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Research Report

Antimicrobial effect of chlorine dioxide on *Actinobacillus actinomycetemcomitans* in diabetes mellitus rats treated with insulin

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ABSTRACT

Background: *Periodontitis is a chronic inflammatory disease of periodontal tissues. Etiology of periodontal disease includes Actinobacillus actinomycetemcomitans (A. actinomycetemcomitans) which is the most predominant disease-causing bacteria found in the gingival sulcus. Periodontitis can be exacerbated by the systemic disease, such as diabetes mellitus considered as a metabolic disease characterized by hyperglycemia due to insulin deficiency. Treatment of periodontitis is then required in patients with type I diabetes to avoid radical reaction that can not only cause bleeding, but can also prevent infection, as a result, topical antimicrobial therapy and blood glucose control are required. Topical antimicrobial chlorine dioxide is a disinfectant that is effective in killing A. actinomycetemcomitans.* ***Purpose:*** *This study is aimed to determine the effects of topical antimicrobial chlorine dioxide gel or rinse on the number of A. actinomycetemcomitans in DM rats treated with insulin.* ***Methods:*** *20 three month old male Wistar rats with weight of 170–200 grams were divided into four groups. First, periodontitis and DM were manipulated into all groups through aloksan injection with dose of 170 mg/kg. Those rats in group I were treated with insulin and chlorine dioxide gel, those in group II were treated with insulin and chlorine dioxide rinse, those in group III were treated with insulin only, and those in group IV were without treatment. In the third and seventh weeks, the number of A. actinomycetemcomitans was measured. The data was tested by using One-Way ANOVA test followed by LSD test.* ***Results:*** *The study showed that chlorine dioxide gel has a greater ability in reducing the number of A. actinomycetemcomitans than chlorine dioxide rinse although both are antimicrobials.* ***Conclusion:*** *It can be concluded that the use of chlorine dioxide gel can more effective to decrease the number of A. actinomycetemcomitans than chlorine dioxide rinse in DM rats treated with insulin therapy.*

Key words: *Periodontitis, Actinobacillus actinomycetemcomitans, diabetes mellitus, insulin, chlorine dioxide*

ABSTRAK

Latar belakang: *Periodontitis adalah suatu penyakit inflamasi kronis jaringan periodontal. Etiologi penyakit periodontal diantaranya adalah* ***A. actinomycetemcomitans yang merupakan bakteri paling dominan penyebab penyakit yang terdapat pada*** *sulkus gingiva. Periodontitis yang terjadi diperparah adanya penyakit sistemik yaitu diabetes mellitus (DM) yang merupakan penyakit metabolik yang ditandai dengan hiperglikemi akibat defisiensi insulin.* ***Perawatan periodontitis pada penderita DM tipe I adalah untuk*** *menghindari tindakan radikal yang dapat menyebabkan perdarahan dan mencegah terjadinya infeksi, sehingga digunakan terapi antimikroba topikal serta kontrol glukosa darah. Antimikroba topikal chlorine dioxide merupakan desinfektan yang efektif dalam membunuh A. actinomycetemcomitans.* ***Tujuan:*** *Penelitian ini bertujuan untuk mengetahui efek antimikroba topikal chlorine dioxide gel atau rinse terhadap jumlah A. actinomycetemcomitans pada tikus DM dengan insulin.* ***Metode:*** *Dua puluh ekor tikus Wistar, jantan, usia 3 bulan, berat 170–200 gram, dibagi menjadi empat kelompok. Semua kelompok sebelumnya dimanipulasi periodontitis serta DM dengan injeksi aloksan dosis 170 mg/kgBB. Kelompok I adalah tikus yang diterapi insulin dan chlorine dioxide gel, kelompok II diterapi insulin dan chlorine dioxide rinse, kelompok III diterapi insulin, serta kelompok IV adalah tikus tanpa dilakukan terapi apapun. Pada minggu ke-3 dan ke-7 dilakukan penghitungan jumlah A. actinomycetemcomitans. Hasil pengukuran di analisis menggunakan uji Anava 1 jalur dilanjutkan dengan uji LSD.* ***Hasil:*** *Penelitian menunjukkan bahwa chlorine dioxide gel mempunyai kemampuan lebih besar dalam menurunkan jumlah A. actinomycetemcomitans dibandingkan chlorine dioxide rinse, walaupun keduanya bersifat antimikroba.*

