Indonesian Journal of
Tropical and Infectious Disease
Vol. 2. No. 1 January–March 2011

BIOCOMPATIBILITY OF AZITROMICYN ON CONNECTIVE TISSUE

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ABSTRACT

Background: *periodontal disease is commonly caused by bacteria, especially actinomyces actinomycetemcomitans and porphyromonas gingivalis have an abilty enter epithelial cells* **Objectives:** *to investigate systemic azithromycin as the antibiotic of choice for periodontal disease based on biocomptability test in connective tissue.* **Material and Methods:** *BHK 21 cell lines were exposed to 0.025%, 0.050%, 0.075%, and 0.1% azithromycin solution for seven times. Samples were put in incubator for 24 hours.* **Result:** *Azitrromycin 0.050%-0.1% showed significant difference between life cells percentage and control, however, azithromycin 0.025% revealed insignificant difference with control.* **Conclusion:** *0.025% azithromycin was considered biocompatible with connective tissue and 0.050% was not.*

Key words: *azithromycin biocomptability, connective tissue, periodontal disease*

INTRODUCTION

Until nowadays, infectious diseases still become very prominent diseases in many developing countries, including Indonesia, and a lot of effort had been done to eliminate these problems.

Periodontitis is an infectious disease caused by bacterial accumulation on tooth surface that cause inflammation, bleeding on probing, pocket formation, periodontal attachment loss, tooth mobility, and tooth lost[1].

Since it was known that most periodontal disease was caused by bacteria, the idea of antibiotics treatment was emerged, the periodontal pathogenic bacteria in the oral cavity will recolonize rapidly after scaling and root planning. The ability of Actinobacillus actinomycetemcomitans to penetrate the soft tissue make it protected from scaling and root planing.[1] This is the rationale for the need of antibiotics in the successful treatment of peridontal disease.

Some classes of antibiotics like penicillin, amoxicillin, erythromycin, azithromycin, tetracycline, metronidazole, and clyndamisin are widely used in dental treatment.[2]

Azithromycin is the latest generation of macrolides, erythromycin-derived but slightly differs in chemical compounds.[3] Azithromycin is a broad spectrum antibiotics, works effectively to gram-positive aerobic, gram-negative aerobic, anaerobes obligates such as Bacteroides fragilis, Fusobacterium sp, and Peptostreptococcus sp. Azithromycin was stated to be effective againts Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis.[4] Some researches supported this opinion by declaring that azithromycin was effective as adjuntive therapy to patients with advanced periodontitis and deep pocket.[5] Azithromycin was also effective for treating *Porphyromonas gingivalis*-involved refractory periodontitis.[1] Systemically-administered azithromycin was shown to be 4–8 times more effective. Its local usages are also expected to be more effective than erythromycin, and required a lower concentration than eritromisin.[6] In order to minimize the side effects of systemic-administered azithromycin and to make it more economical and affordable, the authors consider to prepare azythromycin as a local preparations. However its biocompatibility test needs to be carried out to determine the optimum concentration of therapeutical doses which will not harm the gingival and the surroundings tissues. Biocompatibility test can be performed in cell culture, tissue culture, or culture organ.[7] Connective tissue cells (fibroblasts) are one of cell types which is suitable for biocompatibility observation.

Connective tissue cells (fibroblasts), which is often used in cell culture techniques are the L-929 cells, from rat's lung fibroblasts derivation, and BHK-21 cells, hamster's kidney fibroblasts derivation.[8] Fibroblasts is the largest component of the pulp and periodontal ligament. In periodontal tissue, fibroblasts synthesize collagen and extracellular matrix that preserve the health of periodontal ligament.[9]

The question is whether the lower the concentration of azithromycin, the more biocompatible to the connective tissue cells? It is hypothetized that the lower the concentration of azithromycin, the more biocompatible to the connective tissue cells. The purpose of this study is to determine the biocompatibility of azithromycin at various concentrations to connective tissue, as the basis to produce local azithromycin preparations

RESEARCH METHOD

Preparation of azithromycin solution

Azithromycin powder was prepared to four different concentrations: 0.025%, 0.050%, 0.075%, and 0.1%. The powder is digitally weighed, and diluted in aqua bidestillata to reach certain concentration. These solutions were sterilized with ultra violet.

