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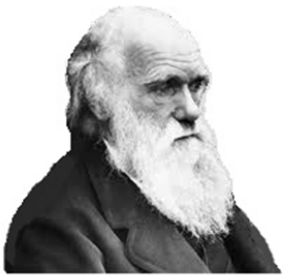

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The usage of antibiotics for prevention of contamination in 3T3-L1 preadipocyte culture

Eko Fuji Ariyanto[1,2,*], Tenny Putri Wikayani[3], Nurul Qomarilla[3]

[1] Division of Biochemistry and Molecular Biology, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Jl. Raya Bandung Sumedang, KM.21, Sumedang, Indonesia

[2] Research Center for Medical Genetics, Faculty of Medicine, Universitas Padjadjaran, Jl. Eyckman No. 38, Bandung, Indonesia

[3] Cell Culture Laboratory, Faculty of Medicine, Universitas Padjadjaran, Jl. Eyckman No. 38, Bandung, Indonesia

*E-mail address: fuji@unpad.ac.id

ABSTRACT

Background: 3T3-L1 preadipocyte is the most commonly used cell line in *in vivo* studies of obesity. One of the main concerns in 3T3-L1 preadipocyte culture is microorganism contaminations. The objective of this study was to determine the appropriate antibiotics to prevent contaminations in 3T3-L1 cultures. Method: This study used descriptive analysis. Frozen 3T3-L1 preadipocytes were thawed and cultured in DMEM-10% FBS-1% penicillin-streptomycin, DMEM-10% FBS-1% penicillin-streptomycin-fungiezone, or DMEM-10% FBS-0.2% ciprofloxacin 200 mg/100 ml. After 24-hour incubation, the cells were observed under the microscope for any change in the medium colour, presence of abnormal structures, and abnormality in cell morphology. Results: The usage of 1% penicillin-streptomycin, 1% penicillin-streptomycin-fungiezone, or 0.2% ciprofloxacin 200 mg/100 ml maintained the clean medium and conserved normal fibroblast-like morphology of the cells. Conclusion: This study suggested that 1% penicillin-streptomycin, 1% penicillin-streptomycin-fungiezone, or 0.2% ciprofloxacin 200 mg/100 ml can be utilized in 3T3-L1 preadipocyte cultures to prevent contaminations.

***Keywords*:** 3T3-L1 preadipocytes, antibiotics, contamination

1. INTRODUCTION

Microorganism contamination, typically caused by virus, bacteria, mycoplasma, or endotoxins, is considered as one of the main problems occurred during cell culture, including

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3T3-L1 preadipocyte culture[1]. Commonly, antibiotics are used in cell culture to prevent contaminations[2]. Inappropriate usage of antibiotics during cell culture, however, can result in less optimal culture and might interfere experimental results[3]. Several antibiotics widely used in cell culture are penicillin-streptomycin, penicillin-streptomycin-fungiezone, and ciprofloxacin.

3T3-L1 is a cell line of preadipocytes which are originated from mouse 3T3 cells[4]. It has fibroblast-like morphology, and, when induced with adipogenic cocktails (isobuthyl-methyl-xanthine, dexamethasone, and insulin), can differentiate into mature adipocyte, characterized by lipid droplets and increased adipogenic gene expressions[5]. One of pathologic conditions associated with abnormal adipocyte differentiation and excess of lipid accumulation is obesity[6].

Obesity is still considered as one of the main health problems around the world. The prevalence of obesity is still increasing globally[7-10]. Many studies revealed that it is closely correlated with several metabolic diseases, such as coronary heart disease, hypertension, diabetes mellitus, and stroke[11]. 3T3-L1 cell line was the most widely used preadipocytes in *in vivo* studies elaborating adipocyte differentiation and its significance to obesity[12]. Optimal culture of 3T3-L1 preadipocytes, therefore, is crucial in conducting various studies to formulate strategies to treat and prevent obesity. The objective of this study was to determine the suitable antibiotics for 3T3-L1 preadipocyte cultures.

2. MATERIALS AND METHODS

This study used descriptive analysis. 3T3-L1 preadipocytes were obtained from Cell Culture Laboratory, Faculty of Medicine, Universitas Padjadjaran. Briefly, frozen cell stocks were thawed and cultured in three different antibiotics-containing Dulbecco's Modified Eagle Medium (DMEM, Gibco)-10% FBS. The cells were then observed for the existence of contamination and abnormal morphology of the cells. Three different antibiotics tested in this study were 1% penicillin-streptomycin (Sigma), 1% penicillin-streptomycin-fungiezone (HyClone), and 0.2% ciprofloxacin 200 mg/100 ml (Finusolprima Farma).