Kesimpulan: *Dapat disimpulkan bahwa pemberian chlorine dioxide gel lebih efektif menurunkan jumlah A. actinomycetemcomitans dibandingkan kelompok yang diberi chlorine dioxide rinse pada tikus DM dengan terapi insulin.*

Kata kunci: *Periodontitis, Actinobacillus actinomycetemcomitans, diabetes mellitus, insulin, chlorine dioxide*

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INTRODUCTION

Periodontitis is common disease in oral cavity and often associated with various diseases is periodontitis. Periodontitis can be defined as an infectious disease leading to local inflammation of tissues supporting teeth that later can cause the destruction of periodontal ligament and alveolar bone.[1] Bacteria that have a major role on such periodontal tissue destruction are *Actinobacillus actinomycetemcomitans* (*A. actinomycetemcomitans*), *Porphyromonas gingivalis* (*P. gingivalis*), *Prevotella intermedia*, and *Fusobacterium nucleatum*.[2] Early symptoms of periodontitis is caused by the colonization of pathogenic species in periodontal tissue. The fact that bacteria or their products move into the tissue then can cause destruction due to their direct interaction with hospes cells.[3]

Periodontal disease can be exacerbated by systemic diseases, such as diabetes mellitus (DM). The severity of periodontitis in patients with DM is greater than those wihout DM, especially with poor glycemic control, indicated by the increasing of plaque index, gingival index, probing depth, attachment damage, and dental loss. These conditions of patients with DM then lead to the decreasing function of polymorphonuclear (PMN) that can increase the severity of periodontal tissue destruction. Under these conditions, there will be impaired fatty acid metabolism later that causes the damage of the cell functions and homeostasis.[4,5]

As a result, the content of glucose in gingival crevicular fluid of DM patients is higher than in that of non-DM ones. This increasing then may not only alter the microflora environment of oral cavity, and but also be considered as a good growing medium for bacteria, such as *A. actinomycetemcomitans*. Bacteria are actually also found in non-DM patients, but their number is lower than in DM ones.[6]

The level of virulence of *A. actinomycetemcomitans,* furthermore, is characterized by the presence of inflammation modulation, tissue damage induction, and tissue repair inhibition. *A. actinomycetemcomitans* actually produces a virulence factor by producing leucotoxin in the form of lipoproteins that are secreted, especially for killing PMN and macrophages by producing citolethal distending toxin (CDT).[7,8]

The purpose of periodontitis treatment in type I DM patients is not only to avoid radical reaction that can cause bleeding, but also to prevent infection either by eliminating plaque and calculus, or by using antibacterial drugs and blood glucose control.[9] Thus, systemic therapy for type I DM patients is insulin therapy that can make blood glucose level normal.

DM rats treated with insulin can decrease their blood glucose levels close to normal and also the number of bacterial colonies of *A. actinomycetemcomitans* in the gingival sulcus fluid. It means that there is an improvement for periodontal condition, but the healing process of the tissue takes longer time. Thus, antimicrobial therapy is required to accelerate the improvement of the periodontal tissue conditions.[10,11]

Topical antimicrobial drug used is chlorine dioxide. This antimicrobial material is effective to kill bacteria in oral cavity, especially gram-negative bacteria causing periodontitis. Chlorine dioxide used for periodontal therapy is offered in the forms of gel, rinse, solution, or paste. However, antimicrobial therapy used is often in the form of gel or rinse.[12] Therefore, this study is aimed to determine the effects of topical antimicrobial agents, *chlorine dioxide* gel or rinse, on the number of *A. actinomycetemcomitans* in diabetes mellitus rats treated with insulin therapy.

