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Research Article

Antibacterial Effectiveness of Chloramphenicol Ophthalmic Hydrogel Against *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633

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ABSTRACT

Background: Hydrogels are the unique three-dimensional polymeric materials that can hold a large fraction of water thus aims to release the drug in a controlled manner. Controlled drug delivery systems that are meant to deliver the drugs at predetermined rate for a pre-programmed period is a good alternative to accomplish and overcome the inadequacy of low bioavailability of conventional dosage form. Aims and Objectives: To determine the effectiveness of antibacterial activity of chloramphenicol in ophthalmic hydrogel preparations against *S. aureus* and *B. subtilis* comparing with eye drops dosage form. Materials and Methods: Ophthalmic hydrogel and eye drops of chloramphenicol were used for comparing the effectiveness of antibacterial activity against *S. aureus* and *B. subtillis* using agar diffusion methods with perforation technique. The observations were made for 28 days with evaluation of the physical preparation includes organoleptic, pH, viscosity. Result: The chloramphenicol eye drops preparation showed that the largest inhibition diameter at concentrations 20%,10% and 5% were 2.87-2.90; 2.64-2.76 and 2.48-2.55 cm. During comparison with opthalmic hyrdogel preparations there was not a very significant difference observed at opthalmic hydrogel preparation of 20% , 10% and 5% inhibition diameter obtained were 2.85-2.98; 2.58-2.69 and 2.42-2.46 cm. This showed that both preparations were equally effective in inhibition of *S. aureus* and *B. subtilis* growth. The minimum inhibitory concentration growth of the hydrogel opthalmic preparations against the bacteria *B. subtilis* was 10% and *S. aureus* at concentration 20%. In the evaluation of the physical preparation includes organoleptic, pH, viscosity showed good results, and still within the range of requirements. Conclusion: The effectiveness of antibacterial preparations in chloramphenicol ophthalmic hydrogel were not much of a difference compared to the form of eye drops preparations against *S. aureus* and *B. subtilis*.

Keywords: ophthalmic hydrogel, chloramphenicol, *Staphylococcus aureus*, *Bacillus subtilis*, minimum inhibitory concentration

INTRODUCTION

Both Gram-positive and Gram-negative bacteria are increasingly becoming important both clinically and therapeutically as biological agents of ocular infections throughout the world. Various forms of ocular infections caused by pathogenic bacteria have been reported by different investigators. Gram-positive bacteria such as *Staphylococcus aureus*, non-coagulase-positive *Staphylococci*, *Bacillus* sp., *Corynebacterium* sp., *Streptococcuspneumoniae*, *Streptococcus pyogenes*, and *Streptococcus viridans* have been implicated as aetiologies of conjunctivitis in patients[1,2]. Conjunctivitis treatment depends on the identification of the cause. Bacterial conjunctivitis may be treated with sulfonamides (15% sulfasetamide) or antibiotics (gentamicin 0.3%, 0.5% chloramphenicol)[3]. Treatment of eye infections is done by administering eye drug preparations containing antibiotic, such as chloramphenicol, that can overcome the acute conjunctivitis in the eye, caused by microorganisms[4,5]. The chloramphenicol gives a good distribution of antibiotic drugs on the eye[6]. The treatment involves the application of ophthalmic dosage forms such as eye drops, suspensions or ointments. Unfortunately, some of the therapeutic approaches have major shortcomings, especially in the treatment of the posterior segment of the eye, where many vision-threatening diseases originate[7].

For comfort in eye drug admistration, sterile hydrogel formulation can be choosen. The comfort of such formulation is the same as eye drops form. The gel form gives a profit on an increased retention time of drug on the surface of the eye as well as increased permeability of drug in the cornea[8-10]. The drug release is slow moreover, can fix bioavaibilty value of drug in the eye[10-12].

MATERIALS AND METHODS

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Table 1: Formula of hydrogel ophthalmic preparations.

Ingredients (%)	Concentration (% b/v)
Chloramphenicol	0.5
Poloxamer 188	10
Poloxamer 407	10
Propylenglycol	10
Nipagin	0.02
Aquadestilata	ad 100

Table 2: Formula of eye drops preparations.