The details of the procedures were described as follows. One tube of cell stock (1 ml volume, containing approximately 1×10^4 cells), previously frozen at -80 °C in fetal bovine serum (FBS, Gibco)-10% DMSO (Sigma) medium, was firstly heated at 37 °C at waterbath until it got thawed. 300 ul of cell solution (containing approximately 3×10^3 cells) was then added to 15 ml tube containing 2 ml of DMEM-10% FBS-1% penicillin-streptomycin, DMEM-10% FBS-1% penicillin-streptomycin-fungiezone, or DMEM-10% FBS-0.2% ciprofloxacin 200 mg/100 ml.

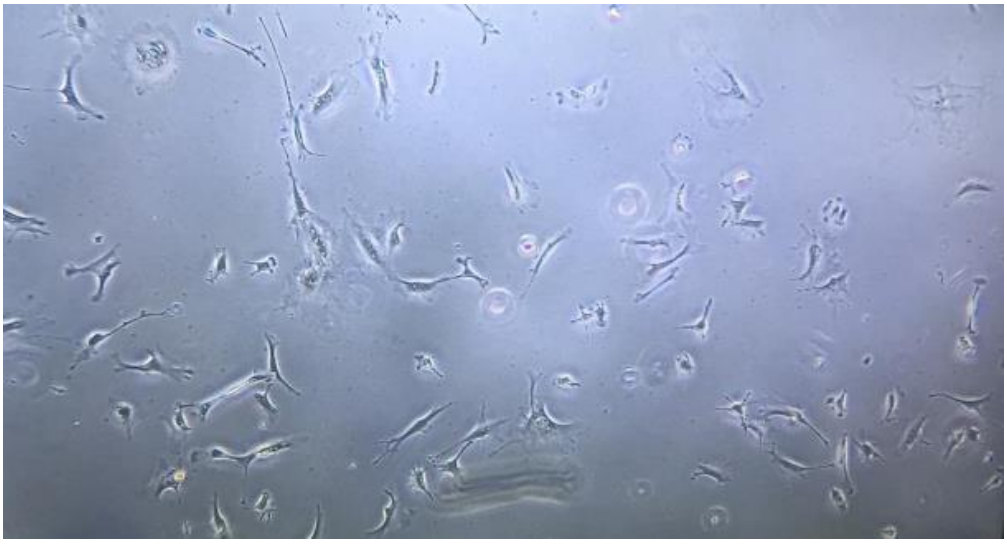
The tubes were centrifuged at 800 rpm for 5 minutes to remove the DMSO. The medium was discarded and the cell pellet was then suspended in 5 ml of DMEM-10% FBS-1% penicillin-streptomycin, DMEM-10% FBS-1% penicillin-streptomycin-fungiezone, or DMEM-10% FBS-0.2% ciprofloxacin 200 mg/100 ml, by rattling the tubes and pipetting. The cell suspensions were transferred to cell culture flasks (surface area 25 cm^2, TPP). The cells were incubated at 37 °C, 5% CO_2 for 24 hours.

The flasks were then observed under the microscope for the existence of contamination in the medium (characterized by cloudy medium and prominent black dots in the medium). The cells attached to the base of the flasks were also observed to see any abnormal morphology.

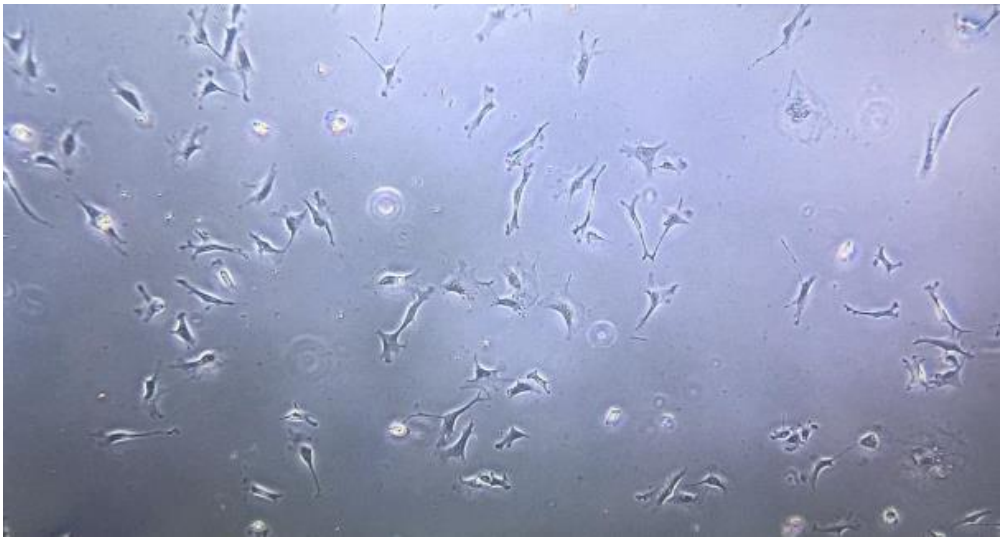
3. RESULTS

The result showed that whether1% penicillin-streptomycin, 1% penicillin-streptomycin-fungiezone, or 0.2% ciprofloxacin 200 mg/100 ml prevented the samples from microorganism contamination. Microscopic pictures showed clean culture medium without prominent black dots in the medium (Figure 1).

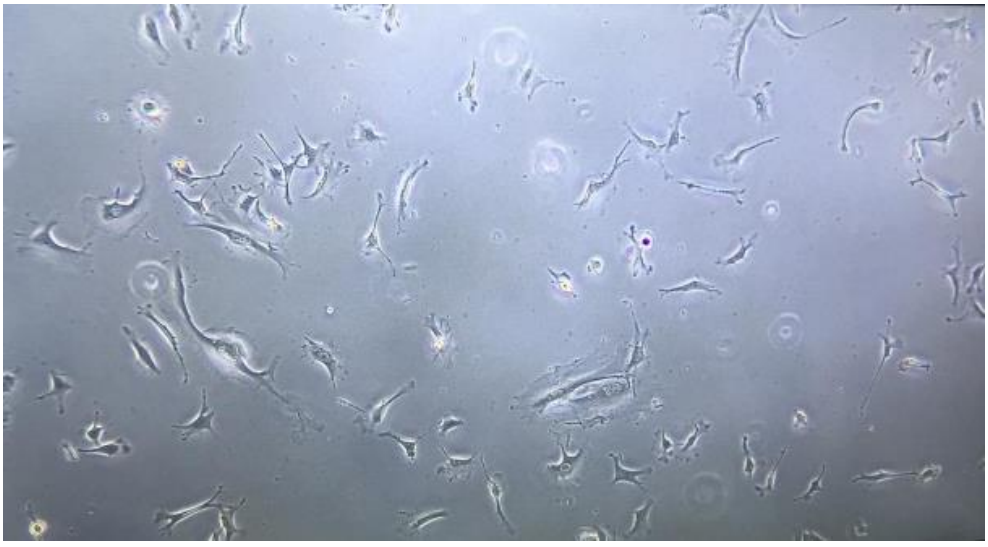
Observation on the cell morphology concluded that the antibiotics supplementation using 1% penicillin-streptomycin, 1% penicillin-streptomycin-fungiezone, or 0.2% ciprofloxacin 200 mg/100 ml into medium conserved the normal fibroblast-like morphology of 3T3-L1 preadipocytes (Figure 1).



(a) 1% penicillin-streptomycin



(b) 1% penicillin-streptomycin-fungiezone



(c) 0.2% ciprofloxacin 200 mg/100 ml

Figure 1(a,b,c). Microscopic pictures of 3T3-L1 after 24 hour incubation. The cells were cultured in DMEM-10% FBS supplemented with three different antibiotics as indicated. The microscopic view used 100× magnification.

4. DISCUSSION

The purpose of this study was to determine the antibiotics which can optimally be used in 3T3-L1 culture to prevent microorganism contamination. Our result found that 1% penicillin-streptomycin, 1% penicillin-streptomycin-fungiezone, or 0.2% ciprofloxacin 200 mg/100 ml can prevent contamination in 3T3-L1 preadipocyte culture. Furthermore, all three antibiotics maintained the normal morphology of preadipocytes.

Contamination in cell culture can be caused by virus, bacteria, mycoplasma, or endotoxins[1]. It is often accompanied by cloudy medium and prominent black dots which can be observed under the microscope. Previous study revealed that microorganism contamination can affect cells' gene expressions, cellular metabolism, and cell growth[13]. Our study unravelled that supplementing culture medium with 1% penicillin-streptomycin, 1% penicillin-streptomycin-fungiezone, or 0.2% ciprofloxacin 200 mg/100 ml can maintain clean medium without the presence of black dots suggesting prevention of contamination in the culture medium.

Our study also found that addition of 1% penicillin-streptomycin, 1% penicillin-streptomycin-fungiezone, or 0.2% ciprofloxacin 200 mg/100 ml maintained the normal morphology of 3T3-L1 preadipocytes. This finding suggested that these antibiotics were not toxic for 3T3-L1 cells, so it might be safe to use them for culturing this cell line. However, further analysis on gene expression is very important to confirm this hypothesis, since previous study reported antibiotic-induced changes in gene expression in liver cell line HepG2[3].

Our study revealed that 1% penicillin-streptomycin, 1% penicillin-streptomycin-fungiezone, or 0.2% ciprofloxacin 200 mg/100 ml had similar effect on preventing 3T3-L1 culture from microorganism contamination and maintaining normal morphology of the cells.

Further study is required to evaluate whether these antibiotics affect adipocyte differentiation under adipogenic induction.

5. CONCLUSION

As the conclusion, 1% penicillin-streptomycin, 1% penicillin-streptomycin-fungiezone, or 0.2% ciprofloxacin 200 mg/100 ml can be used to prevent contaminations during 3T3-L1 preadipocyte culture.

Acknowledgement

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