Preparation of cell culture

The monolayer cell lines of BHK 21 with Eagle's MEM medium was grown in culture bottles, then incubated with 37° for 2 × 24 hours. The goal was that cells can live and repopulate. After 2 × 24 hours, 21 BHK cells are harvested and in re-suspensed in Eagles MEM medium with approximate density 2 × 106 cells / ml. Then it was divided in 35 petri dishes, for each concentration of azithromycin and control needed seven petri dishes. Prepared azithromycin solution respectively 0.025%, 0.050%, 0.075%, and 0.1% was introduced to the petri dishes. For each concentration of azithromycin was 7 times replicated. A cell culture with Eagles MEM medium without azithromycin was used as a control. Then the petri dishes was incubated for 24 hours.

After 24 hours, Eagle's MEM was discarded, then the petri dishes were washed with 20 ml PBS (Phosphate Buffer Saline) twice to clean up the waste products of cell metabolism, only the cells would be left on the petri dishes.

In order to observe and count the changes, the cells attached to the Petri dishes ought to be removed with 0.1 ml trypsyn versene 0.25%. Eagle's MEM medium were used again to obtain cell suspension. To count the cells, 0,1 ml were taken from each suspensions, and added with 0.9 ml added tryphan blue, then brough into the hemocytometer.[7]

The results were obtained by calculating the average number of living cells and dead cells of each box. The calculation were performed with the aid of a microscope with 100 times macnification. The living cells were brightly colored, while the dead cells will absorb the blue color. This calculation were performed for all concentration of azithromycin and control groups. The calculations of each concentration obtained were compared to control group, to determine the biocompatibility azithromycin at different concentrations

RESULTS AND DATA ANALYSIS

The precentage of total cells in azithromycin administered with various concentrations after 24 hours can be seen in table 1 below (the results can be seen in appendix I)

Table 1. The average value and standard intersection number of living cells after treatment for 24 hours

Group	N	Average (%)	deviation
Control	7	100	0,00
0,025%	7	95,11	3,69
0,050%	7	94,76	3,25
0,075%	7	94,61	3,02
0,1%	7	86,23	12,18

The test results showed that after 24 hours the group with 0.025% azithromycin, had the highest percentage of living cells while the 0.1% group had the lowest.

Summary of test results can be seen in table 2 (see Annex III).

Table 2. Summary of test results t-test

KGroup	Kontrol	0,025%	0,050%	0,075%	0,1%
KCOntrol					
0,025%	-				
0,050%	X	-			
0,075%	X	-	-		
0,1%	X	-	-	-	

Description: "x" means no significant difference

From Table 2 it might be observed that treatment with Azithromycin concentration of 0.050%–0.1% showed no significant difference in the percentage of living cells to control, whereas treatment with azithromycin 0.025% showed no significant difference against control. This means that azithromycin at concentrations of 0.025% was biocompatible to connective tissue cells, whereas azithromycin concentrations above 0.050% tend not to be biocompatible.

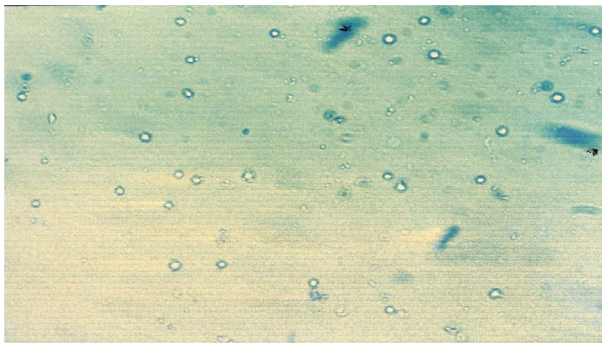

Figure 1 Image of BHK-21 cell lines when a head count by hemositometer
Description: A live cell = B = cell death

