JOSS:
Journal of Social Science
Volume 2 Number 4 April 2023
p- ISSN 2963-1866- e-ISSN 2963-8909

EFFECT OF GENTAMICIN AND NEOMYCIN+BACITRACIN ON DEOXYRIBONUCLEASE ACTIVITY I ON THE QUALITY OF HUMAN DNA OF SALIVARY ORIGIN

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KEYWORDS
deoxyribonucleic acid; deoxyribonuclease ;gentamicin, neomycin, saliva

ABSTRACT
Deoxyribonucleic acid (DNA) can be found in saliva and various other sources. Saliva contains epithelial cells and leukocytes that are released into the oral cavity. Cells in saliva are potential sources of DNA for diagnostic purposes. DNA in saliva can be degraded due to the activity of deoxyribonuclease (DNase), an enzyme that catalyzes the hydrolysis of DNA. DNase I is expressed in exocrine cells while DNase II is expressed in macrophages. Inhibition of DNase activity will preserve DNA. Besides being able to kill bacteria, aminoglycosides (i.e. gentamicin and neomycin) can inhibit DNase. Methods: Saliva was collected from 8 subjects. Furthermore, saliva samples from each subject were divided into 4 groups, namely negative control (K1), positive control (K2), gentamicin (K3), and neomycin + bacitracin (K4). DNA was extracted from saliva using the spin column method. DNA from groups K3 and K4 were added to 1 mg/mL gentamicin and 20 mM neomycin + bacitracin, respectively, before adding 2.5 g/mL DNase I (DNA degradation assay). The quality of human genomic DNA from saliva was determined by the presence of the NOTCH2 gene amplicons (~704 bp). DNA was separated by 1% agarose gel electrophoresis and recorded using the gel documentation system. Results: The DNA extracted from saliva using the spin column method had an average concentration of 26.49 + 30.70 ng/mL and an average purity of 1.819 + 0.122. The administration of distilled water as a negative control in the DNA degradation assay showed that no DNA was digested. Gentamicin inhibited the activity of 2.5 mg/mL of DNase I at a concentration of 35 g/mL while neomycin + bacitracin inhibited it at a concentration of 0.8 mM. Conclusion: Certain levels of gentamicin and neomycin+ bacitracin inhibited the activity of 2.5 mg/mL DNase I and maintained the quality of human genomic DNA from saliva.

INTRODUCTION

Deoxyribonucleic acid (DNA) is a macromolecule in the form of a linear polymer composed of nucleotide monomers. The process of transcription and translation of DNA produces products in the form of proteins. DNA can be found in saliva, skin, sweat, blood, teeth, hair, mucus, sperm, and vaginal fluids (Syaifiatul, 2017).

Saliva is an acidic oral liquid (pH 6-7). Three major glands and thousands of minor

salivary glands in the oral cavity are the main components of salivary development (Kaczor-Urbanowicz et al., 2017). Saliva consists of 99% water and the rest is protein, inorganic, and organic substances. Gingival crevicular fluid, cell debris, plaque, bacteria, nasal secretions, epithelial cells, blood, and exogenous substances are also present in saliva. Some oral microbiota are present in saliva and consist of bacteria, archaea, fungi (Valentijn-Benz et al., 2015), protozoa (Khairnar & Parija, 2008), and viruses (Samaranayake & Matsubara, 2017). Most of the organisms in the salivary microbiota can be found in almost all individuals as normal flora. This normal flora consists of Streptococcus, Neisseria, Rothia, Prevotella, Actinomyces, Granulicatella, Porphyromonia, Haemophilus, and Porphyromonas species (Yamashita & Takeshita, 2017). In saliva can be found epithelial cells that are released mucosa of the oral cavity (Garbieri et al., 2017).

