MICROBIAL ASSAY OF CYPROFLOXACIN IN A BONE IMPLANT (CHITOSAN –BOVINE HYDROXYAPATITE WITH CROSS-LINKER GLUTARALDEHYDE) TOWARDS *Staphilococcus aureus* ATCC25923

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INTRODUCTION

Bone is one part of the body has an important role to support the body's physiological functions (Porter et al., 2009). Complications of bone diseases and bone disorders caused by traumatic accidents may result in a gap (defect) on the bone. The healing process of damage or fracture is determined by the level of trauma and soft tissue damage (Strobel et al., 2011). Some cases of damage or injury to the bone can not undergo natural recovery (Porter et al., 2009). Therefore, clinical rehabilitation to overcome the defect on the bone is expected to increase in line with population growth (Mourino et al., 2010). Treatment rehabilitation of bone cannot be separated from the risk of infection complications. Complications of bacterial infections can be treated with antibiotics. However, in the case of a crack (defect) occurs devascularity of bone tissue so that the delivery of antibiotics to the target tissue to be blocked. This resulted in the concentration of the antibiotic to the target so low that it cannot penetrate the bacteria. The condition can lead to bacterial resistance to antibiotics (Li et al., 2010). A high dose of antibiotics in the long term experienced problems because it can cause systemic toxicity and side effects (Mourino et al., 2010). To overcome these problems, antibiotics can be done locally using a certain drug delivery systems. The purpose of such delivery systems is to provide drug concentration in a specific location and ensure the drug release profile for a certain time period (Dubnika et al., 2012). Drug delivery locally has several advantages, among others, (a) the systemic effects can be avoided, (b) the amount of drugs used less and secure, and (c) the efficacy and efficiency of drug delivery locally can be achieved (Harmankaya et al., 2013). Administration of antibiotics locally also to minimize side effects and risk of toxicity compared to administration of systemic antibiotics. In addition, antibiotics locally also allows conduction in target tissues with high concentration (Mourino et al., 2010). The release of antibiotics on the target network is expected to last continuously for a certain time and achieve a greater concentration than the minimum inhibitory concentration (MIC). Drug delivery systems in a controlled manner (controlled release system) can help increase the bioavailability of antibiotics in target tissues. The system is designed to release the drug at the expected location at a rate appropriate for a certain time period (Mourino et al., 2010). In a previous study showed that a good composite is Ciprofloxacin: BHA: Chitosan = 10:30:60. Cross linker with glutaraldehyde (GA) 0.7% and with 10% active ingredient Ciprofloxacin can release Cyprofloxacin for 30 days (Hendradi et al, 2015). This research will be seen potency against Staphilococcus aureus ATCC25923 Ciprofloxacin for 30 days.

MATERIAL AND METHODS

Materials

Ciprofloxacin (Shangyu Jingxin Pharmaceutical Co. Ltd) ; Bovine Hydroxyapatite (BHA) diperoleh dari Bank Jaringan RSUD DR Soetomo Surabaya; Chitosan (Biotech Indonesia); glutaraldehid 25% p.a (Merck Milipore-German); asam asetat glacial p.a (Merck), Na_2HPO_4 p.a (Merck), K_2HPO_4 p.a, KH_2PO_4 p.a, NaCl p.a (Merck-German) and Aquabidest

Methods

1.Formulation of Bovine Hydroxyapatite - chitosan-ciprofloxacin implant

The composition of formulations of implant before adding glutaraldehyde was mentioned in Table 1. The implant produced by compression method. Ciprofloxacin were dissolved in aquabidest, Bovine Hydroxyapatite added gradually and mixed until homogen with ciprofloxacin. Chitosan powder were added to ciprofloxacin-Bovine Hydroxyapatite blend and mixed until homogen. Aquabidest were added gradually with continous stirring until form wet granules mass. Wet granules mass were sieved using 1 mm siever and dried overnight (24 hours) at 40 °C to obtain dried granules. Dried granules were immersed in glutaraldehyde solution (0.7% concentration) for 24 hours until the colour was change. Granules were washed with aquabidestilata to remove the residual glutaraldehyde. At the final stage, granules were washed

with phosphate buffer saline (PBS) pH 7.40. Granules were dried in oven at 40 °C for 24 hours. Dried granules were weighed 100 mg, pressed using tablet press machine with 4.0 mm diameter and the compression pressure was 2 tons (Hendradi et al, 2015).

