Rhinology, 43, 185-189, 2005

Determination of reactive oxygen species in nasal polyps*

Cüneyed Üneri[1], Özmen Öztürk[1], Şenol Polat[1], Meral Yüksel[2], Goncagül Haklar[2]

[1] Department of Otorhinolaryngology, Head and Neck Surgery, Marmara University School of Medicine, Istanbul, Turkey
[2] Department of Biochemistry, Marmara University School of Medicine, Istanbul, Turkey

SUMMARY

A strong relationship between tissue damage and reactive oxygen species (ROS) has been established by previous studies. The aim of the current study was to evaluate the presence of ROS in nasal polyps (NP) by measuring luminol and lucigenin amplified chemiluminescence (CL). Three groups of specimens were studied. Group 1 composed of NPs of 15 patients, and group 2 constituted of healthy appearing nonpolypoid nasal mucosa of the same patients. Group 3 specimens (control group) were obtained from 15 patients who underwent septoplasty and/or inferior turbinectomy operations, and detected to be free of rhinosinusitis. None of the patients had a history of allergy, asthma, or aspirin sensitivity, and all patients showed negative results to a skin prick test. The ROS levels were measured directly with luminol and lucigenin amplified CL. CL measurements revealed significant differences between ROS constituents of NP (group 1) and control (group 3) tissue samples. CL measurements of healthy appearing nonpolypoid nasal mucosa (group 2) of the NP patients revealed values that were scattered between the values of the other two groups. Although insignificant, ROS levels of the nonpolypoid nasal mucosa (group 2) were found to be higher than normal controls (group 3). In this study, ROS levels of NP tissue samples were directly measured. In our study, by measuring high ROS levels in NP samples, a strong relationship between tissue damage in NPs and ROS has been demonstrated, and the contribution of ROS in the pathophysiology of nasal polyposis has been emphasized.

Key words: nasal polyp, reactive oxygen species, free radicals, chemiluminescence.

INTRODUCTION

A nasal polyp (NP) is defined as a pedunculated benign mucosal protuberance emanating from mucoperiosteal or mucoperichondrial tissue, and filled with an edematous and fibrous stroma enclosing blood vessels, mucous glands, and inflammatory cells [1,2]. NPs, most commonly originate from the lateral nasal wall in the area of the ethmoidal clefts, tend to be bilateral, and obstruct the nasal airway [1,2]. They are often associated with chronic rhinosinusitis, and have a tendency to recur despite medical and surgical treatment [1,2].

For NPs, chronic persistent inflammatory reaction of the nasal and paranasal sinus mucosa is a major determinant irrespective of the proposed etiologic factors [3,4]. Histological studies revealed various inflammatory cells (eosinophils, neutrophils, lymphocytes and their subgroups, plasma cells, mast cells, and macrophages), and immune mediators (adhesion molecules, transforming growth factor-β, and various cytokines/ chemokines) in the development and life cycle of NPs [4-7].

It is well known that inflammatory cells are important sources of reactive oxygen species (ROS) [8-10]. ROS may cause tissue

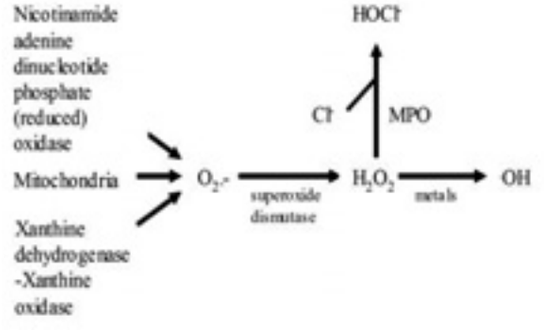

Figure 1. Origins of reactive oxygen species (ROS) in biological systems were shown. The major sources of O_2.- include mitochondrial respiration, "nicotinamide adenine dinucleotide phosphate oxidase" catalyzed reaction for bacterial killing, and xanthine dehydrogenase conversion to xanthine oxidase during ischemia and reperfusion injury. ROS may contribute to the pathogenesis of diseases directly or through oxidation products of lipids, proteins, and nucleic acids (O_2.-, superoxide radical; H_2O_2, hydrogen peroxide; MPO, myeloperoxidase; .OH, hydroxyl radical).

