Development and In-Vitro Clinical Study of Tinidazole - Chlorhexidine Gluconate ophthalmic suspension for Treatment of Keratitis

Hiba Muaffak Salmo*, Haider Kadum Abbas, Faiz I. Al-Shakarchi***, Jamal Abdul-Rahman******

الخلاصة :

استحداث تركيبة جديدة من المعلق المائي الصغير الجزيئات للتينيدازول مع الكلورهيكزدين كلوكونيت لتكون مفيدة في علاج الالتهابات او التقرحات الفطرية لقرنية العين.

اثبتت الدراسة المختبرية للتينيدازول بعض الفعالية ضد انواع معينة من الفطريات المعزولة من العين المصابة بتقرح القرنية نتيجة للالتهاب الفطري . اظهرت النتائج ان التركيبات المحضرة باستخدام البروبلين كلايكول كمذيب جيد وكمادة ضابطة للملوحة وغير سامة , بعض الفوائد العملية مقارنة بالتركيبة التي تحتوي على البروبلين كلايكول مع المادة الغير آيونية الفعالة سطحيا (توين ٨٠) وذلك بتحسينها للذوبانية والتحرر المختبري لمعلق التينيدازول ,وان جميع التركيبات المحضرة اظهرت تلائم فسلجي عالي على عين الارنب . اضافة الى ذلك ان استعمال المثيل سيليلوز ذو الوزن الكتلي العالي كان فعال في اعطاء لزوجة ملائمة والتي تبقى حتى بعد التعقيم بالحرارة.

ABSTRACT

A new model aqueous micro fine suspension of tinidazole (TND) was developed on the basis of the combination with chlorhexidine gluconate to be useful in treatment of fungal corneal infection or ulcer (fungal keratitis). *In vitro* clinical study for TND - chlorhexidine gluconate showed a significant antifungal activity against certain species of fungi isolated from patients with corneal ulcer . Propylene glycol (PG) was chosen because it is known as a good solubilizer , it could be used as a tonicity adjustment agent in appropriate concentration & it is nontoxic . An enhancement in the solubility of TND was achieved also by interaction with a non-ionic surfactant polysorbate 80 (Tween80) and PG. .

*** M.Sc. College of Pharmacy / Baghdad University ** M.Sc College of Pharmacy /Al Kufa University *** M.D. Ibn Al Haitham Teaching Eye Hospital **** M.Sc. College of Science / Al-Anbar University**

The models prepared by using PG were showed some practical advantages over the models containing a combination of PG and non –ionic surfactant polysorbate 80 (Tween 80) , by enhancement the *in vitro* dissolution & releasing of TND suspension .

Previous histological studies have shown that these additives used not cause eye irritation. The prepared models showed high physiological tolerance on rabbit eye. Moreover high molecular weight methylcellulose (MC) was effective to create appropriate viscosity that was maintained unchanged after heat sterilization.

INTRODUCTION

The object of the present investigation was to develop a new ophthalmic preparation containing TND and chlorhexidine gluconate to be used in treatment of fungal corneal ulcer. Fungal keratitis is an important cause of corneal blindness in the developing countries at the tropical area(1) . Trauma, by organic materials, is the main predisposing factor for fungal keratitis (2,3) . Fungal keratitis is the third most common cause of suppurative keratitis in Iraq (4), due to semitropical weather and high number of agricultural workers . Previous study had shown that , TND - chlorhexidine gluconate mouth gargle preparation exhibit a physical , chemical compatibility and a maximum stability at pH4-5 for about 2.44 years as an expiration date(5).

TND is antiprotozoal & antibacterial agent (6,7) , while chlorhexidine gluconate had been proved its antifungal activity in previous study (4) . In vitro clinical studies has proved that ,a combination of these drugs showed a significant effect against some species of fungi isolated from corneal ulcer , as well as , it might be also subjected to further in vitro clinical study in the future to show its antiprotozoal effect in treatment of Acanthamoeba keratitis (protozoal infection of the eye) (8).