Ingredients (%)	Concentration (% b/v)
Chloramphenicol	0.5
Acidum boricum	1.5
Sodium tetraborate	0.3
Phenyl mercury nitrate	0.002
Aquadestilata	ad 100

Table 3: Evaluation of chloramphenicol hydrogel ophthalmic preparations

Observ-ation day	Evaluation of preparation		
	Performance	pH	Viscos ity
0	Liquid, colorless, odorless	6.34	8.2
1	Liquid, colorless, odorless	6.34	8.2
3	Liquid, colorless, odorless	6.25	8.0
5	Liquid, colorless, odorless	6.21	7.5
7	Liquid, colorless, odorless	6.18	7.5
14	Liquid, colorless, odorless	6.18	7.3
21	Liquid, colorless, odorless	6.13	7.2
28	Liquid, colorless, odorless	6.10	7.2

Table 4: The optimization of chloramphenicol concentration of ophthalmic hydrogel preparations and eye drops.

Preparations	Concentration	Drags zone average (cm)	
		B. subtilis	*S. aureus*
Hydrogel	50%	3.11	3.28
	40%	3.02	3.15
	30%	2.76	2.94
	20%	2.63	2.76
	10%	2.59	2.61
	5%	1.89	2.28
Eye drops	50%	3.92	2.86
	40%	3.84	2.88
	30%	3.01	2.85
	20%	2.68	2.80
	10%	2.66	2.76
	5%	1.86	2.21

Instruments/equipment/apparatus

pH meter (Methrom 744™ USA), viscometer (Rion™ VT-04 F Japan), Laminar air flow cabinet (Esco™ USA), analytical balance (Mettler Toledo™ Canada), autoclave (All American™ USA), incubator (Memmert™ USA), oven (Memmert™ USA), membrane filters, cotton, muslin, eyedropper, micropipette (Biohit® Finland), perforator, ose, test tubes (Pyrex® Indonesia), racks of test tubes, petri dish (Pyrex® Indonesia).

Chemicals and reagents

Chloramphenicol (BioBasic© Canada, Batch: CB0118, ED: 09/2018), poloxamer 188 (Pluracare® F68 NF Prill), poloxamer 407 (Pluracare® F127 NF Prill), propylenglicol (Brataco® Indonesia), aquadestilata (Brataco® Indonesia), nipagin (Brataco® Indonesia), sodium tetraborate (E-Merck® USA), boric acid (E-Merck® USA), Phenyl mercury nitrate (E-Merck® USA), standard sol. pH 7 and pH 4 (E-Merck® USA), sodium chloride 0.9 % (IPHA® Indonesia), Trypticase Soy Agar (E-Merck® USA), Trypticase Soy Broth (E-Merck® USA), Mueller-Hinton Agar (E-Merck® USA), Mueller-Hinton Broth (E-Merck® USA), and blood.

METHODS

Formulation of chloramphenicol ophthalmic hydrogel

The formulation was prepared according to Table 1.

Each of Poloxamer 188 and poloxamer 407 was weighed and dissolved with distilled water, then stored in the refrigerator for 24 h. The chloramphenicol was dissolved with propylenglicol, and added with nipagin. The mixture of chloramphenicol was stirred until the entire dissolved and homogeneous. The materials were ready on each put in a bottle 100 mL size vial, then sterilized by autoclave for 15 min at 121^0C[7,13].

Formulation of chloramphenicol eye drops

The formulation was prepared according to Table 2[14].

Evaluation of chloramphenicol hydrogel ophthalmic preparations

Organoleptic test

Organoleptic hydrogel was checked by observing changes in color, odor and clarity. Clarity was checked visually by examination of the formulation against white and black background. Organoleptic observations were performed on the day-to-1, 3, 7, 14, 21 and 28.

pH measurement

pH was measured by using the pH meter tool calibrated with pH 4 and 7 of standard solutions. pH hydrogel ophthalmic preparations that have been made was measured by dipping the rod cathode pH meter into the preparation. pH call button was pressed, accordingly, the screen will appear on the pH of the preparation. pH measurement was performed on days 1, 3, 7, 14, 21 and 28.