* Received for publication: May 17, 2004; accepted: December 1, 2004

damage by chemical modification of cellular proteins, carbohydrates, nucleotides, and lipids (Figure 1) [8-10]. ROS mediated damage has been implicated in the pathogenesis of several disease processes, including cataract formation, stroke, atherosclerosis, arthritis, tympanosclerosis, and reperfusion injury [8,9,11,12].

The presence and the role of ROS in NP pathophysiology was investigated in a study by indirectly measuring NP tissue levels of malondialdehyde (MDA), an aldehyde end product formed from the decomposition of lipid peroxides by ROS [13]. In this study, MDA level, an indicator of ROS presence, was measured to be higher when compared to normal nasal mucosa, and it was proposed that ROS should have been considered in the pathophysiology of NPs [13].

In another study, the relationship between nitric oxide (NO), which probably plays a major role in the nonspecific defense of the nasal cavity; and superoxide anion, an important ROS component was evaluated in NP tissue. It was shown that phagocytic-derived superoxide anion production could contribute to a decrease in sinus NO concentration, and thus alter the natural local defence [14]. It was argued that these events could participate in chronic mucosal inflammation, and contribute to the pathophysiology of NP [14].

Chemiluminescence (CL) is a well established and sensitive technique of estimating ROS generation, which measures light production as a by-product of oxidative metabolism, and has been utilized to demonstrate the involvement of ROS in various disease states [14-16].

The aim of the present study was to directly detect ROS in NP by measuring luminol and lucigenin amplified CL.

MATERIALS AND METHODS

Patients

This study involved 30 new patients operated on in our department due to nasal polyposis (n=15), and septal deviation and/or turbinate hypertrophy (n=15). The diagnosis of nasal polyposis was based for all patients on the following criteria: 1) the visualization of bilateral polyps in the nasal cavities on anterior rhinoscopic and endoscopic examinations and 2) the existence of bilateral opaque areas located in the anterior and/or posterior ethmoidal sinuses on paranasal computed tomography scans (coronal planes). The examination was performed without contrast medium. None of the patients had a history of allergy, asthma, or aspirin sensitivity. All patients (groups 1-3) showed negative results to skin prick test with 43 common allergens. The patients who had received either oral or nasal corticosteroids or oral antihistamines were excluded from the study.

Biopsies

The tissue specimens were harvested before topical anesthesia and decongestant applications during the endonasal interventions. Punch biopsy from the superficial layer of the nasal polyp (group 1) and macroscopically uninvolved nasal mucosa (groups 2 and 3) provided a representative tissue of approximately 1x1x0.5 mm3.

NP specimens (group 1) were obtained from 15 patients (9 men and 6 women; average age, 36; age range, 21-64) undergoing endoscopic nasal surgery due to nasal polyposis. Besides polypoid tissue specimens, healthy appearing nonpolypoid nasal mucosa of the same patients (group 2) was also studied. The tissue specimens of group 2 were obtained from inferior meatal mucosa.

Control specimens (group 3) were obtained from 15 patients (8 men and 7 women; average age, 29; range, 19-49) undergoing septoplasty and/or inferior turbinectomy operations. The nasal mucosal specimens were harvested from inferior meatal mucosa. None of these patients in group 3 revealed any findings related to rhinosinusitis by anterior rhinoscopic, endoscopic, radiologic, and intraoperative examinations.

The specimens were collected immediately, washed with ice-cold saline, and transferred to counting vials in 10 minutes without mincing, as decribed [17].

Chemiluminesence

CL measurements were made at room temperature using "Mini Lumat LB 9506 luminometer" (EG&G Berthold, Germany) in the presence of 0.2 mM luminol and 0.2 mM lucigenin. Each vial was observed continually, and counts were obtained at 5-second intervals for a counting period of 5 minutes. The results were given as the area under curve (AUC) to assess the diagnostic accuracy of lucigenin and luminol amplified CL. Counts were corrected for wet tissue weight [rlu/mg tissue, (rlu: relative light unit)] after CL measurements, as decribed [18].

