Tropical Journal of Pharmaceutical Research February 2018; 17 (2): 233-237
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v17i2.6

Original Research Article

Effect of electrolyzed reduced water on malondialdehyde levels and neutrophil cells in aggressive periodontitis

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Sent for review: 25 October 2017 *Revised accepted: 21 January 2018*

Abstract

Purpose: *To evaluate the effects of electrolyzed reduced water (ERW) on malondialdehyde (MDA) levels and neutrophil cells in Wistar rats suffering from aggressive periodontitis.*
Methods: *Wistar rats (Rattus norvegicus) were infected with A. actinomycetemcomitans before being divided into a control group and a treatment group. The control group was treated orally with distilled water (pH 7.0), while the treatment group was administered with ERW (pH 9.8, 20 ml/day) for three successive days. Gingival tissue taken post-treatment was examined to determine the number of neutrophils and MDA level. Hematoxylin eosin staining was performed to identify neutrophil cells while MDA levels were determined by colorimetric assay.*
Results: *The number of neutrophils in the control group (30.04 ± 15.30) was higher than that of the treatment group (7.76 ± 2.52), while the MDA levels of the control and treatment groups were 4.58 ± 0.66 and 4.30 ± 0.81, respectively ($p < 0.05$).*
Conclusion: *ERW demonstrates the ability to reduce neutrophil cells without altering MDA levels in Wistar rats suffering from aggressive periodontitis. This is an indication that ERW was an effective material to manage aggressive periodontitis.*

Keywords: *Periodontitis, Electrolyzed reduced water, Malondialdehyde, Neutrophil cells*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Periodontitis is an inflammatory disease resulting in the destruction of tissues and structures surrounding the teeth and represents a major worldwide oral disease [1]. Data issued by the Health Research and Development Department of the Indonesian Ministry of Health (Riskesdas) in 2014 confirmed that the prevalence of periodontitis within the country remained high [2], constituting the second most prevalent oral disease after caries [3].

Aggressive periodontitis is a destructive inflammatory disease characterized by periodontal attachment loss and bone resorption [4]. Patients with this condition often demonstrate an inadequate immune response to pathogenic organisms. Periodontal pathogens will induce a significantly elevated production of inflammatory mediators [5]. Aggressive periodontitis is

predominantly caused by the gram-negative bacteria, *A. actinomycetemcomitans* one virulent factor of which is leukotoxin, an inflammatory mediator activating inflammatory cells, such as leukocytes, polymorphonuclear (PMN), and macrophages [6,7].

The superoxide of reactive oxygen species (ROS) is produced by inflammatory cells as a defense mechanism response to infection. An increased level of ROS can be destructive to the body since it has the same properties as interleukin which damages tissue if produced in excessive amount. High levels of ROS can lead to lipid peroxidation a secondary product of which is malondialdehyde (MDA), a biomarker of ROS activities [8,9]. Excessive ROS must be eliminated in order to avoid tissue damage. It can be reduced by a component such as active hydrogen which is able to bind to the free electrons of ROS. In addition, active hydrogen atoms can inhibit the expressions of proinflammatory cytokine [10].

Electrolyzed reduced water (ERW) is also known as alkaline electrolyzed water, alkali-ionic water, alkaline cathodic water and alkaline ionized water. ERW can be defined as water with an alkaline pH which contains hydrogen-rich molecules [11]. Active hydrogen atoms can be obtained from ERW at the cathode by means of an electrolysis process.

ERW contains low levels of dissolved oxygen and high levels of dissolved hydrogen, while demonstrating a negative redox potential. ERW at a pH of 9.8 has been reported to reduce levels of ROS in the blood plasma of patients with kidney disorders [12]. Previous studies have also confirmed active hydrogen atoms as demonstrating the ability to decrease the activities of ROS [11,12].