MATERIALS AND METHODS

This study is a experimental study using 20 three-month old Wistar rats with weighing between 170–200 grams. To create periodontitis condition, all of those rats were then bond around the cervical of their lower incisors for 7 days. To prevent the loss of the binding, the thread was sewed on the gingiva. After 7 days, the thread was removed and evaluated in order to know whether there had been any signs of periodontitis involving the changes of tooth servical color into darker red (compared with the normal one, pink), the enlargement or swelling of the gingiva, and the increasing of the depth of the gingival sulcus.

The identification of bacteria was then conducted by using sample cultures of pure *A. actinomycetemcomitans* (Lab. Veterianary Faculty, Gadjah Mada University). The medium used was a modified agar-Tood Hewit Broth + bacitracin 5 units/ml media, in which biochemical test was then conducted for identifying *A. actinomycetemcomitans*. Diabetic rats were then obtained by injecting aloxan with dose of 170 mg/kg dissolved in 0.9% NaCl (physiological saline), and then given subcutaneously. Next, those rats were fasted for 12 hours before and after the injection of aloksan.[13]

Table 1. The mean and standard deviation of the number of *A. actinomycetemcomitans* (CFU/ml) based on the treatment groups and the observation week

Groups	The mean and standard deviation of the number of *A. actinomycetemcomitans*		Decreasing
	Week 3	Week 7	
I	65.000 ± 28.133	1.000 ± 1.414	64.000 ± 28.204
II	58.200 ± 30.449	28.000 ± 5.147	30.200 ± 25.606
III	70.000 ± 16.324	51.000 ± 12.708	19.000 ± 13.583
IV	109.400 ± 18.270	198.800 ± 8.467	-89.400 ± 20.354

Note:
Group I : DM rats treated with insulin + chlorine dioxide gel
Group II : DM rats treated with insulin + chlorine dioxide rinse
Group III : DM rats treated only with insulin
Group IV : DM rats treated without any therapy

Those rats were then divided into four groups, each of which consisted of 5 rats (group I, II, III, IV) randomly. After 2 weeks, blood glucose levels were measured. When blood glucose levels had reached ≥ 250 mg/dl, then the rats indicated suffered from DM. Those rats in Group I were then injected with insulin (5 IU dose of suspension-insulin isophan) subcutaneously every day for 28 days, and then treated with chlorine dioxide gel (oxygene dental gel) with dose of 0.27 ml in the gingival sulcus. Meanwhile, those rats in Group II were injected with insulin, and then treated with chlorine dioxide rinse (oxygene mouthrinse) with dose of 0.27 ml. Those rats in Group III, moreover, were injected with insulin, while those in group IV, as control group, were not treated with any therapies.

The number of bacteria was measured twice, in the third week when those rats were indicated to suffer from DM rats (before being treated with any therapies), and in the seventh week after the rats were treated. Initially, those rats were anesthetized by using ketamine, and then a sterile paperpoint was inserted into the gingival sulcus of the incisors for 10 seconds. Samples were suspended into vortex for 10 seconds. Each 0.1 ml of the solution was diluted into 10^{-2} ml. 1 ml of the dilution result was then planted in agar Tood-Hewit Broth + bacitracin 5 units/ml (modification) media. Next it is known that small round-shaped, convex, and translucent white color *A. actinomycetemcomitans* attached to agar Tood-Hewit Broth + bacitracin 5 units/ml (modification) media. Then,