Table 1. The composition of implant formulation

Compound	Concentration (%)
Cyprofloxacin	10
BHA	30
Chitosan	60

2. Released Study of Cyprofloxacin

The release study of cyprofloxacin from implant was done as sample for test potential. Implant was placed in a vial containing 5 ml of phosphate buffer saline (PBS) pH 7.4. Vial was placed in a shelf and incubated in waterbath at 37 °C ± 0.5 °C. Sampling was conducted by pippeting 1 ml of elution fluids at predetermined time intervals (1, 2, 3, until ….30) day and replaced with fresh buffer to maintain sink condition. Appropriate dilution was prepared using phosphate buffer saline (PBS) pH 7.4. The release of ciprofloxacin HCL from the implants was determined in triplicate using microbial assay.

3. Optimization of the minimum inhibitory concentration (MIC)

Made six standard solution with a concentration of cyprofloxacin 0.06 ug/ml - 2 µg/ml. Cyprofloxacin standard solution that has been created is inserted into the hole that has formed on nutrient agar, which had previously been inoculated with Staphylococcus aureus ATCC 25923. Incubation for 24 hours at a temperature of 37°C. Observe
the inhibition zone is formed. Lowest levels of inhibitory zone where there is a minimum inhibitory concentration of cyprofloxacin.

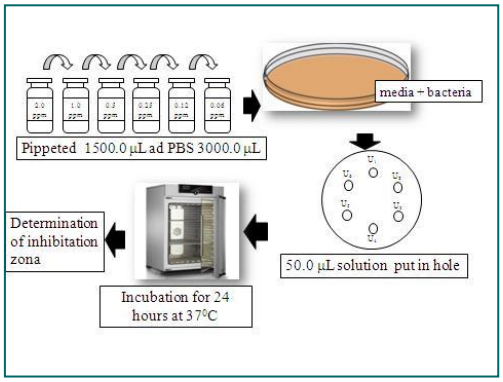

Figure 1. MIC Determination of yiprofloxacin towards Staphylococcus aureus ATCC 25923

4. Test potency dilution method of antibiotic

Test antibiotic poteny dilution method prints holes (wells) design 3-3. A total of 10 ml of inoculum of Staphylococcus aureus ATCC 25923 was inserted into the tube containing the seed layer 8 ml media Nutrient Agar that had thawed and then allowed to stand up to a temperature of 45 - 50ºC. Homogenized with a vortex, then poured evenly over the surface of the base layer has been solidified in a petri dish, allowed to solidify. Hole was made in order to use the printer for sterile. Each hole was filled with the test solution and standard solution as 50,0µl for each hole and then incubated at 37ºC for 24 hours. Diameter of inhibition zone formed at each hole was measured by using a caliper. The resulting inhibition zone diameter compared with the border of the effective inhibition zone, the minimum range of 14-16 mm (Depkes RI, 2014)

RESULT AND DISCUSSION

1. Implant of cyprofloxacin

The implant formulation showed in figure 2.

