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IN VIVO EVALUATION OF AZAPROPAZONE CYCLODEXTRIN COMPLEXES FROM OPHTHALMIC FORMULATIONS / OCULAR TOLERANCE AND DISPOSITION IN EYE TISSUES

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تناول هذا البحث دراسة تأثير السيكلودكسترينات (البيتاسيكلودكسترين والهيدروكسى بروبيل بيتاسيكلودكسترين) على الإتاحة الحيوية للدواء فى الأنسجة المختلفة لعيون الأرانب (الملتحمة ، القرنية ، القزحية–الجسم الهدبى والسائل المائى) فى أشكال صيدلية مختلفة مثل القطرات ، المستحضرات الهلامية والرقائق المحتوية على العقار أو معقداته. تم تحضير المعقدات الصلبة للدواء مع السيكلودكسترينات بطريقة العجن بنسبة ١:١ كما تم تقييم ذوبانية المعقدات المحضرة. أوضحت النتائج أن تأثير هيدروكسى بروبيل بيتاسيكلودكسترين على نفاذية العقار وتوزيعه داخل أنسجة العين المختلفة أكبر من البيتاسيكلودكسترين ، وكان ترتيب تركيز الدواء فى أنسجة العين واتاحته الحيوية على الوجة التالى: الملتحمة > القرنية > القزحية – الجسم الهدبى > السائل المائى. كما أظهرت النتائج أن الوقت الذى وصل إليه أعلى تركيز للعقار (Tmax) فى أنسجة العين هو ٤ ساعات وذلك لجميع الصواغات المحضرة كما أن المساحة تحت المنحنى للتوزيع الكلى للعقار (AUC) فى أنسجة العين كانت ٦١٧,٤ ، ٨٨٢,٤ ، ٩٩٣,٥ ، ١٢٩٢,٧ ، ١٤٦٢,٦ ، ١٩٦٨,٢ و ٢١٤١,٣ ميكروجرام. ساعة/جرام بالنسبة للدواء فى القطرة ، المستحضر الهلامى ، الرقائق ، معقد البيتاسيكلودكسترين فى المستحضر الهلامى ، معقد البيتاسيكلودكسترين فى الرقائق ، معقد هيدروكسى بروبيل بيتاسيكلودكسترين فى المستحضر الهلامى ومعقد هيدروكسى بروبيل بيتاسيكلودكسترين فى الرقائق على التوالى. من النتائج السابقة يتضح أن توزيع الدواء داخل أنسجة عيون الأرانب يعتمد بدرجة كبيرة على المعقدات المستخدمة مع السيكلودكسترينات بالإضافة الى الشكل الصيدلى المصاغ.

The effect of inclusion complex formation of azapropazone (Az) with cyclodextrins (CyDs), hydroxypropyl beta cyclodextrin (HP-β-CyD) and beta cyclodextrin (β-CyD) on the bioavailability of the drug in rabbits eye tissues (conjunctiva, cornea, iris-ciliary body and aqueous humor) from ophthalmic preparations (drops, gels and inserts) containing the drug or it's complexes were studied. The solid inclusion complexes of Az were prepared by the kneading method in molar ratios of 1:1 (guest/host) with HP-β-CyD or β-CyD. The prepared complexes were investigated by the solubility method. The enhancing effect of HP-β-CyD on the penetration of Az in various eye tissues was greater than that of β-CyD. The in-vivo availability of Az from the tested ophthalmic preparations in eye tissues were arranged as following; conjunctiva > cornea > iris-ciliary body > aqueous humor. The peak time of Az for all formulations in the various eye tissues was 4 hours. Meanwhile, AUCs of total drug disposition in the different eye tissues were 617.4, 882.4, 993.5, 1292.7, 1462.6, 1968.2 and 2141.3 μg. hr/g for Az drops, Az gel, Az inserts, Az-β-CyD gel, Az-β-CyD inserts, Az–HP-β-CyD gel and Az-HP-β-CyD inserts respectively. The disposition of the drug in eye tissues showed that the distribution was greatly affected by the presence of CyDs. Moreover, the uptake of Az by different eye tissues was quite different from the selected formulations.