Statistical analysis

The results are given as median values. The significance of differences between groups were estimated by analysis of Wilcoxon signed rank test and Mann Whitney U test. The differences were considered significant when the probability was p 0.05.

RESULTS

Luminol CL measurements revealed significant differences between ROS constituents of NP (group 1) and control (group 3) samples. Luminol CL level was 59.6 rlu/mg tissue in the NP group (group 1), and 20.3 rlu/mg tissue in the control group (group 3) (p 0.0005) (Figures 2 and 3).

Median value of luminol CL level was found to be 32.00 rlu/mg tissue in group 2, with no significant difference (p 0.05) when compared with group 3 (p 0.05); and with a statistically significant difference, when compared with group 1 (p 0.01), although the values were found to be significant between group 1 and 3 (Figures 2 and 3).

Similiar findings were obtained with lucigenin CL measurements. There was a significant difference between ROS constituents of NP (group 1), and control (group 3) tissue samples.

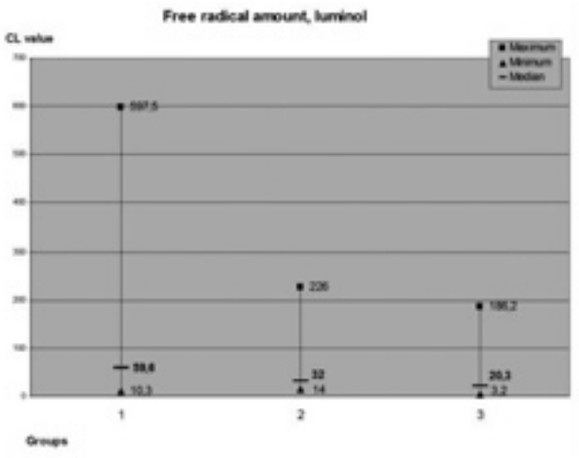

Figure 2. Comparison of luminol chemiluminescence (CL) median values.

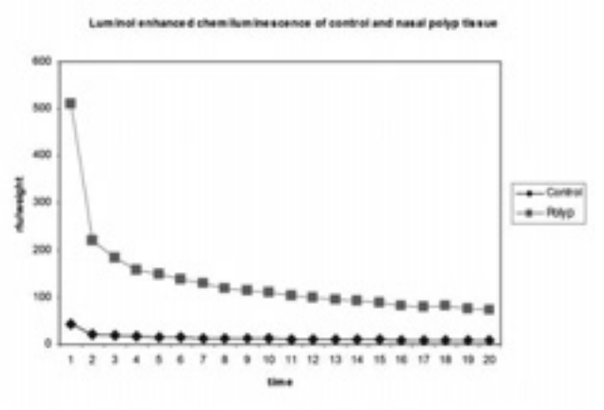

Figure 3. Correlation between luminol enhanced chemiluminescence of control and nasal polyp tissue versus time (each time interval= 15 sec) (rlu, relative light unit; weight= mg tissue).

Lucigenin CL level was 36.0 rlu/mg tissue in the NP group (group 1), and 15.7 rlu/mg tissue in the control group (group 3) (p 0.0005) (Figures 4 and 5).

The measurement of median value of lucigenin CL level in group 2 was found to be 29.7 rlu/mg tissue, although this level was higher than group 3 (15.7 rlu/mg tissue), there was no statistically significant difference between group 2 and 3. There was a significant difference, when compared with group 1 (p 0.0005) (Figure 4).

DISCUSSION

The current study demonstrated increased levels of ROS in NP. High levels of these reactive species indicated the propagation of inflammation and tissue damage.

The presence of inflammatory cells such as eosinophils, neutrophils, macrophages, lymphocytes, and myofibroblasts were proposed to be closely related to the development epithelial cell injury in NPs [4-7]. Eosinophils represent the most predominant infiltrating inflammatory cell type in NPs, and eosinophilia were found to be more prevalent in rhinosinusitis associated with nasal polyposis [6-8]. In a recent study, it was found that chronic rhinosinusitis patients with serum eosinophilia have worse prognosis when compared with control patients after endoscopic sinus surgery [20]. In another study, it was shown that the peripheral eosinophil count increased with increasing severity of nasal disease [21]. Eosinophils in the systemic circulation are activated to migrate from the peripheral vascular system into the nasal tissue in nasal polyposis and chronic rhinosinusitis [6,22].