Viscosity measurement

The viscosity measurement was done using Rion viscometer VT-04 by No.3 spindle to dip into a container containing hydrogel preparations up to the mark. Safety valve was released nevertheless the rotor turned until stable (± 2 min) that was appointed by needle pointer. A measurement was achieved during storage days 1, 3, 7, 14, 21 and 28.

Antibacterial effectiveness test of chloramphenicol opthalmic hydrogel and eye drops against S. aureus and B. subtilis

The optimization of chloramphenicol concentration of ophthalmic hydrogel preparations and eye drops

The bacterial suspension of 0.50 mL was mixed with 50

Table 5: Drags zone measurement average of chloramphenicol eye drops and opthalmic hydrogel at the storage time.

Days	Drags zone average (cm)					
	Opthalmic hydrogel			Eye drops		
	S. aureus					
Preparation concentration (%v/v)	20	10	5	20	10	5
0	2.98	2.69	2.46	2.90	2.64	2.55
1	2.95	2.66	2.45	2.87	2.63	2.42
3	2.78	2.64	2.43	2.85	2.62	2.33
5	2.74	2.58	2.29	2.84	2.60	2.32
7	2.73	2.54	2.27	2.81	2.60	2.23
14	2.70	2.52	2.25	2.79	2.53	2.21
21	2.67	2.49	2.24	2.75	2.49	2.20
28	2.62	2.28	1.83	2.68	2.44	1.98
	B. subtilis					
Preparation concentration (% v/v)	20	10	5	20	10	5
0	2.85	2.58	2.42	2.87	2.76	2.48
1	2.83	2.58	2.37	2.84	2.72	2.46
3	2.73	2.52	2.15	2.75	2.67	2.44
5	2.70	2.46	2.15	2.71	2.57	2.32
7	2.68	2.45	2.13	2.63	2.48	2.31
14	2.64	2.44	2.12	2.62	2.47	2.27
21	2.62	2.40	2.10	2.61	2.45	2.23
28	2.55	2.34	1.76	2.59	2.30	1.88
Control +ve	+					
Control –ve	-					

Description:+ = Growth of microorganism
- = No growth of microorganism

Table 6: MIC of chloramphenicol opthalmic hydrogel preparations.

Concentration (% v/v)	*S. aureus*	*B. subtilis*
40	-	-
20	-	-
10	+	-
5	+	+
2.5	+	+
1.12	+	+
0.625	+	+

Description:
+ = Growth of microorganism
- = No growth of microorganism

mL of diluted Mueller-Hinton Agar (MHA) then homogenized and allowed to solidify and then made holes using a sterile perforator and each hole was filled with a solution of the eye drops and a hydrogel solution ophthalmic (with 30%, 20%, 10% and 5% concentration variations) of 50 µL using micropipet in each hole. After that it was incubated at 37^0C for 18-24 h. The inhibit zone formed was measured using a calipers. After that can be determined the diameter of the inhibition on each dosage with the variation of concentration having the diameter of the inhibition is almost the same[15,16].

The appeal value of chloramphenicol effectiveness on ophthalmic hydrogel preparations and eye drops

The inoculated media of the test bacteria was prepared by using a sterile perforator. Each hole was inserted 50 µL chloramphenicol from an ophthalmic hydrogel preparation and eye drops having almost the same inhibition diameter, at concentrations of 20%, 10% and 5%. Afterwards the medium was incubated for 18-24 h at 37^0C, then measured the diameter of the inhibition area using calipers.[15,16]

Determination of minimum inhibitory growth concentration (MIC)

Determination of minimum inhibitory growth concentration (MIC) was performed by the tube dilution method of the ophthalmic chloramphenicol hydrogel reaction tube. In the process of determining MIC, an initial dilution of the hydrogel preparation was prepared by 80%. Thereafter, a 40%, 20%, 10%, 5%, 2.5% and 1.25% dilution of an ophthalmic chloramphenicol hydrogel preparation was added in 1 mL MHB using a micropipette and a bacterial suspension using ose, then incubated at a temperature 37^0C for 18-24 h. MIC values were obtained from the last cloudy tube to the clear tube[15,16].

RESULTS AND DISCUSSION

Preparations of chloramphenicol opthalmic hydrogel

The results of clear ophthalmic hydrogel preparations with a clear and odorless consistency with a pH of 6.34 and a viscosity of 7.5 cP. This result has met the requirements of eye preparation that is clear, clear, odorless and free of particles[15,17].