INTRODUCTION

The rapid elimination of conventional eye drops from the eye is considered as one of the main problems in ophthalmic drug delivery. A number of factors, namely rapid tear turnover, induction of tear flow due to irritation caused by the drug preparations, as well as the relatively

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large volume of the administered eye drops (~50 µl versus 7 µl of corneal tear film), lead to a high rate of lacrimal drainage.[1]

Various systems have been designed to maximize ocular absorption of ophthalmic drugs; increasing the corneal permeability and prolonging the contact time on the ocular surface.[2] Ocular drug inserts are one possibility to reach these goals and major improvements of drug delivery compared to the eye drops.[3]

The use of non-steroidal anti-inflammatory drugs (NSAIDs) in ocular therapy is consequent to the well-recognized dangers associated with the use of corticosteroids.[4] Piroxicam was used in the topical treatment of ocular inflammation as an alternative to topical anti-inflammatory steroids.[5,6] Also, the topically administered indomethacin as anti-inflammatory has been reported to reduce post-operative inflammation occurring after cataract surgery.[7]

Cyclodextrins affect many of the physicochemical properties of drug molecules and can result in increased aqueous solubility and stability.[8] Some recent reviews summarize the factors which affect corneal penetration of the drugs after administration with CyDs containing ophthalmic formulations.[2,9] They have been used to increase the corneal penetration, ocular absorption or efficacy of poorly water soluble drugs. This is may be attributed to the higher concentration of the drug that may be administered when aqueous solubility of the drug is substantially increased with CyDs.[10] Moreover, CyDs can be used to reduce the side effects such as irritation and local pain and improve the bioavailability of anti-inflammatory drugs.[11]

The objective of the present work is, to study the effect of Az complex formation with CyDs on the bioavailability of the drug as an effective anti-inflammatory that may be recommended in ophthalmic formulations used in the eye treatment.

EXPERIMENTAL

Materials

- HP-β-CyD (an average degree of substitution, 4.8) and β-CyD were supplied by Nippon Shokuhin Kako Co., Tokyo, Japan. The former was used as received, while, the latter was used after recrystallization from water.
- Azapropazone was supplied by Siegfried, Zofingen, Switzerland.
  Tetracaine hydrochloride, fluorescein sodium, hydrochloric acid, citric acid and sodium dibasic phosphate, n-octanol were supplied by Prolabo, Paris, France.
- Carbopol 941 was supplied by BF Goodrich Co. Specialty & Chemical Division 6100 Oak Tree Bivd., Cleveland, Ohio 44131, USA.
- Sodium carboxymethyl cellulose and benzalkonium chloride, *o*-phosphoric acid, were supplied by BDH Chemical Ltd, GB. Liverpool, England.
- 0.22 µm PTFE filters was supplied by Whatman Ltd, Maidstone, UK.
- All other chemicals and solvents were of analytical reagent grade and used as received.

Apparatus

- Unicam SP 1800 U.V. Spectrophotometer (Pye Unicam Ltd Cambridge, England).
- Beckman pH-meter (Beckman instruments Fullerton, CA 92634, USA).
- MSE Minor centrifuge (MSE Scientific instruments, Manor Royal, Crawley RH_{10} 2QQ Sussex, England).
- High performance liquid chromatography (HPLC) (Gilson Model 302, Anachem Ltd, Luton, UK).

Preparation of inserts

Azapropazone 0.1% or its equivalent weight of HP-β-CyD or β-CyD complexes were dissolved in 5 ml propylene glycol. These solutions were added separately to sodium carboxymethyl cellulose (1.2% W/V) and carbopol 941 (0.5% W/V) in distilled water containing 0.01% benzalkonium chloride, (0.03%) sodium metabisulphite and 0.1% edetate disodium. Equal volumes of the prepared solutions were transferred into polytetrafluorethylene (PTFE) moulds. Each mould was covered with an inverted funnel (stem orifice diameter 6.9 mm) to control solvent evaporation and placed in a laminar flow hood (micro flow laminar air flow station) with an air speed of 90-150 feet/min. The solvent was permitted to evaporate for 48 hours at

ambient temperature. The formed film was transferred to a desiccator containing silica gel, where it was stored for another 24 hours before use.[12] The prepared inserts (0.4-0.5 mm thickness) were cut in the form of circular discs, of one cm diameter, Then, analyzed for azapropazone content spectrophotometrically at 255 nm.[13] The inserts were individually sealed in foil sachets until used.

