference in IOP between the treated and control eye of the same rabbit[11,12].

Results and Discussion

OCULAR SAFETY STUDY OF DORSOLAMIDE HCL OCULAR INSERT

Table 1: Twenty fourth Hour Scores for Grading the Severity of the Ocular Irritation of Dorsolamide HCl ocusert

Sr. No.	Observations		Rabbit No.		
			1	2	3
1	CORNEA		1	2	3
	A	Opacity - Degree of density (area which is more dense taken for reading)	0	0	0
		Scattered or diffused area-details of iris clearly visible			
		Easily discernible translucent areas, details of iris slightly obscured			
		Opalescent areas, no details of iris visible, size of pupil barely discernible			
		Opaque, iris invisible			
	B	Area of cornea involved	0	0	0
		One quarter (or less) but not zero			
		Greater than one quarter-less than one-half			
		Greater than one half less than three quarters			
		Greater than three quarters up to whole area			
		Score equals – A×B×5 Total possible maximum = 80	0	0	0
2	IRIS				
	A	Values	0	0	0
		Folds above normal, congestion, swelling, circumcorneal injection (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)			
		No reaction to light hemorrhage; gross destruction (if any or all of these)			
		Score equals – A×5 Total possible maximum = 10	0	0	0
3	CONJUNCTIVA				
	A	Redness (refers to palpebral conjunctiva only)	0	0	0
		Vessels definitely injected above normal			
		More diffuse, deeper crimson red, individual vessel not easily discernible			
		Diffuse beefy red			
	B	Chemosis	0	0	0
		Any swelling above normal (include nictitating membrane)			
		Obvious swelling with partial aversion of the lids			
		Swelling with lids about half closed			
		Swelling with lids about half closed to completely closed			
	C	Discharge			
		Any amount different from normal (doesn't include small amount observed in inner cul-de-sac)	0	0	0
		Discharge with moistening of the lids and hairs just adjacent to the lids			
		Discharge with moistening of the lids and considerable area around the eye			
		Score equals – (A+B+C)×2 Total possible maximum = 20	0	0	0

Table 2: Thirty sixth hour Scores for Grading the Severity of the Ocular Irritation of Dorsolamide HCl ocusert

Sr. No.	Observations				
1	CORNEA		1	2	3
	A	Opacity - Degree of density (area which is more dense taken for reading)	0	0	0
		Scattered or diffused area-details of iris clearly visible			
		Easily discernible translucent areas, details of iris slightly obscured			
		Opalescent areas, no details of iris visible, size of pupil barely discernible			
		Opaque, iris invisible			
	B	Area of cornea involved	0	0	0
		One quarter (or less) but not zero			
		Greater than one quarter-less than one-half			
		Greater than one half less than three quarters			
		Greater than three quarters up to whole area			
		Score equals – A×B×5 Total possible maximum = 80	0	0	0
2	IRIS				
	A	Values	0	0	0
		Folds above normal, congestion, swelling, circumcorneal injection (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)			
		No reaction to light hemorrhage; gross destruction (if any or all of these)			
		Score equals – A×5 Total possible maximum = 10	0	0	0
3	CONJUNCTIVA				
	A	Redness (refers to palpebral conjunctiva only)	0	1	1
		Vessels definitely injected above normal			
		More diffuse, deeper crimson red, individual vessel not easily discernible			
		Diffuse beefy red			
	B	Chemosis	0	0	0
		Any swelling above normal (include nictitating membrane)			
		Obvious swelling with partial aversion of the lids			
		Swelling with lids about half closed			
		Swelling with lids about half closed to completely closed			
	C	Discharge	0	0	0
		Any amount different from normal (doesn't include small amount observed in inner cul-de-sac)			
		Discharge with moistening of the lids and hairs just adjacent to the lids			
		Discharge with moistening of the lids and considerable area around the eye			
		Score equals – (A+B+C)×2 Total possible maximum = 20	0	2	2

Table 3: Seventy two hour Scores for Grading the Severity of the Ocular Irritation of Dorsolamide HCl ocusert