Epithelial cells released into saliva could potentially be used as a source of DNA for diagnostic purposes using nucleic acid sources. Saliva is easy to collect because it is non-invasive and there are many (Garbieri et al., 2017). Unfortunately, efforts to obtain human DNA from saliva are not easy because contaminants in saliva have the potential to damage human DNA. Normal flora, enzymes, hormones, immunoglobulins and other biomolecules secreted in saliva have the potential to immediately damage human DNA in saliva (Garbieri et al., 2017).

Deoxyribonuclease (DNase) is an enzyme capable of catalyzing DNA (Kolarevic et al., 2014). There are 2 types of DNase. DNase I is expressed by exocrine cells in the gastrointestinal tract. DNase II is expressed by macrophage cells located throughout the tissue (Keyel, 2017). Inhibition of DNase activity can use aminoglycosides (Krause et al., 2016).

The use of aminoglycosides to preserve human DNA from saliva will be beneficial because aminoglycosides inhibit DNase activity as well as bactericidal properties that kill bacteria in saliva (Kolarevic et al., 2014), (Krause et al., 2016). Aminoglycosides inhibit bacterial protein synthesis by blocking the initiation process or by changing the conformation of amino acids (Krause et al., 2016). In addition, aminoglycosides can enter bacterial cells and increase membrane permeability by involving the electrostatic binding of polycationic aminoglycosides to negatively charged components, such as phospholipids, Gram-positive bacterial teichoic acid, and Gram-negative bacterial phospholipids and lipopolysaccharides (Krause et al., 2016).

The aminoglycosides used in the form of gentamicin and neomycin + bacitracin are types of aminoglycosides that can be purchased cheaply and are also easy to obtain. In addition, gentamicin works as an antibiotic and DNase inhibitor (McGuire et al., 2015).

Preservation of human DNA of salivary origin is possible using aminoglycosides. The assessment of the success of preservation can be assessed in quantity and quality. This study aims to see the effect of aminoglycosides on DNase activity on the quality of human DNA of salivary origin (Alipour et al., 2009).

METHOD RESEARCH

This study used experimental methods. The study was conducted from December 2021

to January 2022. This research was conducted at the Integrated Laboratory of the Faculty of Medicine, University of North Sumatra (Kolarevic et al., 2014). This study used saliva and salivary DNA from 8 subjects. Saliva was divided into four groups, each consisting of Negative control (K1) given Aquades, positive control (K2) given DNAse I, treatment group 1 given gentamicin (K3), and treatment group 2 given neomycin + bacitracin (K4) (Woegerbauer Et Al., 2000).

Research procedure

a. Saliva collection from 10 subjects was collected in one room at 07.00 WIB and was not allowed to brush their teeth and gargle with oral cleaning liquid. Subjects were instructed not to eat and drink for 90 minutes before saliva collection. Next, the subject was given 10 minutes to spit in a salivary pot.
b. DNA isolation preparations. Each saliva of as much as 2 mL is inserted in a microtube then DNA isolation using the spin column method by looking at DNA purity with UV-Vis spectrophotometry and calculated absorbance value of 260 nm divided by absorbance value of 280 nm (A260 / A280), and DNA purity value ranges from 1.8-2.0.
c. Negative control (K1). A total of 1 µg of salivary DNA was given the addition of aqua dest.
d. Positive control (K2). A total of 1 µg of salivary DNA was treated *with DNA degradation assay* with the addition of 2.5 µg/mL DNase I (DN25, Merck).
e. Treatment group (K3). A total of 1 µg of salivary DNA will be added with 1 mg / mL of gentamicin antibiotics and 2.5 µg / mL DNase I (DNA *degradation assay*).
f. Treatment group (K4). A total of 1 µg of salivary DNA will be added with 20 mM of neomycin + bacitracin antibiotics and 2.5 µg / mL DNase I (DNA *degradation assay*)
g. DNA testing with 1% agarose gel. A total of 1.3 g of 1% agarose was included in the Erlenmeyer flask and given 130 mL of 1X TAE buffer.
h. PCR preparation. The PCR method was run using 25 µL of PCR reaction mixture containing 1 ng of salivary DNA as *a template*, 1 µM for each primer, and 200 µM for each dNTP, 2.5 mM MgCl2, 5 µL 5X PCR buffer and also 5 U/µL Taq DNA polymerase in PCR *master mix* (2GFRMKB, Kapa2G Fast ReadyMix, Merck).

RESULT AND DISCUSSION

The following research data are data obtained from 4 test groups, namely the negative control group (K1), positive control (K2), the treatment group given gentamicin (K3), and the treatment group given neomycin + bacitracin (K4) which have each been given salivary DNA of 1 µg.

a. Negative Control (K1) and Positive Control (K2)

Figure 1
DNA degradation assay

Figure 1 is a treatment with a concentration of 10 µL. Lane 1 contains a DNA ladder of 100 bp. Lane 2 is a negative control that is 1 µg DNA + equates. Lane 3 was a positive control containing 1 µg of DNA + 2.5 µg/mL DNase I.

b. Kelompok perlakuan 1 (K3)

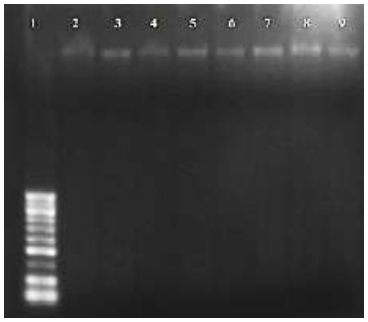

Figure 2
Effect of gentamicin in *DNA degradation assay*

The presence of gentamicin has prevented DNA digestion by DNase I Lane 1 contains a DNA ladder of 100 bp. Each lane 2-9 contains 1 µg of DNA that has been added with gentamicin 35, 70, 140, 280, 560, 1120, 1500, and 2240 µg / mL, and received 2.5 mg / mL DNase I. DNA electrophoresis was carried out 1% agarose gel with conditions of 80 mV, 400 mA, and 90 minutes

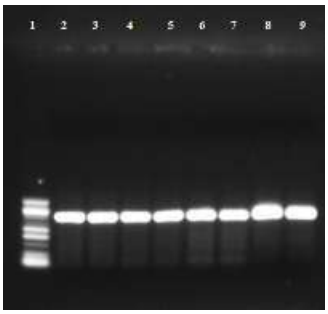

Figure 3

PCR results of human NOTCH2 genes from DNA samples that received additional concentrations of gentamicin and 2.5 mg / mL DNase I.

The human NOTCH2 gene (~704 bp) was successfully amplified from all DNA samples. Lane 1 contains a 100 bp DNA ladder. Each lane 2-9 contains 1 µg of DNA that has been added with gentamicin 35, 70, 140, 280, 560, 1120, 1500, 2240 µg / mL, and received 2.5 mg / mL DNase I. DNA electrophoresis was carried out 1% agarose gel with conditions of 80 mV, 400 mA, and 90 minutes.

c. Treatment group 2 (K4)

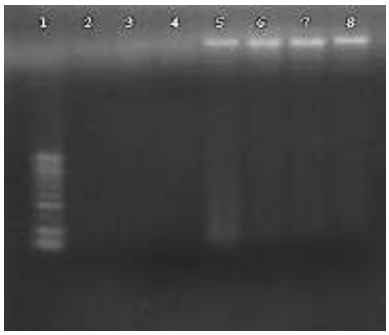

Figure 4

Effect of neomycin in DNA degradation assay.

The concentration of neomycin determines the digestion of DNA by DNase I. A concentration of 0.8 mM has provided minimal inhibition of the activity of 2.5 mg/mL DNase I. Lane 1 contains a DNA ladder of 100 bp. Each lane 2-8 contains 1 µg of DNA that has been added with Nebacetin® 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 mM, and received 2.5 mg / mL DNase I. DNA electrophoresis was carried out 1% agarose gel with conditions of 80 mV, 400 mA, and 90 minutes.

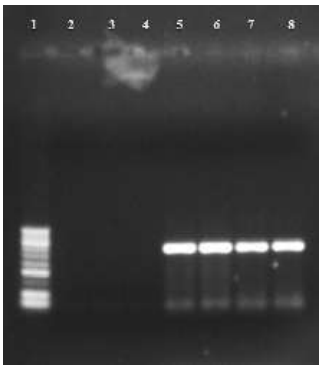

Figure 5
Human NOTCH2 gene with DNA preservation influence with the addition of neomycin in DNA degradation assay.

Presence of human NOTCH2 gene (~704 bp) in agarose gel electrophoresis 1%. Lane 1 contains 100 bp DNA ladder, Lane 2 contains 0.1 mM nebacetin, Lane 3 contains 0.2 mM nebacetin, Lane 4 contains 0.4 mM nebacetin, Lane 5 contains 0.8 mM nebacetin, Lane 6 contains 1.6 mM nebacetin, Lane 7 contains mM nebacetin. 2.0 mM, Lane 8 contains 3.2.

This study aims to determine the effect of aminoglycoside antibiotics on DNase I activity on the quality of human DNA of salivary origin using the spin column method as a DNA isolation method. As well as knowing the effect of gentamicin and neomycin + bacitracin on DNase I activity in human NOTCH2 genes and determining the minimum dose of gentamicin and neomycin + bacitracin in inhibition of DNase I activity in human genome DNA of salivary origin assessed through 1% agarose gel electrophoresis (b / v) and followed by visualization under UV light after ethidium bromide (EtBr) staining was carried out with considering the presence of PCR results in the form of a band measuring ~704 bp.

Based on the results of the research presented above, DNA extraction carried out by the spin column method is following the average purity rule of 1.8-2.0. Then the use of gentamicin with a concentration of 35 µg/mL was sufficient to prevent the digestion of 1 µg of DNA by 2.5 mg/mL DNase I and the use of neomycin + bacitracin with a concentration of 0.8-2.0 mM was sufficient in preventing the digestion of 1 µg of DNA by 2.5 mg/mL DNase I.

The results of this study are different from previous research conducted by McGuire AL in 2015 using antibiotics in the form of gentamicin. McGuire Al stated at a concentration of >35 µg / mL gentamicin can inhibit 2.5 mg / mL Dnase 1.13 Then the results of research conducted by (Alipour et al., 2009) stated that the combination of DNAse and gentamicin can increase the work of gentamicin with a minimum concentration of 64 µg / mL.14 The use of antibiotics in the form of neomycin + bacitracin was also carried out by Ana Kolarevic in 2014 and Markus Woegerbauer in 2000 who states that neomycin can inhibit DNAse I activity at neomycin concentrations of 2 mM.15,16 While bacitracin does not have a major effect on DNAse I. This is reinforced by research conducted by Jerzy Ciesiolka in 2014 which states bacitracin can induce DNA degradation. However, the use of bacitracin in inducing DNA degradation should be greater in concentration, even with a concentration of 23 mM does not change the migration of plasmid DNA in agarose gel by 1%. So in this study, the

use of neomycin was much more instrumental than bacitracin which did not affect 2.5 mg / mL DNAse I.

CONCLUSION

Gentamicin and neomycin + bacitracin can inhibit the activity of 2.5 mg / mL DNase I to maintain the quality of intact DNA of the human genome from saliva. Gentamicin and neomycin + bacitracin inhibit the activity of 2.5 mg / mL DNase I to maintain the NOTCH2 gene (704 bp) Gentamicin can inhibit the activity of 2.5 mg / mL DNase I in the DNA quality of the human genome of salivary origin at a concentration of 35 µg / mL while neomycin + bacitracin inhibits at a concentration of 0.8 mM.

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