Preparation of azapropazone eye drops

Aqueous solutions of sodium carboxymethyl cellulose (0.5% W/V) and carbopol 941 (0.2% W/V) containing 0.01% benzalkonium chloride, 0.03% sodium metabisulphite and 0.1% edetate disodium were prepared.[14] Then, azapropazone 0.1% (W/V) was dissolved in 5 ml propylene glycol and added to the previous mixture. The solutions were then completed to 100 ml and filled in a clean, dry and sterile glass containers. The sterilization of the prepared solutions was induced by autoclaving at 125-130° for 30 min, then left until cooling. The sterile products were tested for drug content, pH, color change and degradation products using UV scanning and TLC.[13]

Preparation of azapropazone eye gels

The calculated amounts of sodium carboxymethyl cellulose (2.1% W/V) and carbopol 941 (0.6% W/V) containing 0.01% benzalkonium chloride, 0.03% sodium metabisulphite and 0.1% edetate disodium were prepared. Then, azapropazone 0.1% (W/V) or its equivalent weight of HP-β-CyD or β-CyD complexes were dissolved using 5 ml propylene glycol and added to the previous mixtures. The mixtures were completed to 100 g, then filled in clean, dry and sterile glass containers. The sterilization of the prepared formulations was induced by autoclaving at 125-130° for 30 min, then cooled. The sterile products were subjected to the determination of drug content, pH, color change and degradation products using U.V. scanning and TLC.[13]

Tested formulations

On the basis of the obtained results from the *in-vitro* evaluation of the prepared formulations, the following formulations were selected for the bioavailability study.[15]

1- Azapropazone drops.
2- Azapropazone gel.
3- Azapropazone inserts
4- Azapropazone-β-CyD gel
5- Azapropazone-β-CyD inserts
6- Azapropazone-HP-β-CyD gel
7- Azapropazone-HP-β-CyD inserts

Ocular irritation study

Seven groups of albino rabbits, each of three male weighing 2.0 to 2.5 kg were used. The rabbits were subjected to normal feeding, ventilation and illumination. In each animal, one of the eyes served as a test and the second as a control. An accurately, 100 mg of each formula were applied to the test eye. While, plain bases were used in the other eye which served as a control. The application of each formula was done using special applicator twice daily for two weeks. The eyes were examined each morning and evening before the application of selected formulations as described by Berens *et al.*[16] The eye lids, cornea, conjunctiva and anterior chamber were inspected for inflammation. In addition, the eyes were stained with few drops of 2% solution of fluorescein. Following the final examination, the eyes were examined with a magnifying lens and with focal diffuse illumination. The symptoms of iritis, conjunctivitis and keratitis have been observed. However, another group of three rabbits was not received either azapropazone formulations or plain bases and considered in the observation of any changes for the eye tissues. The gross appearance of control and tested eyes was photographed.

Application of azapropazone ophthalmic formulations on rabbits eyes

During the experiments, all rabbits were kept in a normal upright position in restraining boxes. Three rabbits were used for the determination of the drug in the different eye tissues at each time interval. Five eyes were used for the determination of azapropazone from different formulations and the sixth eye was used as a control. Accurately, individual doses of 100 mg of each formula containing the drug or its complexes were applied to the center of the lower eyelid of the test eyes of rabbits with a microspatula. Plain bases were used in the other eye which served as control. During dosing, care

was taken not to irritate the eyes or touch the corneal surface with the spatula. The lower eyelid was gently moved upward to spread the dose over the corneal surface and then released.

Determination of azapropazone in the ocular tissues of rabbits

Extraction of azapropazone from the ocular tissues of rabbits

The rabbits were sacrificed at 1, 2, 3, 4, 5 and 6 hr after the application of the medicated and plain formulations. The eyes were separated, conjunctiva, cornea and iris-ciliary body of each eye were separated immediately, weighed and grounded with a powdered glass in a mortar.[17] Certain weights of the ground tissues were centrifuged for 20 min, then, extracted with 20 ml of HPLC grade methanol pH 3 and assayed using the validated HPLC method for drug determination in biological fluids.[18] The aqueous humor was aspirated from the anterior chamber of the eyes and treated similarly as the other eye tissues.

HPLC method of assay

HPLC assay was performed using a 25 cm long x 4.5 mm i.d. Lichrosorb RP-18 pre-packed reverse phase column (Jones Chromatography Ltd, Llanbradach, Glamorgan, UK) coupled to either Gilson model 302 (Anachem Ltd, Luton, UK) or ARL constant flow HPLC (Applied Chromatography Systems, Luton, UK). Pump set at flow rate of 1 ml min^{-1}. The solvent system was 80% HPLC grade methanol (Blackford Wells Ltd, Coalville, Leics UK) in triple distilled water adjusted to pH 3 with *o*-phosphoric acid, filtered through 0.22 μm PTFE filters and degassed before use. Samples were injected in 20 μl sample loop (Rheodyne In., Cotati, CA., USA). For assay of azapropazone, the elute was monitored by UV detection (Waters Lambda Max Model 480; Waters Assoc., Milford, Mass., USA) at 250 nm. Samples from the control eyes were prepared similarly, where, indomethacin was dissolved and used as an internal standard.

RESULTS AND DISCUSSION

All of the tested formulations were well tolerated by rabbits. No physical signs of irritation (e.g. redness, lacrimation or ulceration) were observed during or after the experimental period (2 weeks).

The concentration of Az in various eye tissues after the application of Az drops, gels and inserts were illustrated in Figures (1-5) and Table 1. The disposition of the drug in various eye tissues was different during the first hour of the application. The concentrations of the drug in conjunctiva and cornea were significantly higher than that in iris-ciliary and aqueous humor from all applied formulations. This increment may be due to the direct contact of conjunctiva and cornea with the tear pool which housing the drug. Also, the concentrations of the drug in iris-ciliary body was higher than that in aqueous humor at different time intervals. This implies that the drug which appears in aqueous humor was absorbed through the iris-ciliary body and/or endothelial surface of cornea.[19]

The concentration of the drug in rabbits eye tissues after 2 hours of application was affected by the type of the ophthalmic dosage forms and the position of eye tissue. Moreover, the peak time of Az in all tissues with respect to all preparations was 4 hours after application. Meanwhile, the total amounts deposited of Az in all eye tissues after 4 hours were; 172.8, 224.5, 269.4, 318.8, 369.2, 482.4 and 511.2 μg/g for Az drops, Az gel, Az inserts, Az-β-CyD gel, Az-β-CyD inserts, Az–HP-β-CyD gel and Az-HP-β-CyD inserts respectively (Table 1 and Fig. 5).

Meanwhile, AUCs of total drug disposition in the different eye tissues were 617.4, 882.4, 993.5, 1292.7, 1462.6, 1968.2 and 2141.3 μg. hr/g for the tested dosage forms respectively (Table 1).

However, the peak concentrations of the drug in conjunctiva were; 62.0, 76.0, 90.4, 104.8, 118.4, 153.6 and 169.6 μg/g using the same bases respectively (Fig. 1). Concerning the maximum concentrations of the drug in cornea, these were; 49.6, 61.5, 72.2, 84.0, 97.6, 130.8 and 136.0 μg/g using the same bases respectively (Table 1 and Fig. 2).

In deeper tissues (iris-ciliary body and aqueous humor), azapropazone-HP-β-CyD complexes either in the form of gel or insert is the most effective in providing the higher drug concentrations than β-CyD complexes, followed by the drug alone (Figs. 3 and 4). This can be explained on the basis that, HP-β-CyD

Table 1: Area under curve (AUC), peak concentration and peak time of azapropazone after the application of ophthalmic Formulations in rabbits eyes.

Variable \ Type of preparations / Tissue		Concentration of azapropazone (μg / gm)						
		Az drops	Az gel	Az insert	Az-β-CyD gel	Az-β-CyD insert	Az-HP-β-CyD gel	Az-HP-β-CyD insert
Peak Concentration (μg/gm)	Conjunctiva	62±1.32	76±0.64	90.4±3.04	104.8±1.81	118.4±3.14	153.6±1.78	155.6±2.31
	Cornea	49.6±0.99	61.5±1.23	72.2±2.17	84±2.52	97.6±2.88	130.8±1.57	133±3.08
	Iris-ciliary body	39.6±0.78	48.8±1.46	59.6±1.19	76.4±0.76	94±3.01	114.4±3.43	115.8±3.56
	Aqueous humor	21.6±0.71	38.2±1.15	47.2±1.42	53.6±1.61	59.2±1.18	83.6±2.09	85.8±1.74
	Total availability	172.8±3.46	224.5±4.15	269.4±5.01	318.8±6.38	369.2±5.41	482.4±11.21	490.2±8.01
AUC (μg.hr/gm)	Conjunctiva	200.5±4.01	305.8± 3.06	340.6±3.81	436.4±3.72	482.8±4.83	651.4±6.01	723.6±3.78
	Cornea	170.1±1.71	234.7±2.35	261.8±2.62	345.8±3.46	402.3±4.01	544±5.44	538.8±5.39
	Iris-ciliary body	154.3±1.54	193.9±1.94	215.2±2.15	287.8±5.76	324.2±6.48	436.1±3.72	462.3±2.99
	Aqueous humor	92.5±0.93	148±1.92	175.9±2.73	223±2.72	251.8±1.98	336.8±2.71	364±3.77
	Total availability	617.4±7.12	882.4±9.81	993.5±7.12	1292.7±8.71	1462.6±11.21	1968.2±13.74	2141. 3±13.11
Peak time (hr)	------	4	4	4	4	4	4	4

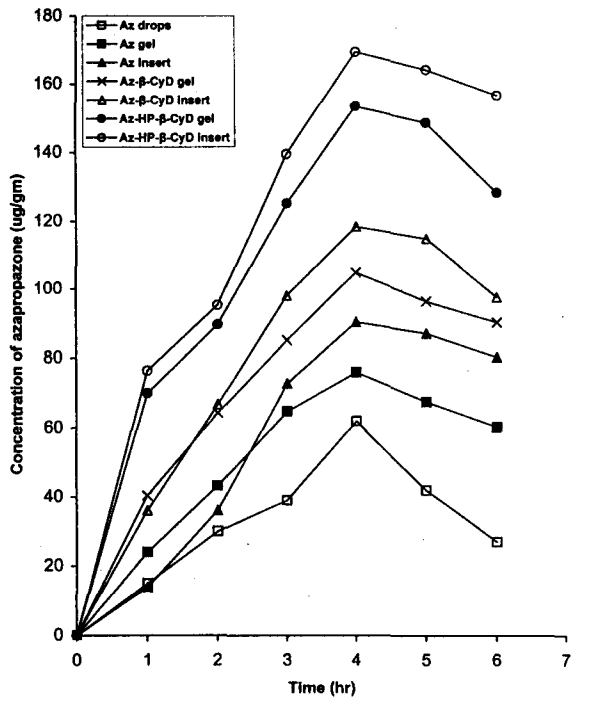

Fig. 1: Disposition of azapropazone in conjunctive after application of ophthalmic preparations.

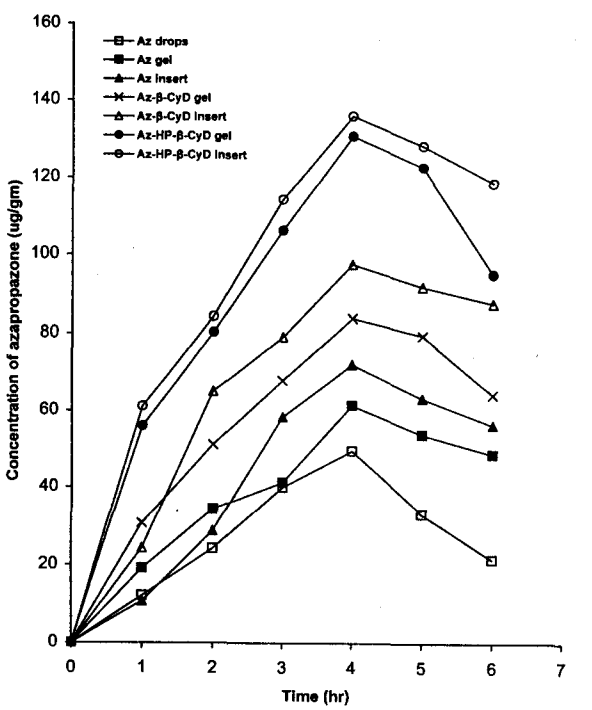

Fig. 2: Disposition of azapropazone in cornea after application of ophthalmic preparations.

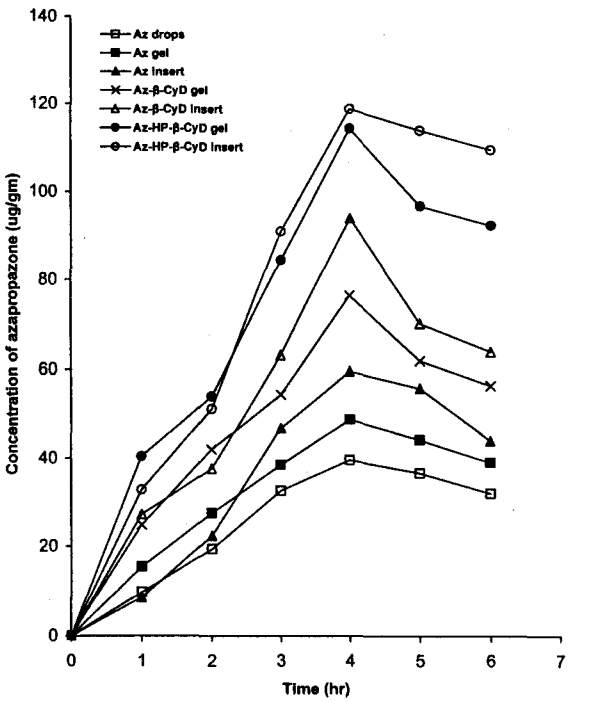

Fig. 3: Disposition of azapropazone in iris-cilliary body after application of ophthalmic preparations.

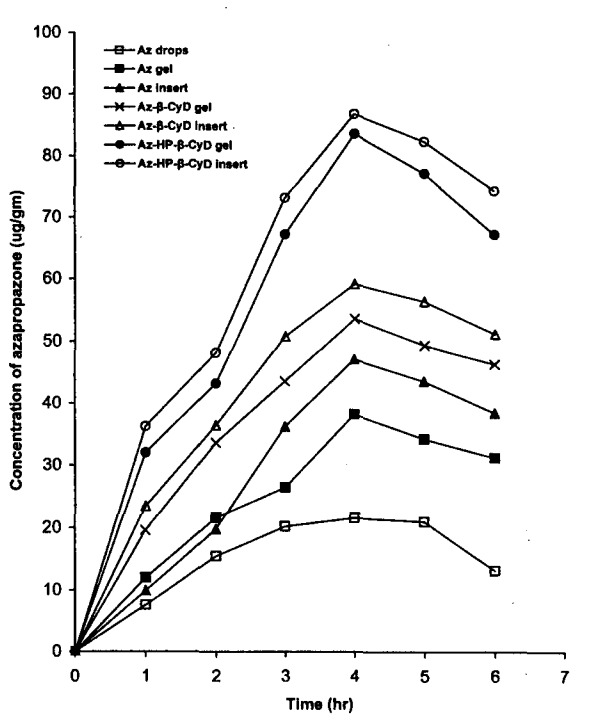

Fig. 4: Disposition of azapropazone in aqueous humor after application of ophthalmic preparations.

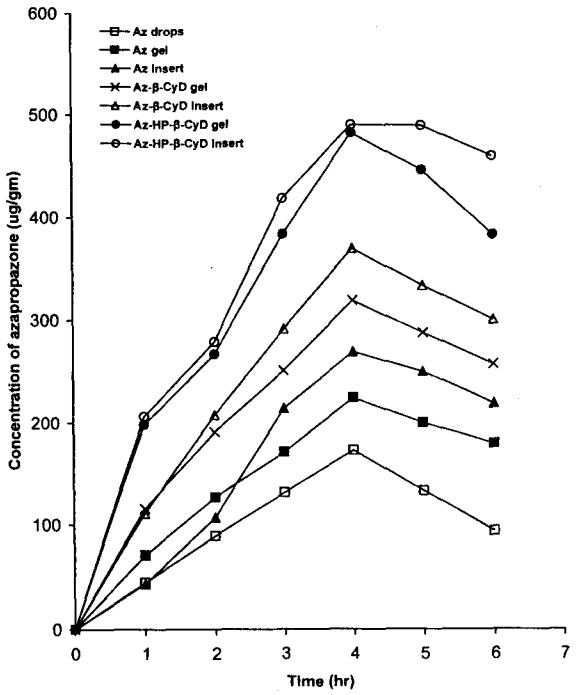

Fig. 5: Disposition of azapropazone in eye tissues after the application of various ophthalmic preparations.

increased the aqueous solubility of the drug, furthermore, it has the ability of solubilization of poorly water soluble drugs intended for the ophthalmic therapy,[20] and so, a fast penetration of the drug from the complexes of HP-β-CyD in the gel or insert through the cornea to the deeper tissues with the aid of blinking has been occurred.[21]

The inserts containing HP-β-CyD or β-CyD complexes provided a higher drug bioavailability than gels containing the same complexes or the drops containing the drug alone. This high efficacy of the inserts compared to the drops may be due to; upon instillation of eye drops, a fraction of the instilled dose is lost because the fluid runs off over the lid margin and spillage, or the instilled dose that gets to the cul-de-sac is being released immediately in the lacrimal fluid and as a consequence, is rapidly removed. An immediate spike followed by relatively short contact time results in a short duration of activity. HP-β-CyD complex in the inserts increased the bioavailability of Az in eye tissues 3.47, 2.43 and 2.16 times greater than Az in drops, gels and inserts in the untreated form. The improved bioavailability of the drug from CyDs complexes may be attributed to the faster dissolution of the complexes containing the drug and the lower binding affinity of the inclusion complexes to the gel or the insert bases.

Regarding the results of Az bioavailability, the formulations can be arranged as follows; Az drops < Az gel < Az inserts < Az-β-CyD gel < Az-β-CyD inserts < Az–HP-β-CyD gel < Az-HP-β-CyD inserts. Also, the bioavailability of Az from the tested ophthalmic preparations in eye tissues were arranged as follows; conjunctiva > cornea > iris-ciliary body > aqueous humor. The obtained results suggest that an improvement of the bioavailability of Az can be obtained through the topical use of inclusion complexes. The results of statistical analysis of the obtained data using "t" test showed that, there is a high significant differences were present between the tested formulations.

CONCLUSION

From the obtained results it could be concluded that, the selected cyclodextrins do not cause any signs of irritation to the eye tissues. Also, they protect the eye from side effects of the topically applied Az used for the treatment of the eye inflammation. HP-β-CyD was the most effective complexing agent with respect to the bioavailability improvement of azapropazone in various eye tissues. Ophthalmic inserts are considered as a promising ocular delivery systems for azapropazone. The tested formulations are suitable and effective formulations in comparison with other available dosage forms containing the tested drug.

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