The aim of this work is to develop an ophthalmic suspension containing a combination of antimicrobial agents have an antifungal activity against some species of fungi isolated from corneal ulcer in case of fungal keratitis.

MATERIALS & METHODS

MATERIALS

TND was obtained from Sigma chemical co . Chlorhexidin gluconate was gift from AL Mansour factory in Baghdad. All other materials used were of analytical reagents.

METHODS

Preparation of simulant eye fluid

Simulant eye fluid (S.E.F) was prepared from the following materials : 0.67 % NaCL , 0.2 % Na bicarbonate, 0.008% CaCL2.2H2O and distilled water up to100ml. The pH was adjusted at 7.4 (9).

Particle size measurement

The microscopical measurement of the particle size of TND powder was carried out by putting the powder on the surface of clean slide then diluted with little amount of water and covered with a small clean slide cover. It was about 7.6 µm in diameter, which is in agreement with U.S.P. (XXII) requirement for the preparation of micro fine ophthalmic suspension.

Preparation of TND micro fine suspension

Preparation of 1% TND micro fine suspension models were carried out by dispersing the micro fine powder of TND into 24 hours hydrated mixture of all other ingredients. Chlorhexidine gluconate was act also as antiseptic & preservative , but because of its incompatibility with salts (7) , therefore any salts including the buffering agents were excluded from this preparation & the formulas were prepared in water , MC was used as a thickening agent also it have a property to be act as a suspending agent (10).

Table -1 represent s the prepared models of TND - Chlorhexidine gluconate suspension .The pH of the prepared models were in a range of 4.65-5.1 .

All freshly prepared suspension were sterilized at 100°C (water steam) for 30 minute(11).

Table -1-

Models of 1% TND aqueous micro fine suspension

Formula (w/v)	Model suspension no. 1	2	3	4	5	6
TND	1	1	1	1	1	1
Chlorhexidine gluconate	0.1	0.1	0.1	0.1	0.1	0.1
Tween 80	1	1	1	-	-	-
PG	10	10	10	10	10	10
MC (high molecular weight)	0.3	0.5	1	0.3	0.5	1
Distilled water up to pH =4.65-5.1	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

Viscosity measurements

The viscosity measurements were carried out in triplicate at 25°C using Brookfield Viscometer LV1 spindle L2 at 200 sec-¹ shearing rate .

Solubility measurement

The solubility of TND in water, Tween80:water , PG:water and PG:Tween80:water
were measured as follows : After a measured amount of micro fine powder of TND was added to the mixture of these additives ,each mixture was shaken in an incubator at 35°C for 48 hours. It was then passed through a Millipore filter (0.45µm) .The solubility of TND in the mixed solvent measured by U.V spectrophotometer after an appropriate dilution with water at 230 nm (12).

In vitro clinical test

This test was carried out by using disc diffusion method in which the diameter of the inhibition zone of antimicrobial agent was measured in (mm) for a number of fungi species isolated from patients with corneal ulcer.

In vitro dissolution& releasing studies

For dissolution studies:

The dissolution study was carried out in a USP dissolution apparatus type I ,a basket of 2.5 cm in diameter was enclosed with a multifold filter paper (dialysis cell) in order to be filled with 2ml of each sample. After connecting to a stirrer motor , the basket was immersed in 500ml of S.E.F pH 7.4 (collecting medium) containing in a flask of the dissolution apparatus . The system maintained at 37°C, the collecting medium was stirred at 120 r.p.m during the studying period (13).

For releasing studies

Also the same technique was used except that the dialysis cell was immersed to about 1 cm of its surface in 100ml S.E.F pH 7.4 (collecting medium) containing in flask .The system maintained at 37°C, the collecting medium was stirred at 120 r.p.m during studying period (11,14).

The concentration of the dissolution and released TND was determined spectrophotometrically at 320 nm.

In vivo studies of the local tolerance

In vivo studies of the local tolerance were carried out on the eyes of white male rabbits after a single treatment.

Two drops from the prepared suspensions were instilled into the right eyes .The left eyes were left as references. The reactions of the folds, the conjunctiva, the cornea and the iris wereobserved30 minute after the instillation.

RESULTS& DISCUSSIONS

Viscosity evaluation

It was noticed that the apparent viscosity values of samples were increased with MC concentration and remained unchanged after heat sterilization . Table-2- shows the viscosity values of these samples . This fact can be considered as a practical advantage to increase the contact time of the medicinal agents to the eye surface and it to prolong the therapeutic activity in vivo(15). can be expected

The optimum viscosity for topically applied ophthalmic suspension is in the rang of 15-25 c.p (16), increasing the viscosity may present a problem with respect to accuracy of instillation and blurring in vision (17). Therefore 0.5% MC can be concentration that giving the optimum viscosity value. considered as a best

Table -2- The viscosity values of the prepared samples

% MC	Viscosity centipoises(c.p)
0.3	12-14
0.5	20-22.5
1	121-125

Solubility study

Table -3- shows the effect of additives on the solubility of TND

Table -3-

Solvent/additive	Solubility mg/ml
water	6.1
Tween 80:water	9
PG:Tween 80 :water	12
PG: water	16

The solubility of the drug was increased as follows:

PG: water > PG:Tween80: water > Tween80: water > water alone.

The results indicated that PG is a good solvent for TND .The non-ionic surfactant Tween80 act as a solubilizing agent, the non polar hydrocarbon chains would be adsorbed by the hydrophobic particle surfaces of the drug, where as the polar groups project into the aqueous medium and become hydrate (18), as a result this phenomena could be limit or decrease the effect of PG as a co solvent when used in combination.

In vitro dissolution studies of TND

The dissolution profiles are depicted in figures 1&2 and clearly show a slow dissolution with models 3&6 containing 1% MC, this result can be related mainly to the marked ability of MC to increase the viscosity . Models 2&5 containing 0.5% MC and models 1&4 containing 0.3% MC show a rapid dissolution , hence , it can be expected that the models of 0.5% MC with an optimum viscosity value can be well prolong the therapeutic effectiveness of antifungal agents in vivo if compared with low viscosity models of 0.3% MC , so the models 2&5 will be subjected to in vitro releasing studies.

In vitro releasing studies of TND

Figure 3 shows the releasing profile for models 2&5. Its clearly indicates that the difference in the releasing profile for the similar viscosity models (20-22.5 c.p) can be related mainly to the solubility of TND in these preparations as shown in table -3-.

In vivo studies of the local tolerance

It was established that the rabbit eye tolerated very well the micro fine TND ophthalmic suspensions of models 2&5.

In vitro clinical test

Table 4 shows the in vitro susceptibility of some fungi species isolated from patients with corneal ulcer to TND -chlorhexidine gluconate compound eye drop (inhibition zone in mm).

Table-4-

Organism	No. of cases have corneal ulcer	Inhibition zone in (mm) to TND-chlorhexidine gluconate compound eye drop
Aspergillus species	4	9
	1	20
	1	21
	3	16
Aspergillus niger	1	13
Aspergillus fumigatus	1	20
Fusarium solani	1	10
	1	8
Fusarium species	1	10
	1	13
Alternaria species	2	20
	2	24
	1	17

	1	**12**
	2	**14**
Penicillium	**1**	**16**
	1	**17**

Quantitative methods of susceptibility testing that required measurement of zone diameters give the most precise estimates of the antimicrobial susceptibility .Interpretations the diameters on the disc of inhibition zone less than 10mm indicates a weak susceptibility ,while the zone of 10-13 mm indicates an intermediate susceptibility &over 14 mm indicates that the infecting organism is respond early to the therapy (19).

Therefore in this study 20% (5 out of 25) cases show a weak susceptibility, 20% (5 out of 25) cases show an intermediate susceptibility & 60% (15 out of 25) cases are very susceptible to this type of preparation .

These results indicate that this compound have a good antifungal activity against some species of fungi isolated from a number of a corneal ulcer cases, when compared with the previous results obtained from the susceptibility study of only chlorehexidine gluconate eye drop used for treatment of fungal keratitis that exhibit its susceptibility for only Aspergillus niger& Aspergillus flavuse (12.5%) 2 out of 16 different types of fungi species cases (4).

CONCLUSION

Ophthalmic drop containing a combination of TND and chlorhexidine gluconate have some effect against species of fungi responsible for fungal keratitis .The dissolution of TND from the micro fine suspension to the S.E.F was affected by the viscosity of the prepared formula which remained unchanged upon heat sterilization, while its releasing was found to be affected by the solubility of the drug in the prepared formula.

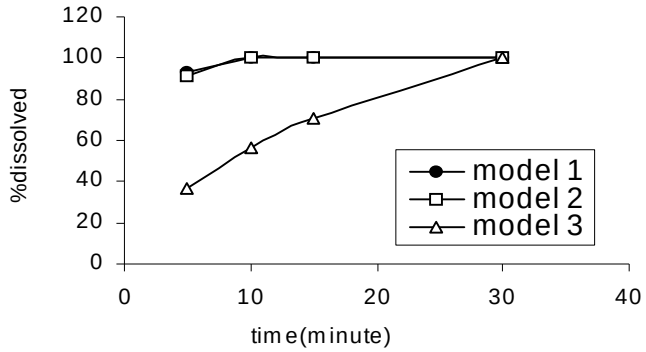

figure1- Dissolution profile of TND micro fine suspension models 1,2,3 in simulant eye fluid

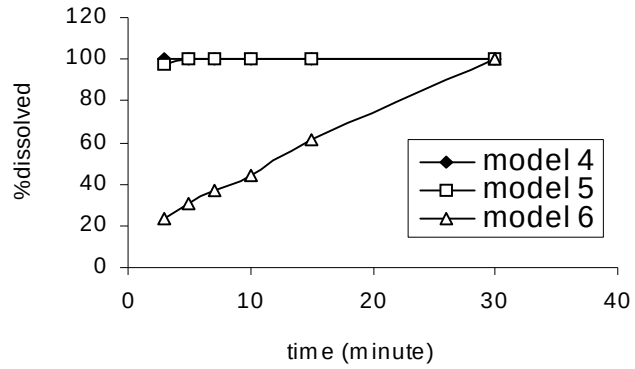

figure 2- Dissolution profile of TND micro fine suspension models 4,5,6 in simulant eye fluid

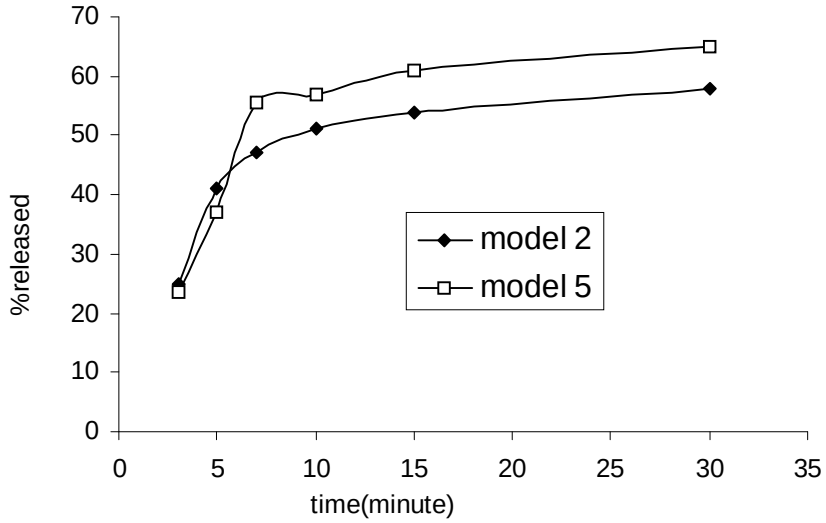

figure 3. Releasing profile of TND micro fine suspension models 2&5 in simulant eye fluid

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