Sr. No.	Observations				
1	CORNEA		1	2	3
	A	Opacity - Degree of density (area which is more dense taken for reading)	0	0	0
		Scattered or diffused area-details of iris clearly visible			
		Easily discernible translucent areas, details of iris slightly obscured			
		Opalescent areas, no details of iris visible, size of pupil barely discernible			
		Opaque, iris invisible			
	B	Area of cornea involved	0	0	0
		One quarter (or less) but not zero			
		Greater than one quarter-less than one-half			
		Greater than one half less than three quarters			
		Greater than three quarters up to whole area			
		Score equals – A×B×5 Total possible maximum = 80	0	0	0
		Total Score			
2	IRIS				
	A	Values	0	0	0
		Folds above normal, congestion, swelling, circumcorneal injection (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)			
		No reaction to light hemorrhage; gross destruction (if any or all of these)			
		Score equals – A×5 Total possible maximum = 10	0	0	0
		Total Score			
3	CONJUNCTIVA				
	A	Redness (refers to palpebral conjunctiva only)	1	1	1
		Vessels definitely injected above normal			
		More diffuse, deeper crimson red, individual vessel not easily discernible			
		Diffuse beefy red			
	B	Chemosis	0	0	0
		Any swelling above normal (include nictitating membrane)			
		Obvious swelling with partial aversion of the lids			
		Swelling with lids about half closed			
		Swelling with lids about half closed to completely closed			
	C	Discharge	0	0	0
		Any amount different from normal (doesn't include small amount observed in inner cul-de-sac)			
		Discharge with moistening of the lids and hairs just adjacent to the lids			
		Discharge with moistening of the lids and considerable area around the eye			
		Score equals – (A+B+C)×2 Total possible maximum = 20	2	2	2
		Total Score			

24th Hour36th Hour72nd Hour

Figure 1: Eye irritancy test of Dorsolamide Hydrochloride ocular insert

The ocular safety study observations are summarized in table – 1, 2 and 3. The ocular safety score of the optimized formulation was found to be 2 at the end of 72 hours and therefore, considered as practically not irritating. Thus, it can be concluded that they were safe for ocular administration.

IN VIVO RELEASE STUDY OF DORSOLAMIDE HCL OCULAR INSERT

Table 4: *In Vivo* Drug Release Data of Optimized Dorsolamide HCl ocular insert.

Time (hrs)	% drug released	<i>In vitro</i> % drug released
1	4.01±0.35	3.26±0.35
2	8.1±0.52	6.7±0.51
4	15.8±0.48	13.01±0.79
6	23.56±0.89	20.36±0.87
10	39.25±1.15	35.26±1.01
22	90.78±0.45	86.47±0.85
24	100.01±0.69	95.56±0.76

Labeled Claim=0.450 mg

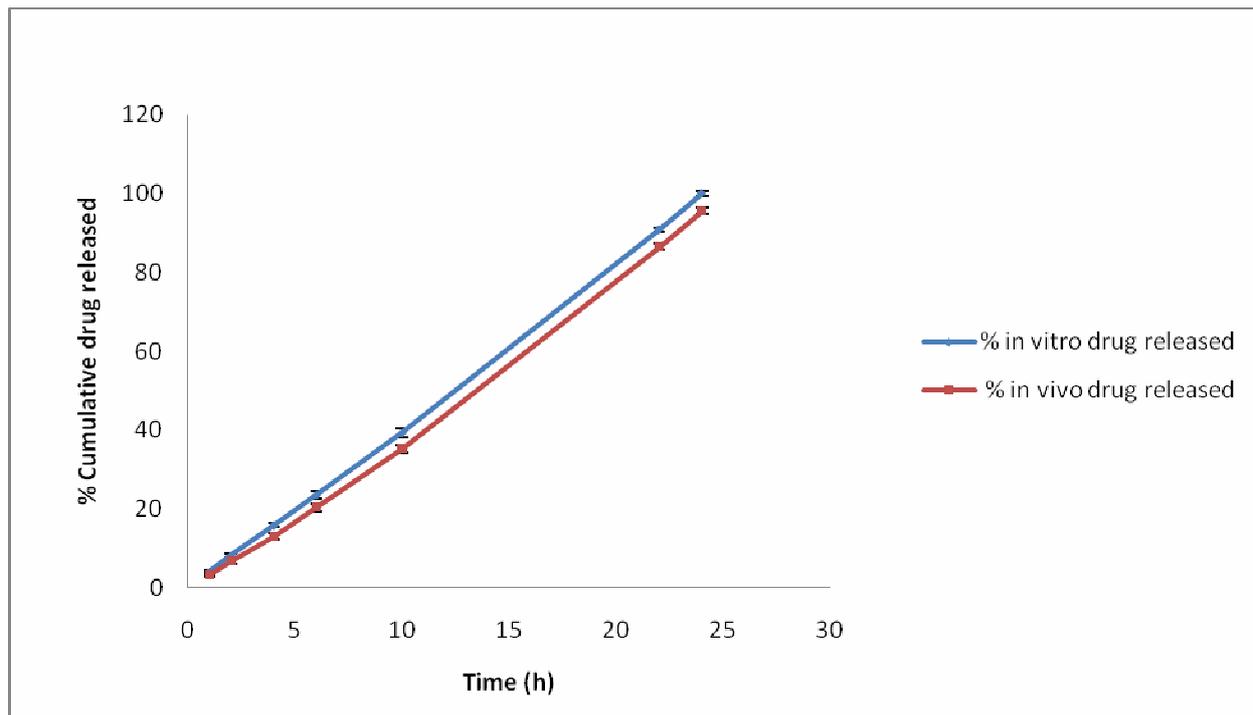


Figure 2 : In Vitro – In Vivo Correlation for optimized Dorsolamide HCl ocular insert.

The results of *in vivo* release study of the optimized formulation is shown in Table 4 and figure 2. The ocusert showed 95.56% of drug release after 24 hours which was comparable to *in vitro* drug release (table 4). Thus there was good *in vitro* – *in vivo* correlation for the optimized formulation (figure - 2) indicating the effectiveness of the formulation to be used *in vivo*.

INTRAOCULAR PRESSURE LOWERING ACTIVITY

Table 5: Intraocular pressure lowering activity of Dorsolamide HCl ocular insert.

Time (Min)	Control* (mmhg)	Marketed* (mmhg)	Ocusert* (mmhg)
0	33.1±0.10	33.0±0.20	33.1±0.14
30	33.2±0.25	30.4±0.10	30.4±0.19
60	32.8±0.16	23.8±0.25	26.1±0.25
90	32.7±0.36	17.0±0.29	22.3±0.37
120	33.1±0.41	15.4±0.25	20.1±0.45
150	33.1±0.20	18.5±0.16	16.2±0.35
180	32.8±0.13	23.2±0.42	15.5±0.17
210	32.7±0.19	23.8±0.25	15.5±0.18
240	27.1±0.29	17.3±0.25	14.1±0.42
270	26.9±0.25	17.0±0.38	14.1±0.32
300	24.8±0.23	15.6±0.10	12.9±0.14
330	24.5±0.15	15.6±0.19	12.9±0.16

Mean ± SD Mean ± SD (*n=3)

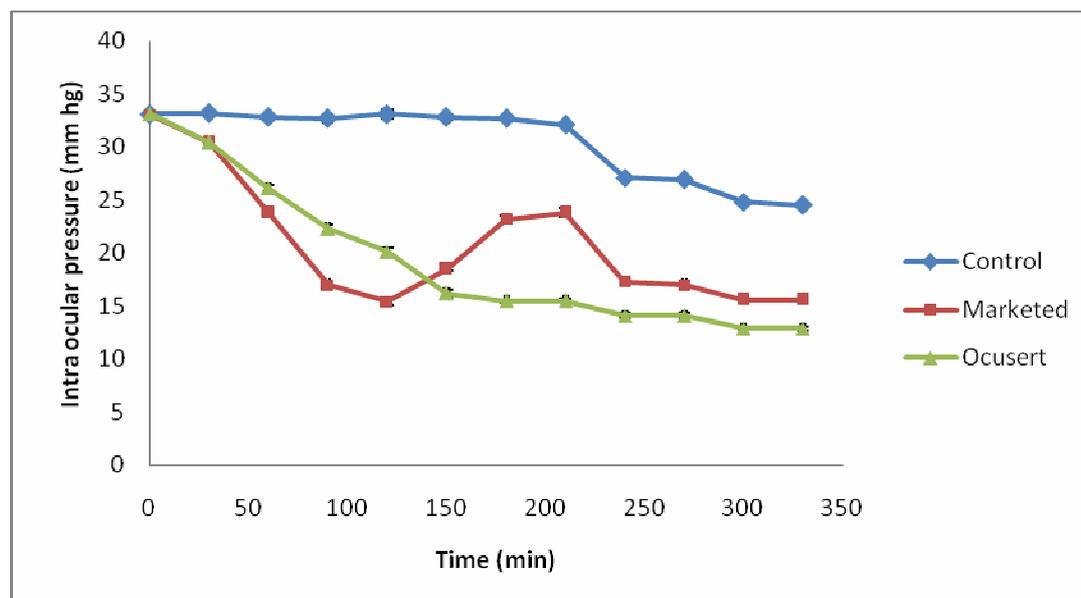


Figure 3: Intraocular pressure lowering activity of Dorsolamide HCl ocular insert.

Dorsolamide HCl prevented acute rise in the intraocular pressure induced by intravenous administration of 5 % 15 ml/kg of dextrose solution. The extent of IOP lowering activity was found to be better with ocuser compared to marketed product and control. Whereas for marketed product the effect was observed immediately but could not sustain for long duration. It was also observed that at the end of 240 min the effect of all the formulation was found to be nil as it was evident from the figure 3.

Conclusion

It was concluded that prepared Dorsolamide HCl ocular insert is safe for ocular administration as it provide good in vitro in vivo correlation and better in vivo intra ocular pressure lowering activity. Still it is required to evaluate its efficacy by other clinical study.

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