*Preparations of chloramphenicol eyedrops*The results of the preparation of eye drops were clear and odorless liquid with pH 6. These results have met the requirements of eye preparation that is clear, clear, odorless and free of particles, while the pH is in the pH range of stability[15].

The evaluation of chloramphenicol opthalmic hydrogel preparations

The evaluation of chloramphenicol opthalmic hydrogel was conducted to knew the changing of physical or chemical in the preparations may occur during storage, which would affect the stability and activity of the opthalmic hydrogel preparations. Physical observation preparations was done on day 1, 3, 5, 7, 14, 21 and 28. The results of evaluation of hydrogel preparation can be seen in Table 3.

The results showed that the preparation was clear, colorless, odorless. Accordingly, pH test results evaluation of preparation demonstrated during 28 days of storage at room temperature were met the requirements of the pH material of opthalmic hydrogel i.e. 5-7.4.[7] Nevertheless the viscosity showed that the preparation was met the requirement i.e. 5-100 cps[18].

Antibacterial effectiveness test of chloramphenicol ophthalmic hydrogel

The optimization of chloramphenicol concentration of ophthalmic hydrogel preparations and eye drops

The optimization of chloramphenicol concentration was performed to obtain three concentrations with inhibitory diameter which were not significantly different between the ophthalmic hydrogel preparation and the eye drops against *B. subtilis* and *S. aureus* bacteria. This test was performed on six concentrations, i.e 50%, 40%, 30%, 20%, 10% and 5%. The diameter inhibitory data can be seen in the following Table 4.

From Table 4, it can be seen at concentrations of 50%, 40%, 30%, 20%, 10% and 5% tested on ophthalmic hydrogel preparations and eye drops, three concentrations having nearly the same diameter of the inhibition between the two preparations - bacterial samples of *B. subtilis* and *S. aureus*, i.e at concentrations of 20%, 10% and 5%.

Antibacterial effectiveness test of chloramphenicol ophthalmic hydrogel

The purpose of the stage was to compare the antibacterial effectiveness of chloramphenicol preparations in the hydrogel against the eye drops dosage forms. The testing was achieved by the diffusion method, whereas conducted for 28 days of storage time. The results of the measurement of the diameter drags zone from opthalmic hydrogel and eye drops preparations can be seen in Table 5.

From the results of the table 5, the preparation of chloramphenicol ophthalmic hydrogel and chloramphenicol eye drops against *S. aureus* bacteria showed that there was a difference between the inhibitory diameters of each eye preparation. Based on the result of measurement of the inhibitory diameter of each dosage give different effect to each other over the storage time for 28 days. The difference in diameter of the inhibitory was not very significant, it can be seen at concentrations of 20%, 10% and 5% of the ophthalmic hydrogel preparations obtained inhibitory diameter of 2.98 cm; 2.69 cm and 2.46 cm, compared with eye drops at concentrations of 20%, 10% and 5%, i.e 2.90 cm; 2.64 cm and 2.55 cm. This showed that more higher concentration of chloramphenicol, the larger the inhibitory diameter was formed.

In chloramphenicol drip preparations it was seen that the largest inhibitory diameter was found at concentrations of 20%, 10% and 5% i.e 2.87 cm; 2.76 cm and 2.48 cm. When compared with the ophthalmic hydrogel preparation there was a difference of non-significant inhibitory diameter, i.e at the concentration of 20%, 10% and 5% of the ophthalmic hydrogel preparations obtained inhibition diameter was 2.85 cm; 2.58 cm and 2.42 cm. This showed that both stocks were equally effective in inhibiting the growth of *B. subtilis* bacteria.

Determination of minimum growth inhibitory concentration (MIC)

The determination of the minimum growth inhibitory concentration (MIC) was performed on a 0.5% chloramphenicol hydrogel preparation by the tube dilution method. In the process of determining MIC, a sample solution with various concentrations was made using multilevel dilutions to establish the smallest concentrations that can still inhibited the growth of test bacteria. The results of MIC determination can be seen in Table 6.