DISCUSSION

Recently, research in antibiotics therapy has developed rapidly especially as an adjuvant in aggresive periodontal treatment. Microbiology research data states that mechanical treatment (scaling and root planing) alone was not able to completely eliminate bacterial pathogens, such as Actinobacillus actinomycetemcomitans from local and subgingiva, therefore antibiotic application was considered to be effective as an adjuvant.[6]

Azithromycin is an effective antibiotic against gram-positive aerobic bacteria, gram negative, and strict anaerobes such as Bacteroides fragilis, Fusobacterium sp, Peptostreptococcus sp. In addition, azithromycin also showed good activity against Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis.[4]

Its antimicrobial activity had been proven against oral infections such as periodontitis, periodontal abscess, acute infection of the oral cavity and others. Azithromycin as adjuvant treatment, had shown to be beneficial in reducing deep pockets (> 6mm) in patients with severe periodontitis.[5]

Azithromycin was used in this study, because its broad spectrum activity and effective for the periodontal disease, especially when resistance to tetracycline and erythromycin had been developed,1 while metronidazole would be effective when combined with the other antibiotics.[3]

However, the side effects of systemic adiministered azithromycin should not be nglected. In order to minimize the side effects and increase its effectivity, a new delivery route shall be developed. Local administration of azythhromicin may be suitable for these purposes to support the periodontitis treatment. The local preparations shall not exceed the biocompatibility dose which had been proven not to harm the exposed tissues.

For these reasons we do the biocompatibility test for azithromycin against connective tissue cells (fibroblasts). Fibroblast cell was chosen as the object of exposure because it consists of 65% of gingival fibroblasts as connective tissue cells. In addition, the largest cellular component of the periodontal ligament is fibroblas.[21]

In this study using fibroblast cell cultures was prepared from Baby Hamster Kidney (BHK-21) because according to Ma'at (1999), the best culture material is derived from young tissue cells or embryonic fibroblasts and BHK-21 cells is able to grow and subcultured easily. In addition, BHK-21 fibroblast cells have been frequently used as materials in the dentistry for biocompatibility test.

The biocompatibility of azithromycin at various concentrations on fibroblast cell cultures are presented in Tables 1 and 2. It may be observed that azithromycin at concentrations more 0.050% were not biocompatible to the fibroblast cell culture. Although at a concentration of 0.050% of the average percentage of living cells is still relatively high at 94.76 ± 3.25, but it was statistically different. In azithromicyn-tretaed groups with concentrations 0.025%, 0.050%, 0.075%, and 0.1% was the average number of living cells in a row is 95.1%, 94.76%, 94.61%, and 86.23%. This shows that the higher concentration of azithromycin, the more toxic to the cells. Intensity of cell death depending on the levels of drugs that come into contact with cells, tissue or organ. Cell death increased as a result of azithromycin at a high concentration can be caused by the nature and chemical structure of azithromycin that may interfere with the living cells[15].

Azithromycin inhibits sythesis of bacterial protein at the ribosomal subunits 50 S[16]. Increased doses of chemicals and drugs have altered some vital functions of cells, which manifests as changes in homeostatic mechanisms associated with protein synthesis and cause changes in membrane permeability.[15] If the permeability change, it will cause an increase in intra-cell movement of to the extra cell, so that the it may lose metabolites necessary to preserve life. Azithromycin dissolve in water which cause it to be more easily in penetrating the cell's membrane, and may cause intra-cell disturbances and may cause cell death[16]. In this study, the dead cells absorb the blue color from blue tryphan because of the disruption in cells' membrane permeability. Azithromycin concentrations below 0.050% was shown to be biocompatible. Azithromycin toxicity was increasing as the concentration increase. This means that the drug has the potential capability to cause tissue destruction. This is in accordance with the opinion stating that all substances may be considered toxic, depend on its dosages.[20]

CONCLUSIONS AND SUGGESTIONS

Conclusion

Azithromycin was shown to be biocompatible to tissues at concentrations below 0.050%.

Suggestions

More researches shall be done to demonstrate the effectivity of azithromycin in biocompatible concentration to inhibit bacteria that cause periodontal disease before preparing local azithromycin for clinical usages.

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