ERW is produced through a process of electrolysis or ionization. The result of water electrolysis is either acid or base form. Acidic water, while unsuitable for human consumption is appropriate for the treatment of the human body and maintaining levels of hygiene. Meanwhile, ERW or alkaline water has certain health benefits when consumed [13]. The aim of this study is to evaluate the effects of ERW on MDA levels and neutrophil cells in Wistar rats suffering from aggressive periodontitis.

EXPERIMENTAL

Animals

This study consisted of laboratory-based experimental research involving the use of rats (*Rattus norvegicus*) as animal subjects. The inclusion criteria included the following; male, aged 2-5 months, in healthy condition and weighing between 150 and 250 g. Research subjects were given seven days to acclimatize. Ethical permission for the study was obtained in accordance with the guidelines of the Board for Animal Experiments at the Faculty of Dental Medicine, Universitas Airlangga (no. 176/KKEPK.FKG/VIII/2016) which followed the Guidelines on the Care and Use of Animals for Scientific Purposes [14].

Induction of aggressive periodontitis

The research subjects were divided into two groups: control and treatment (n = 7), both of which were induced with *A. actinomycetemcomitans* bacteria 0.5 mL at a dose of 10^8 CFU/mL once a day for five days or until their mandibular right and left first molar regions were inflamed.

Experimental design

The animals in the control group were treated orally with distilled water of neutral pH (pH 7.0) at a dose of 20 mL per day (5 mL x 4) for three days. In contrast, those constituting the treatment group were orally treated with ERW with a pH of 9.8 at a dose of 20 mL per day (5 mL x 4) over the same period.

Histological staining

After three days of ERW supplementation, the experiment animals were sacrificed by injecting a 50 mg/kg dose of ketamine (Combipar, Jakarta, Indonesia). The gingival tissues were extracted and fixed with buffered formalin solution (P4417, Sigma, Indonesia) for 24 hours before being dehydrated and cleared with alcohol. Samples were then embedded in paraffin prior to HE staining. Neutrophil cell identification was performed by means of a light microscope at 1000x magnification.

Determination of MDA levels

300 mg of gingival tissues were pulverized and homogenized using 1 mL of cold phosphate buffer, (P4417, Sigma, Indonesia) and 10 µL of probucol solution. Samples of 200 µL were placed in assay tubes, and R1 reagents of MDA kit (MDA-586 Bioxytech, OXIS Health Products, Portland, USA) were added. The samples were vortexed and 150 µL of R2 reagents were added to each tube. All control and treatment group assay tubes were incubated at a temperature of

45 ^{o}C for 60 minutes. The samples were subsequently centrifuged (DSC-200A Dslab, Taiwan) at 6,000 rpm for ten minutes. The supernatants were removed and the absorbance values were read using a spectrophotometer (Shimadzu UV-1800, Japan) at a wavelength of 586 nm.

Statistical analysis

All the data obtained are presented as mean ± standard deviation (SD) and were analyzed using independent *t*-test using SPSS 21. Differences were considered significant if $p < 0.05$.

RESULTS

The levels of MDA and neutrophil are shown in Table 1. There was no significant difference in MDA levels ($p = 0.489$) between control and treatment groups. The numbers of neutrophils in the control and treatment groups were significantly different ($p = 0.003$). The HE staining results for the control and treatment group neutrophils are shown in Figure 1.

Table 1: Mean MDA levels and neutrophils in the rats

Group	MDA levels (mean ± SD)	Neutrophil (mean ± SD)
Control	4.58 ± 0.66	30.04 ± 15.30
Treatment	4.30 ± 0.81	7.76 ± 2.52*

*Significant different ($p < 0.05$)

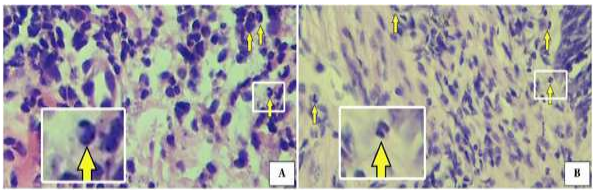

Figure 1: N*eutrophil expression* (yellow arrow) after HE staining using light microscope 1000x in treatment (A) and control (B) group

DISCUSSION

Aggressive periodontitis is a periodontal disease which demonstrates a high level of *A. actinomycetemcomitans*. The host response to bacterial infection plays a critical role in periodontal pathogenesis. PMN is a first-line host defense, against the bacteria which utilizes oxygen-dependent and oxygen-independent mechanisms. The oxygen-dependent pathway involves the production of ROS [15], the excessive presence of which may induce periodontal tissue destruction [15,16].