Eosinophils were found to be major sources of tissue damage via generating ROS in asthmatic patients [23]. It was shown that NADPH oxidase complex, the mechanism involved in superoxide anion production activity, had been more abundant in eosinophils than in neutrophils [24]. Thus, epithelial cell damage and mucosal edema predisposing to NP development could be related to generation of ROS by eosinophils. In NP samples, lucigenin amplified CL was significantly correlated with eosinophils abundance [14].

ROS are capable of causing irreversible and prolonged tissue damage, and injury [10]. In acute otitis media, increased ROS production was measured indirectly with MDA and lipid peroxide levels, and it was proposed that increased lipid peroxide levels could be related to mucosal damage [25,26].

ROS was considered in the development and life cycle of NP by showing increased MDA levels in polypoid tissues [13]. It was argued that superoxide anion production could decrease sinus NO concentration, and could further contribute to the pathophysiology of NP [14]. In our study, we used the CL method to measure ROS levels directly, as described [12,16].

CL is a universal property of organic substances that are able to undergo an oxidation reaction sufficiently exothermic to produce a light emitting state, and may be utilized as a direct method for measuring ROS [27]. Due to potential variability and low intensity of native CL, investigators introduced the use of enhancer compounds, luminol, and lucigenin [28]. When added to an in vitro biological system, luminol and lucigenin function as bystander-substrates for oxygenation, and form high levels of excited-state products and CL [28]. Although, the reliability of this sensitive technique has been recently questioned [29], luminol detects hydrogen peroxide, hydroxyl radical, hypochloride, and peroxynitrite, whereas lucigenin is particularly sensitive to superoxide radical [17,28].

ROS mediated tissue damage was well documented in other disease states, but the role of these highly reactive species in NP etiopathogenesis was obscure. In our study, both luminol and lucigenin CL measurements revealed significant increases in ROS levels in NP specimens when compared to control tissues. Although ROS may not be involved in the starting point of inflammation, they could be fundamental in an ongoing inflammation, and may cause additional tissue damage. It was shown that oxygen mediated cell damage had been an important feature of eosinophils which are abundant in NP [21,23,24]. Additionally, it is known that ROS are also important chemoattractants for inflammatory cells [4,6,22].

Figure 4. Comparison of lucigenin chemiluminescence (CL) median values.

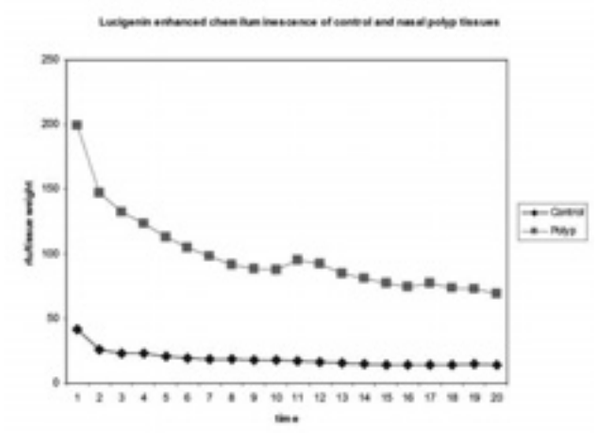

Figure 5. Correlation between lucigenin enhanced chemiluminescence of control and nasal polyp tissue versus time (each time interval= 15 sec) (rlu, relative light unit; weight= mg tissue).

The integrity of the nasal mucosa could be changed in NPs by local mechanisms triggered by multiple factors. Hypersensitivity states (allergy, asthma, and aspirin sensitivity) might be related to atopic NP development by having an impact on eosinophilic recruitment and activation [4]. ROS mediated tissue damage and autonomicity of inflammation may be one of the local mechanisms in nonatopic NP development. The hypersensitive patients are expected to produce differences in local ROS levels. They are excluded from the study to prevent their contribution to the local inflammatory processes, and to bring an explanation to NP development by providing an isolated ROS mediated injury mechanism in nonatopic states.