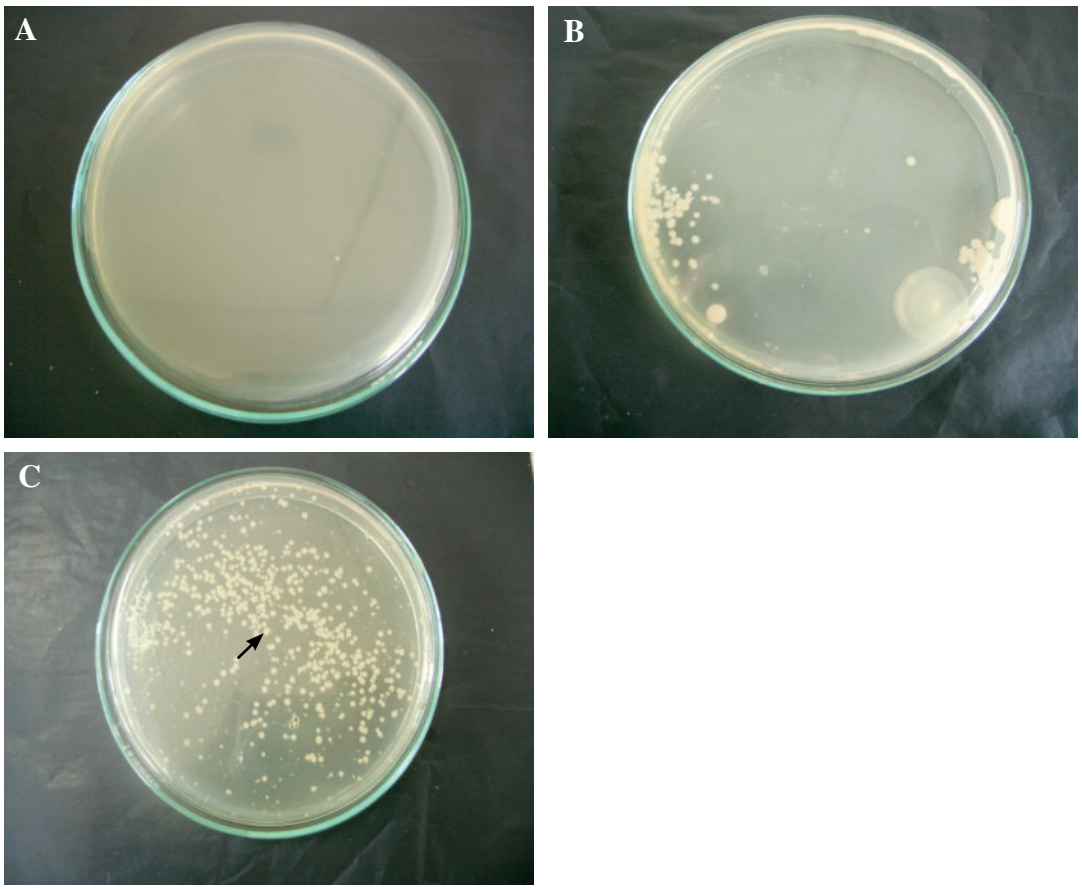

Figure 1. The colonies of *A. actinomycetemcomitans* after 28 days of the treatment.
Note: A) The colonies of *A. actinomycetemcomitans* in DM rats treated with insulin and chlorine dioxide gel; B) The colonies of *A. actinomycetemcomitans* in DM rats treated with insulin and chlorine dioxide rinse; C) The colonies of *A. actinomycetemcomitans* in DM rats treated without any therapy.

to simplify the calculation process of the number of *A. actinomycetemcomitans*, each petridish was divided into four parts. The number of bacteria was then calculated in each petridish. Data obtained from the observations were finally analyzed by using One-Way ANOVA test and then with Least Significant Different (LSD) test.

RESULTS

Calculating the number of *A. actinomycetemcomitans* in DM rats was conducted twice, in the third week (before the therapy) and in the seventh week (after the therapy). The purpose of this calculating of the number of the bacteria is to determine the effects of insulin therapy using chlorine dioxide gel or rinse on the number of *A. actinomycetemcomitans*. The morphology of *A. actinomycetemcomitans* is round, slightly convex, and transparent white color as seen in each treatment group in figure 1.