This study used ERW as an alternative therapy for aggressive periodontal disease and was conducted to evaluate its effectiveness in this regard. The subjects were divided into two groups, control and treatment. The control group, serving as a reference standard for comparing MDA and neutrophil levels with those of the treatment group, was treated with distilled water of neutral pH. Distilled water is a clear, colorless, odorless, non-toxic liquid with a molecular weight of 18.0 g/mol and pH of 5 - 7. The treatment group was treated using ERW with a pH of 9.8. Previous studies have shown pH 9.8 to be capable of reducing ROS and inflammation in kidney disease [17].

The average level of MDA in the treatment group was lower than that of the control group. However, the results of the analysis confirmed there was no significant difference in the level of MDA between the two groups. This was possibly due to a decrease in the antioxidant activities of ERW. ERW is affected by heat and cannot be consumed immediately since this would lead to the instability of active hydrogen atoms and a decrease in antioxidant activities [18]. Furthermore, the antioxidant capabilities of ERW did not reach their maximum level due to the comparatively short treatment period (three days). Previous results indicated that ERW can reduce the inflammatory process in atopic dermatitis when consumed for 25 days [19]. Furthermore, ERW can decrease ROS activities in blood plasma after being consumed for one month [17].

The level of neutrophil in the treatment group was significantly lower than that of control group. This is related to the anti-inflammatory activities of ERW [17]. For example, ERW with a pH of 9.8 promotes anti-inflammatory activities by lowering the levels of cytokines in mice suffering from atopic dermatitis triggered by *Dermatophagoides farinae*. Active hydrogen atoms can also reduce pro-inflammatory expressions, including: tumor necrotizing factor-alpha (TNF-α), interleukin-6 (IL-6), IL-1, IL-10, IL-12, chemokine (C-C motif) ligand 2 (CCL2), intercellular adhesion molecule 1 (ICAM-1), high-mobility group protein 1 (HMGB-1), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), and prostaglandin E2 (PGE2). ERW also inhibits cyclooxygenase (Cox-2) expressions by decreasing IL-1 and TNF-α. The inhibition of IL-1 and TNF-α synthesis leads to reduced stimulation of cell membrane phospholipids. Consequently, arachidonic acid is not released from the cell membrane phospholipids by phospholipase activation. This condition leads to a reduction in both cox-2 protein synthesis and prostaglandin biosynthesis which, in turn, results in a decreased inflammatory response [20]. The

inhibition of IL-1 and TNF α synthesis also leads to capillary vasoconstriction and increased capillary permeability. According to Ohta, ERW can also inhibit the release of PGE2 resulting in a decrease in the pain threshold and inflammatory response [10].

CONCLUSION

The findings of this study indicate that ERW reduces the number of neutrophils without affecting MDA levels in Wistar rats suffering from aggressive periodontitis. These results confirm that ERW was an effective material to manage aggressive periodontitis.

DECLARATIONS

Acknowledgement

The Indonesian Ministry of Research, Technology, and Higher Education for a grant for this research in 2015, including the publication of this manuscript.

Conflict of interest

No conflict of interest is associated with this study.

Contribution of authors

The authors declare that this work was undertaken by the author(s) named in this article and all liabilities pertaining to claims relating to its contents will be borne by the authors. All the authors made substantial contributions to this study and/or manuscript, approved the final draft of the paper prior to its submission.

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