The presence of ROS in NP tissue is particularly important for two reasons; First, they may lead to autonomicity of inflammation with their cheomoattracting properties. Second, they cause tissue damage directly by peroxidating the cellular membrane structural lipids and leading cell death. The latter action is also an important step in the propagation of local tissue inflammation by itself.

In our study, CL measurements of healthy appearing nonpolypoid nasal mucosa (group 2) of the NP patients revealed values that were scattered between the other two groups. Although insignificant, ROS levels of the nonpolypoid nasal mucosa were found to be higher than normal controls. From this point of view, it is conceivable that, there could be a slowly progressive silent inflammation and mucosal damage in these regions, and it seems that these regions could be candidates for NP development in the future. Nevertheless, although the inflammatory reactions seem to be very intense in the NPs, when compared with nonpolypoid nasal mucosa, it is logical to say that, inflammatory reactions and ROS mediated cellular injury may cover the whole mucosal lining of the nasal cavity, despite changes in intensity in different nasal regions. Due to the pathological content of the polypoid nasal mucosa, some of the specimens had values scattered within the normal range (Figures 2 and 4).

In the treatment of NP, steroids decrease the inflammatory response of nasal mucosa, particularly by lowering the number and the activity of eosinophils, thus lowering the ROS levels. It could be proposed that, in the future, intranasal medication with antioxidants in addition to steroids may aid in decreasing epithelial cell damage by lowering ROS levels directly [12]. So, following studies should concentrate on the clinical usage of antioxidant agents in the medical management of nasal polyposis.

In our study, by measuring high ROS levels in NP samples, a strong relationship between tissue damage in NPs and ROS has been demonstrated. This study shows the presence of ROS in nasal polyps but further studies will probably define the role of ROS in nasal polyposis by demonstrating NP development after a change in the nasal mucosal levels of ROS.

ACKNOWLEDGMENT

We thank Nural Bekiroglu, PhD from the Department of Biostatistics for her support and statistical analysis of this manuscript.

REFERENCES

1. Bachert C, Hormann K, Mosges R, Rasp G, Riechelmann H, Muller R, et al. (2003) An update on the diagnosis and treatment of sinusitis and nasal polyposis. Allergy 58: 176-191.
2. Tos M, Larsen PL (2003) Nasal polyps: origin, etiology, pathogenesis, and structure. In: Kennedy DW, Bolger WE, Zinreich SJ (Eds) Diseases of the sinuses, diagnosis & management. B.C. Decker Inc., Hamilton, Canada, pp. 69-75.
3. Pawankar R (2003) Nasal polyposis: an update: editorial review. Curr Opin Allergy Clin Immunol 3: 1-6.
4. Hirschberg A, Jokuti A, Darvas Z, Almay K, Repassy G, Falus A (2003) The pathogenesis of nasal polyposis by immunoglobulin E and interleukin-5 is completed by transforming growth factor-beta1. Laryngoscope 113: 120-124.
5. Larsen PL, Tingsgaard PK, Harcourt J, Sofsrud G, Tos M (1998) Nasal polyps and their relation to polyps/hypertrophic polypoid mucosa in the paranasal sinuses: a macro-, endo-, and microscopic study of autopsy materials. Am J Rhinol 12: 45-51.

6. Allen JS, Eisma R, LaFreniere D, Leonard G, Kreutzer D (1998) Characterization of the eosinophil chemokine RANTES in nasal polyps. Ann Otol Rhinol Laryngol 107: 416-420.
7. Berger G, Kattan A, Bernheim J, Ophir D (2002) Polypoid mucosa with eosinophilia and glandular hyperplasia in chronic sinusitis: a histopathological and immunohistochemical study. Laryngoscope 112: 738-745.
8. Pryor WA (1986) Oxy-radicals and related species: their formation, lifetimes, and reactions. Annu Rev Physiol 48: 657-667.
9. