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OCULAR BIOAVAILABILITY OF PREDNISOLONE FROM MULTIPLE EMULSION DELIVERY SYSTEMS

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في هذا البحث تم تحضير المستحلبات المتعددة نظام ماء فى زيت فى ماء وكذلك زيت فى ماء فى زيت باستخدام زيت البرافين كوسط زيتى للمستحلبات وتم تقييم هذه المستحضرات كأنظمة مطلقة لعقار البريدنيزولون فى عيون الأرانب الملتهبة. وقد تم تحضير المستحلبات البسيطة ماء فى زيت وكذلك زيت فى ماء كوسيلة للمقارنة وقد تم فحص ودراسة المستحلبات مباشرة بعد تحضيرها بالفحص الميكروسكوبى للتأكد من أنها تحتوى على عدد كبير من الحبيبات داخل الحبيبات الأكبر. تأكيدا للحصول على مستحلبات متعددة.

وقد تم اضافة المستحلبات سطحيا على عين الأرنب بعد عمل التهابات فى القرنية بالتأثير الحرارى وتم تتبع درجة الشفاء والتآم الالتهابات يوميا باضافة قطرة الفلوروسين المائية وحساب عدد القرح المتبقية فى القرنية.

وبمقارنة معدل الشفاء فى المجموعات القياسية والمجموعات المختبرة وجد أن هناك اختلاف بينهم وهذا الاختلاف يظهر فى معدل الشفاء ودرجته ايضا. وقد تم عمل تحليل احصائى بالنسبة لعدد القرح المتبقية خلال فترة الملاحظة لمدة اربعة اسابيع.

أثبتت النتائج أن علاج القرح باستخدام المستحلبات المتعددة والمحتوية على عقار البريدنيزولون مفيد جدا وتأثير العقار واضح جدا وهام وخاصة نوع المستحلب (زيت فى ماء فى زيت).

Studies were conducted to evaluate the performance of prednisolone in multiple emulsions as drug delivery systems on the inflamed rabbit's eye. Multiple emulsions w/o/w and o/w/o were prepared by re-emulsification technique. Evaluation of the emulsions was done immediately after the preparation by microscopical examination. Topical instillation of simple emulsion treatments were also investigated for the purpose of comparison. Follow up of the outcome of control and test treatments was made daily by instillation of fluorescein solution and counting the number of induced ulcers. The comparison of the healing rate in control and test treatments groups showed marked differences between the groups. These differences are not only pronounced with regard to the rate of healing but also to its extent. Statistical analysis of differences between the control group and the different test groups with regard to the number of residual ulcers throughout the observation period of four weeks was made according to the student t-test.

INTRODUCTION

Prednisolone is used topically to treat inflammatory conditions of the conjunctiva, cornea, and anterior segment of the eye[1]. The effectiveness of corticosteroids in the healing of corneal ulcers were investigated by many workers[2-3]. Kathleen *et al.*[4], found that, treatment with prednisolone immediately after a thermal burns on cornea of rabbit's eye was effective and exerts strong antiinflammatory influences. The quantity of collagen remaining in the burned tissue indicating the effectiveness of prednisolone in preventing collagen degradation. Tano *et al.*[5] demonstrated that, corticosteroids may be helpful in the treatment of penetrating or perforating injuries of the eye.

The objective of this study is to prepare delivery systems of the drug in multiple emulsions (w/o/w, o/w/o) and to investigate

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their effect on drug penetration and hence ocular bioavailability.

EXPERIMENTAL

Materials

Prednisolone, Sigma Co. St. Louis, USA; Polysorbate 60, Atlas Chem. Ind, USA; Sorbitan mono stearate (Tween 20); Liquid paraffin, Propylene glycol ROTH, Germany.

Experimental animals

Albino rabbits. 1.8-2.8 Kg receiving green fodder.

Apparatus

Homogenizer (Braun) Polarizing microscope (Carl Ziess, Jena, Germany). Transmitted-light microscope (Leitz, Laborlux, Germany).

Methods

Preparation of simple emulsions

Table 1 shows the composition of the simple o/w and w/o formulation. Simple emulsions were prepared using the homogenizer. Aqueous solutions of the hydrophilic emulsifying agent (Tween 20) or the oily solution of the lipophilic emulsifying agent (Span 60) , in definite concentrations, were placed in the homogenizers. According to the type of the emulsion required, the specified amount of oil or water was then added and the mixture was homogenized for 15 minutes at 7000 rpm. at ambient temperature. Prednisolone 0.1% w/v was dissolved in drops of propylene glycol by stirring and incorporating with the aqueous phase of the w/o emulsion.

Preparation of multiple emulsions

Multiple emulsions (w/o/w, o/w/o) were prepared by a two steps emulsification procedure[6]. Table 1 shows the composition of multiple emulsions. In the first step of the procedure, the oily solution of the lipophilic emulsifier was homogenized with the required amount of water at 7000 rpm for 15 minutes at ambient temperature to give w/o emulsion. In the second step, the aqueous solution of the hydrophilic emulsifier was then added and the mixture was homogenized at 7000 rpm for one minute at ambient temperature. To prepare o/w/o emulsions, the first step in the emulsification provides the o/w emulsion. The hydrophilic emulsifying agent was dissolved in the aqueous phase and the solution was homogenized. In the second emulsification step, the freshly prepared o/w emulsion was mixed with definite volume of oil phase that contains the lipophilic emulsifying agent and the mixture was homogenized. The drug was incorporated in the aqueous phase of the simple emulsions (the inner aqueous phase of the multiple w/o/w emulsions) or in the oily phase of the simple emulsions (the inner oily phase of the o/w/o multiple emulsions).

Evaluation of emulsions

The prepared emulsions were subjected to microscopical examination immediately after preparation. The samples were diluted with the external phase before examination[7]. In all examinations an eye piece of 10 X magnification power and an objective lens of 60 X magnification were used. Photomicrographs of these emulsions were taken using transmitted light microscope.

Testing of prednisolone in different delivery systems as an ophthalmic antiinflammatory drug

The effect of prednisolone in different delivery systems was investigated on the inflamed eye of rabbit post instillation of two drops of 2% xylocaine solution in the eye of rabbit; inflammation was evoked by thermal induction of ulcers reaching in depth the corneal epithelium[8]. Five groups of six male rabbits, were used in this study. In one eye of each rabbit, four ulcers were induced.

The control ulcerated group involved instillation of two drops of chloramphenicol solution every morning throughout the observation period. The test groups involved instillation of two drops of chloramphenicol solution every morning plus a twice instillation

of two drops of 0.1% solution of the drug (group II) in different emulsion systems of o/w (group III) w/o/w (group IV) or o/w/o (group V) two times daily.

Follow-up of the outcome of control and test treatments was made daily by the instillation of 2% fluorescein solution and counting the number of ulcers. The criterion for healing was the complete recovery of the corneal epithelium as evidenced by the disappearance of green fluorescence after staining the cornea with fluorescein solution and decreasing the number of ulcers acquiring a green fluorescence.

RESULTS AND DISCUSSION

Microscopic examination of emulsions was performed to inspect the dispersion state of the innermost phase of multiple emulsions. Photomicrographs of simple emulsions were performed for comparison with multiple ones. (Figures 1,2). Figures 3,4 show photo-micrographs of multiple emulsions of w/o/w and o/w/o based on liquid paraffin prepared with Span 60 (2.5%) and Tween 20 (1%) as emulsifying agents, the phase volume ratios of the internal phase as well as the external phase were 0.5. It is clear from these figures, that the

Table 1: Composition of simple and multiple emulsions.

Emulsion type	Oily phase type	Hydrophilic Emulsifier (Tween) type	Lipophilic Emulsifier (Span) type	Tween Conc.	Span Conc.	External phase Volume fraction ratio of primary Emulsion (ϕ)	External phase volume fraction ratio ϕ
W/O/W and O/W/O	liquid paraffin	Tween 20	Span 60	1%	2.5%	0.5	0.5
		Tween 20	Span 60	1%	2.5%	0.5	0.5
		Tween 20	Span 60	1%	2.5%	0.5	0.5
		Tween 20	Span 60	1%	2.5%	0.5	0.5
		Tween 20	Span 60	1%	2.5%	0.5	0.5

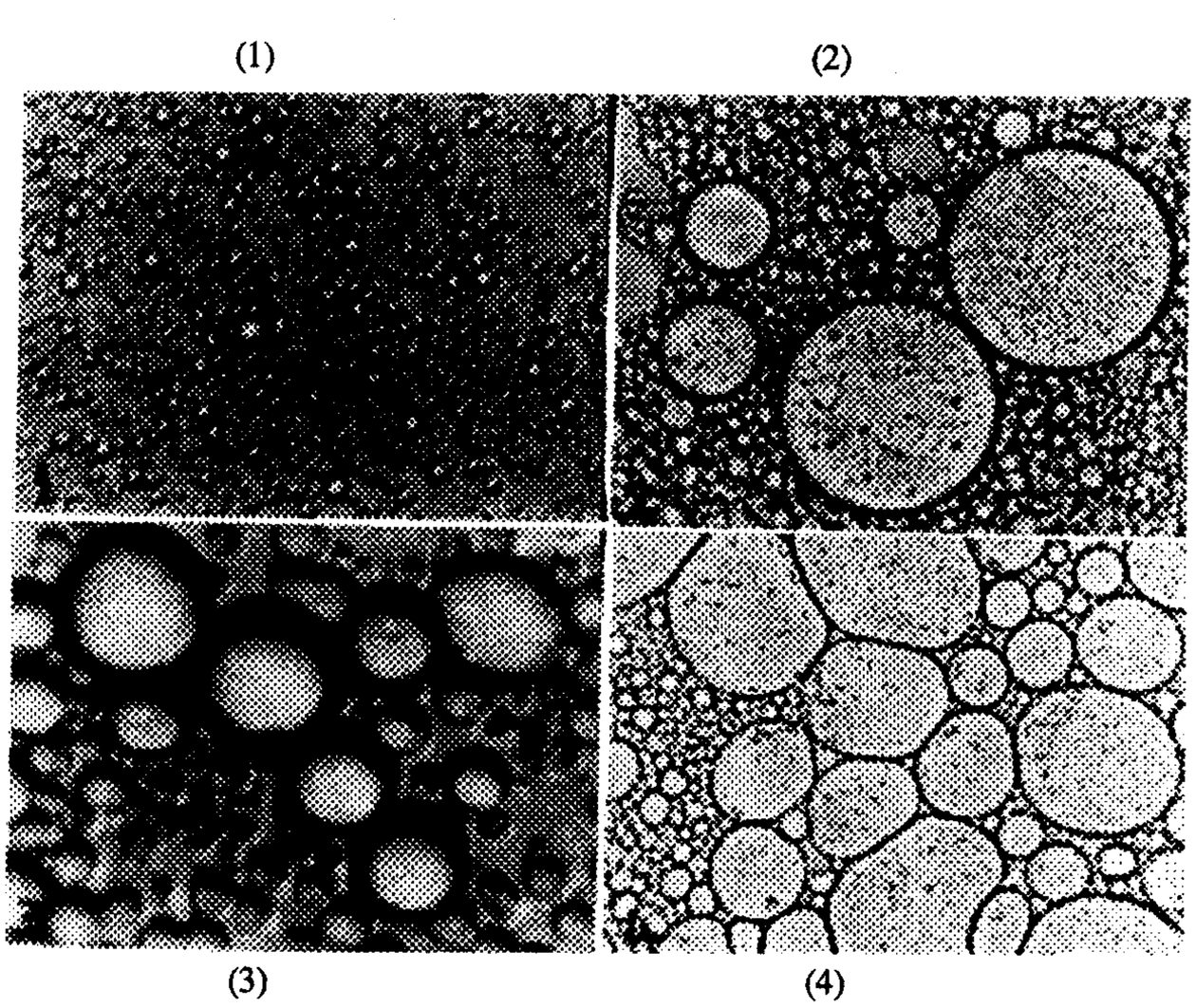

Fig. 1: Photomicrograph of diluted simple o/w emulsion based on liquid paraffin.
2: Diluted simple w/o emulsion based on liquid paraffin.
3: Photomicrograph of multiple w/o/w emulsion based on liquid paraffin.
4: o/w/o Multiple emulsion based on liquid paraffin.

formation of multiple drops contain vast numbers of internal droplets. This observation is in agreement with Florence and Whitehill[6] who claimed that, in photomicrographs of multiple emulsions, three types could be identified, type A, which was composed of small multiple drops containing a few large internal droplets. Type B, in which the emulsion consisted of few larger multiple drops containing smaller internal droplets. Type C, more complex situation, where vast numbers of internal droplets are entrapped. According to figures, 3 and 4, type C drops are more predominant in multiple emulsions.

Anti-inflammatory effect of prednisolone in different delivery systems applied locally to the inflamed rabbit's eye

Evidence for the performance of prednisolone in multiple emulsions as drug delivery systems was studied on the inflamed rabbit's eye. Figure 5 depicts the number of residual ulcers over an observation period of four weeks for control and test treatments. It revealed that, the control treatment did not bring about a healing rate better than 25% after 4 weeks. On the other hand, in case of solution treatment, o/w, w/o/w or o/w/o emulsions, none of the experimental animals showed a healing rate less than 50 %.

Treatment with prednisolone in simple o/w emulsion brings about a healing rate of 75% in almost all animals, at the end of the observation period.

The number of residual ulcers decreased dramatically following treatment with multiple emulsion, where a full healing of 50% and 80% was achieved in case of w/o/w and o/w/o emulsions, respectively after 28 days.

The comparison of the healing rate in control and test groups is depicted in figure 6 and table 2.

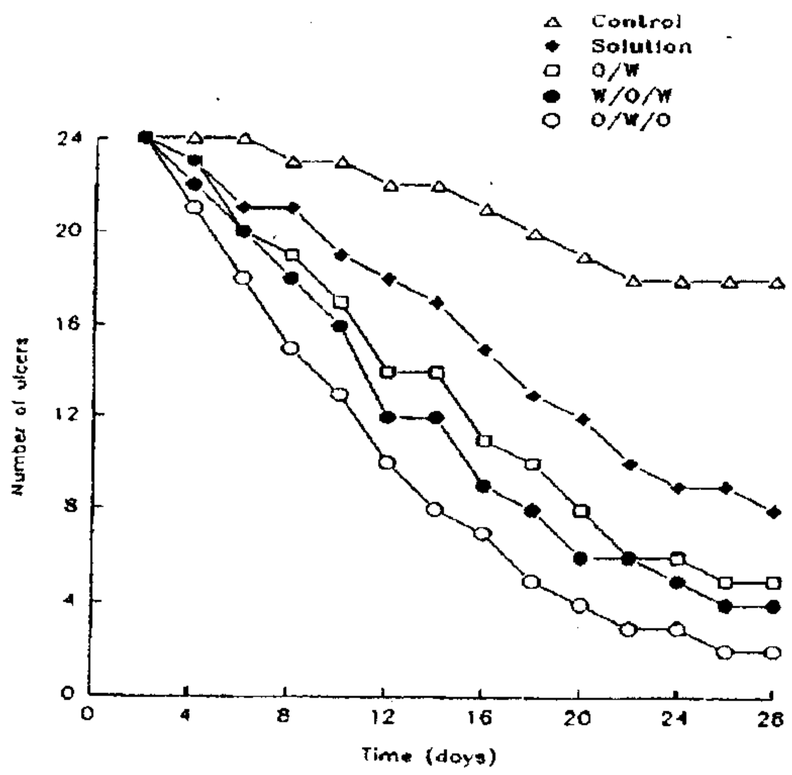

Fig. 5: Effect of prednisolone in solution and in different emulsion delivery systems on healing of ulcers induced in rabbit's eye.

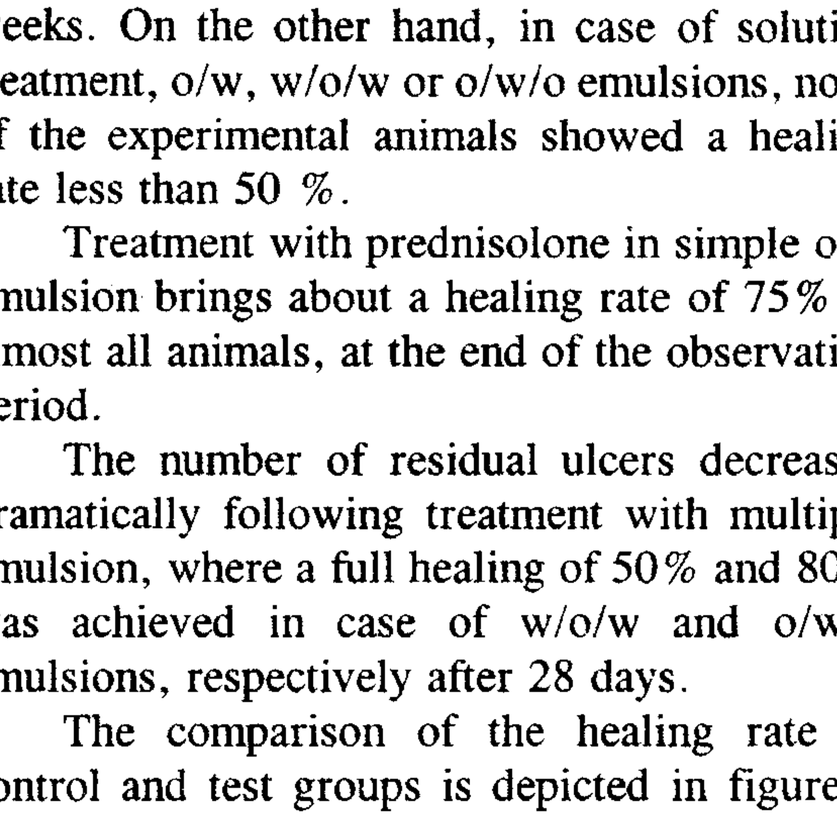

Fig. 6: Effect of prednisolone in solution and in different emulsion delivery systems on healing of ulcers induced in rabbit's eye.

Figure 5 shows marked differences between the groups. These differences are not only pronounced with regard to the rate of healing but also to its extent.

On linearizing the curves presented in figure 5, a fair correlation was obtained for both control and test treatments curves (figure 6). The healing process involves a first-order reaction for which the values of K (healing rate constant) and $t_{1/2}$ (the healing half life in days) were calculated.

Table 2: Effect of prednisolone solution and of different emulsion delivery systems on the healing time of corneal ulcers in the rabbit's eye.

Days	Number of Ulcers				
	Control	Test Treatment			
		Solution	O/W	W/O/W	O/W/O
2	24	24	24	24	24
4	24	23	23	22	21
6	24	21	20	20	18
8	23	21	19	18	15
10	23	19	17	16	13
12	22	18	14	12	10
14	22	17	14	12	8
16	21	15	11	9	7
18	20	13	10	8	5
20	19	12	8	6	4
22	18	10	6	6	3
24	18	9	6	5	3
26	18	9	5	4	2
28	18	8	5	4	2
Cor. coefficient	0.9731	0.9870	0.9892	0.9934	0.9956
Healing rate constant (K^*)	0.0137	0.0448	0.0672	0.0754	0.1029
Half-life time** ($T^{1/2}$)	50.45	15.45	10.32	9.19	6.73

* Day^{-1} ** Day

Table 2 showed that there was a very great difference in healing rate constant for control and test treatments.

Treatment with prednisolone in emulsion systems reduced the healing half life from about 51 days (control treatment) to about 16 days (solution), 10 days (o/w emulsion) about 9 days (w/o/w emulsion) and about 7 days in case of o/w/o emulsion. The results revealed that the healing extent and the healing rate were dependent on the delivery systems used, where they were higher for emulsions compared to the solution. Within the emulsion systems, the healing extent and the healing rate were dependent on the complexity of the emulsion system and the nature of the external phase, they were higher for multiple emulsion systems compared to the corresponding simple one. On the other hand, they were the highest for multiple emulsions with external oily phase (o/w/o emulsions).

Statistical analysis of differences between the control group and the test groups with regard to the number of residual ulcers throughout the observation period of 4 weeks was made according to the student t-test. Table 3 demonstrated that the differences between control and solution groups were significant only after the elapse of two weeks treatment. On the other hand, a highly significant difference ($P = 0.01$) was observed at the end of 3 weeks. Differences between control and either type of multiple emulsion systems were significant in case of w/o/w emulsion and highly significant in case of o/w/o after the elapse of one week treatment and become very highly significant

Table 3: Significance of differences (p= 0.01) between different pairs of control group and test treatment groups (prednisolone in solution and in different emulsion systems based on liquid paraffin).

Pairs of comparison	Parameters of comparison			
	1st week	2nd week	3rd week	4th week
Control with solution	0.1 (1.19)*	0.05 (3.15)	0.01 (3.87)	0.001 (5.00)
Control with O/W	0.1 (1.86)	0.01 (3.35)	0.001 (7.91)	0.001 (7.05)
Control with W/O/W	0.05 (2.83	0.001 (7.91)	0.001 (7.75)	0.001 (7.00)
Control with O/W/O	0.01 (4.24)	0.001 (7.83)	0.001 (11.19)	0.001 (12.66)
Solution with O/W	0.1 (0.54)	0.1 (1.86)	0.1 (2.00)	0.1 (1.10)
Solution with W/O/w	0.1 (1.19)	0.001 (5.00)	0.05 (2.74)	0.1 (1.41)
Solution with O/W/O	0.05 (2.39)	0.001 (5.58)	0.01 (4.39)	0.05 (2.53)
O/w with W/O/W	0.1 (0.62)	0.1 (1.58)	0.1 (1.00)	0.1 (0.32)
O/W with O/W/O	0.1 (1.86)	0.01 (3.36)	0.05 (2.71)	0.1 (1.34)
O/W/O with W/O/W	0.1 (1.41)	0.05 (3.16)	0.1 (1.46)	0.1 (0.84)

Values between parenthesis represent the calculated t-value.

afterwards, indicating a dramatic dependency of the healing rate on the use of multiple emulsions as a drug delivery system.

These findings demonstrate that, prednisolone in multiple emulsions particularly of o/w/o type is very useful in the treatment of inflammatory conditions of the eye.

The effect of the drug is very highly pronounced and significant.

REFERENCES

1- A.Osol, " Remington's Pharmaceutical Sciences", 15th Ed., P. 896 (1975).
2- J.V.Aquavella, A.R.Gassett and C.H.Dohlman, Am. J. Ophthalmol., 58, 621 (1964).
3- S.I.Brown, C.A.Weller and A.M.Vidrich, Ibid., 70, 744 (1970).

4- P.Kathleen, A.Robert and C.Charles, Arch. Ophthalmol., 101, 640 (1983).
5- Y.Tano, D.B.Chandler, B.W.Mc Cuen and R.Machemer, Am. J. Ophthalmol., 91, 184 (1981).
6- A.T.Florence and D.Whitehill, Int. J. Pharm., 11, 277 (1982).
7- S.S.Davis, T.S.Purewall and A.S.Burbage, J. Pharm. Pharmacol. suppl. 28, 60 (1976).
8- D.M.Maurice and S.Mishima, " Ocular Pharmacokinetics " in Hand book of Experimental Pharmacology: Ocular pharmacology vol. 69, London, P.97 (1984).