Figure 2. Implant of cyprofloxacin

2. The Minimal Inhibitory Concentration (MIC) of cyprofloxacin

The Minimal Inhibitory Concentration (MIC) of ciprofloxacin towards *Staphylococcus aureus* ATCC 25923 was 2.0 µg/ml showed in table 2 and Figure 3. In figure 3 showed that the MIC of cyprofloxacin was 2.0 µg/ml (U_1)

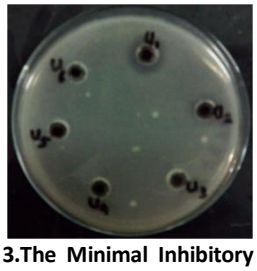

Figure 3.The Minimal Inhibitory Concentration (MIC) of cyprofloxacin towards towards *Staphylococcus aureus* ATCC 25923

Table 2. The Minimal Inhibitory Concentration (MIC) of Cyprofloxacin towards *Staphylococcus aureus* ATCC 25923

Concentration (µg/ml)	Replication 1		Replication 2	
	Zona Inhibition	Diameter of Zona Inhibition	Zona Inhibition	Diameter of Zona Inhibition
2.0	+	14.00 mm	+	14.30 mm
1.0	-		-	
0.5	-		-	
0.25	-		-	
0.12	-		-	
0.06	-		-	

Released Study of Cyprofloxacin

The data of released study was found from the previous study (Hendradi, et.al., 2015). In this data showed that the concentration of Cyprofloxacin release every day was in therapeutics level (2-50 µg/ml). It's showed in figure 4

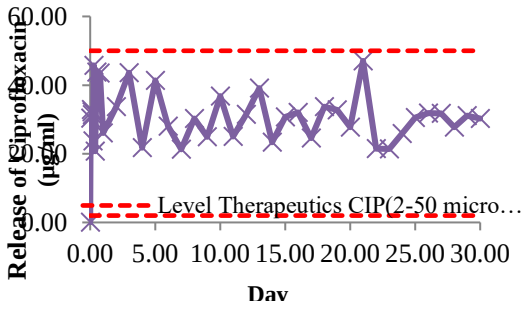

Figure 4. Concentration of cyprofloxacin profil vs time (day) released from implant BHA-chitosan-ciprofloxacin 0.7% glutaraldehyde in phosphate buffer saline Towards Staphylococcus aureus ATCC25923 for 30 days. Each value represents the mean ± S.D. of 3 determinations (Hendradi et al, 2015)

3. *Potency of Cyprofloxacin released to Staphilococcus aureus ATCC 25923*

The result showed that the potency of cyprofloxacin against Staphylococcus aureus ATCC25923 could meet the requirements for antibiotic microbial assay that was 80-125%. The results was similar with the concentration of cyprofloxacin released from implant (Hendradi, et al., 2015). The results of cyprofloxacin towards Staphylococcus aureus ATCC25923 showed in Figure 5. It's mean that the implant formulation of cyprofloxacin had the ability to against Staphylococcus aureus ATCC25923.

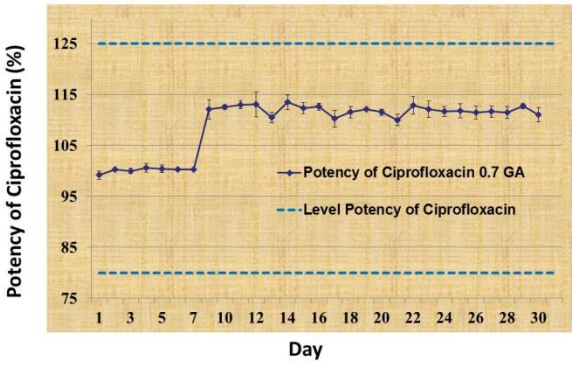

Figure 5. Potency cyprofloxacin profil vs time (day released from implant BHA-chitosan-ciprofloxacin 0.7% glutaraldehyde) in phosphate buffer saline Towards Staphylococcus aureus ATCC25923 for 30 days. Each value represents the mean ± S.D. of 3 determinations

CONCLUSION

The result showed that the potency of cyprofloxacin in the formula could meet the requirements for antibiotic microbial assay that was 80-125% to inhibit the bacteria *Staphylococcus aureus* ATCC 25923 for 30 days.

Acknowledgement

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