Based on Table 6, the results showed that the smallest concentration of chloramphenicol hydrogel preparations against test bacteria *S. aureus* was at the range of 0.625 - 10% and the smallest concentration of chloramphenicol hydrogel preparations against *B. subtilis* was 0.625 - 5%.

CONCLUSION

The chloramphenicol eye drops and hydrogel preparations showed that both preparations were equally effective in inhibition of *Staphylococcus aureus* and *Bacillus subtilis* growth. The minimum inhibitory concentration growth of the hydrogel opthalmic preparations against the bacteria *Bacillus subtilis* was 10% and *Staphylococcus aureus* at concentration 20%. In the evaluation of the physical preparation includes organoleptic, pH, viscosity showed good results, and still within the range of requirements.

REFERENCES

1. Iwalokun BA, O Afolabi, AA Kehinde, MT Niemogha and FO Nwaokorie. Bacteriologic and plasmid analysis of etiologic agentsof conjunctivitis in Lagos, Nigeria. J Ophthal Inflamm Infect. 2011; 1: 95–103.
2. Goodman, Gilman. Manual of Pharmacology and Therapeutics. The Mc Graw Hill, USA. 2008.
3. Friedlaender MH. A review of the causes and treatment of bacterial and allergic conjunctivitis. Clin Ther. 1995; 17(5): 800-810.
4. L. Flis. Asymptomatic and Symptomatic Ocular Infections. Medical University of Lubin. 2006.
5. Siswandono and B Soekardjo. Medicinal Chemistry. Universitas Airlangga Press, Surabaya. 2000.
6. Kirchhof S, Goepferich AM, Brandl FP. Hydrogels in ophthalmic applications. Eur. J. Pharm. Biopharm. 2015; 95: 227–238.
7. Maheswara R, Firoz S, Rajalalakshmi R, Ashok Kumar CK. Design and evaluation of chloramphenicol thermoreversible insitu gels for occular drug delivery. International Journal of Innovative Pharmaceutical Research. 2011; 2(2):131-138.

8. Mundada AS, Avari JG, Mehta SP, Pandit SS, Patil AT. Recent Advances in Ophthalmic Drug Delivery System. Pharm Rev. 2008; 6(1): 93-473
9. Suryawanshi S, Kunjwani HK, Kawade JV, Alkunte MA, Yadav DJ. Novel Polymeric in Situ Gels for Ophthalmic Drug Delivery System. International Journal of Research in Pharmacy and Science. 2012; 2(1): 67-83.
10. Shastri DH, Patel LD. A Novel Alternative to Ocular Drug Delivery System: Hydrogel. International Journal of Pharmaceutical Research. 2010; 2(1): 1-13.
11. Mehta M, Viral S, Upadhay UM. Ophthalmic Drug Delivery with Emphasis on in Situ Gel System. IJPI's Journal of Pharmaceutics and Cosmetology. 2011; 2(7) : 2229-6832.
12. Nanjundswamy NG, Fatima S, Sholapur HN. A Review on Hydrogels and Its Use in In Situ Ocular Drug Delivery. Indian Journal of Novel Drug Delivery. 2009; 1(1): 11-17.
13. Shashank NN, BS Sogali, RS Thakur. Formulation and evaluation of pH triggered in situ ophthalmic gel of moxifloxacin hydrochloride. Int J Pharm Pharm Sci. 2012; 4(2): 452-459.
14. Alfanso R and Gennaro. Ophthalmic preparation: Remington pharmaceutical sciences.18th ed. Mack publishing, Pennsylvania. 1990**.**
15. Ministry of Health RI. Indonesian Pharmacopoeia. Fourth Edition. Indonesia. 1995**.**
16. M. Prescott. Microbiology. 5th Edition. Mc Graw Hill. New York, USA. 2002**.**
17. Nagesh C, M Patil, Chandrashekhara, R Sutar. A novel in situ gel for sustained ophthalmic delivery of Ciprofloxacin hydrochloride and Dexamethasone - design and characterization. *Der Pharmacia Lettre*. 2012; 4(3): 821-827.
18. Hitesh A, Jayvadan K, Kalpesh N, Ravi R. Ophthalmic Drug Delivery System. *Der pharmacia Lettre*. 2010; 2(4): 100-115.