The mean of the calculating results of the number of *A. actinomycetemcomitans* can be seen in table 1. The results show that there were the differences of the number of *A. actinomycetemcomitans* in the group treated with insulin and *chlorine dioxide gel*, that treated with insulin and *chlorine dioxide rinse,* and that treated with insulin therapy alone. The mean values of the reduction were 64.000 CFU/ml; 30.200 CFU/ml; and 19.000 CFU/ ml. Unlike those groups, group IV had a mean value of the increasing number of the bacteria about 89.400 CFU/ml indicating that those DM rats with treatments in group I, II, and III had lower number of the bacteria than those without any treatment.

To determine the effects of the therapy given to the number of *A. actinomycetemcomitans* in the gingival sulcus, One-Way ANOVA test was conducted as shown in table 2. The statistical test results then showed that there was the highly significant difference of the decreasing number of *A. actinomycetemcomitans* in the gingival sulcus ($p < 0.05$) among those different treatment groups.

Next, LSD test was conducted to determine different effects occurred in each treatment group (Table 3). Based on the obtained results of LSD test, it is known that there was significant difference of the mean of the decreasing number of *A. actinomycetemcomitans* among the groups in the third week and in the seventh week ($p<0.05$), except in the group (II-III), since the mean obtained was not significantly different.

Table 2. The summary of test results of One-Way ANOVA concerning with the decreasing of the number of *A. actinomycetemcomitans*

Source of Variance	jk	dk	MS	F	p
Inter Groups	66098.950	3	22032.983	42.991	.001*
Within Groups	8200.000	16	512.500		
Total	74298.950	19			

Note: jk = Sum of square, dk = Degree of freedom, MS = Variance, p = Level of significance; * = Significant

Table 3. The summary of LSD test results concerning with the decreasing of the number of *A. actinomycetemcomitans*

Groups	I	II	III	IV
I	–	33.800*	45.000*	153.400*
II		–	11.200	119.600*
III			–	108.400*
IV				–

Note: * = Significant

DISCUSSION

Based on the data obtained in this study, it is known that the severity of periodontal disease that occurred in those rats was caused by systemic diseases, namely DM. Although it is known that the main factor of periodontal disease was bacterial plaque, the presence of systemic disease could also decrease the immunity of oral cavity, so it could affect the progression of periodontal disease.[3]

Those DM rats were then treated with systemic therapies, which were insulin therapy and topical antimicrobial therapy by using chlorine dioxide gel or chlorine dioxide rinse. The working mechanism of insulin was that after being synthesized, insulin was secreted into the blood circulation in the free forms, and then moved to the target cells. Insulin actually would work after binding to specific receptors. These insulin receptors have two main functions: to distinguish other ingredients from insulin, and then to tie them fast and reversibly, to form complex insulin-receptor formation which could stimulate a series of intracellular events leading to the cellular effects of characteristic insulin.[14] Insulin therapy given regularly to those DM rats could make their metabolism and glucose transport smooth, so their blood glucose levels could be normal and be within safe limits.

Those DM rats which were not treated with insulin therapy would get hyperglycemia condition resulting in the formation of advanced glycation end-product (AGEs) leading to endothelial oxidative stress, so the disruption of blood vessels in periodontal tissues could occur. Without insulin therapy, people with diabetes will easily get infection because there have been damages to the function of neutrophils and monocytes, hypofunction of immune cells, and neuropathy (micro and macro circulation disorders) exacerbating periodontal disease. Therefore, blood glucose control with insulin therapy can prevent infection and complication.[15,16]

Insulin therapy actually also plays a role in lowering the number of *A. actinomycetemcomitans* since with a controlled blood glucose levels the function of leukocytes (PMN) and macrophages as body defense could become normal, so the metabolism of the body could become normal again. This

condition then causes *A. actinomycetemcomitans* become blocked and lack of supports from environment, so its number in the gingival sulcus become lowered. Thus, it indicates that the systemic insulin therapy conducted could also improve the health condition and periodontal tissues of those DM rats, but it would require very long time. Besides that, periodontal treatment given to people with diabetes could also avoid radical acts lead to infection. Therefore, local therapy was conducted by using topical antimicrobial agents, chlorine dioxide gel and chlorine dioxide rinse. These materials are actually the most widely used disinfectants for periodontitis therapy.[11,17] Chlorine dioxide gel and rinse actually contain the same basic material, chlorine dioxide. Chlorine dioxide is antimicrobial, the base material of gel and rinse with high redox capacity.[17]

It is known that there was the difference of the reduction of the number of *A. actinomycetemcomitans* between in chlorine dioxide gel and in chlorine dioxide rinse (Table 3). This difference may be caused by the fact that chlorine dioxide therapy is actually more effective in the form of gel applied into the periodontal pocket. Another reason may also be caused by the fact that chlorine dioxide gel containing chlorine dioxide (stabilized chlorine dioxide) can kill pathogenic bacteria, especially *A. actinomycetemcomitans*. The working mechanism of chlorine dioxide, furthermore, causes reaction with natural organic substances contained in the cell walls of bacteria leading to the impaired cellular mechanisms of those bacteria. It is because chlorine dioxide reacts directly with amino acids and RNA causing the inhibition of protein production in bacterial cells. Chlorine dioxide then affects the bacterial cell membrane by altering protein and fat membranes as well as by inhibiting the process of bacterial respiration.[18] This fact is also supported by a research conducted by Bayaty *et al*.,[19] showing that topical antimicrobial agent, chlorine dioxide gel, is very effective for killing aerobic and anaerobic bacteria in either supra or subgingival plaque. There are two reasons explaining how chlorine dioxide gel can kill bacteria or viruses. First, chlorine dioxide gel can react with specific biomolecules. Second, the effects of chlorine dioxide gel on bacteria are through physiological function since chlorine dioxide gel is a topical antimicrobial agent that is very powerful in killing plaque bacteria.

Therefore, the topical antimicrobial agent, chlorine dioxide gel, is better in reducing the number of *A. actinomycetemcomitans* that chlorine dioxide rinse. Based on its application mechanism, this gel material gives greater advantages in its use that is more resistant in pockets because its consistency is more concentrated towards the flow of gingival and saliva creviculer fluids due to oral activities, such as mastication, speaking, and so forth. The ability of periodotophatic bacteria, especially *A. actinomycetemcomitans*, in conducting tissue attachment and penetration is actually very high, and is even able to penetrate into the gingival connective tissue. It then leads to more resistant periodontal disease. Therefore, chlorine dioxide therapy in the form of gel is considered as disinfectant that either can be resorbed or can kill bacteria inside and outside the tissues, so the reaction of the material with bacteria is more increasing, and then can damage bacterial cell membranes.[12,20]

Chlorine dioxide rinse, on the other hand, can neutralize VSCs in oral cavity. Amino acids, cysteine and methionin (precursors of VSCs), can even be removed by oxidation reactions. Chemically, chlorine dioxide produces oxygen that can reduce both VSCs production and bad breath by breaking the bonds of sulfide hydrogen and mercaptan methyl.[21] Chlorine dioxide rinse is applied into the gingival sulcus by spraying, so the material will be more soluble due to the flow of gingival and saliva creviculer fluids which generally have a short duration. This then causes chlorine dioxide rinse has limited ability to kill bacteria. According to Greenstein *et al.,*[22] the use of antimicrobials in the form of mouth rinse is less effective not only because it is difficult for the antimicrobial agent to achieve the gingival sulcus or periodontal pocket, but also because the agent will be quickly cleared by gingival or saliva creviculer fluids from the mucosal surface. This then causes chlorine dioxide in the form of rinse is less effective in killing *A. actinomycetemcomitans* than that in the form of gel. It can be concluded that the induction of topical antimicrobial chlorine dioxide gel can reduce the number of *A. actinomycetemcomitans* more than the induction of chlorine dioxide rinse in DM rats treated with insulin therapy.

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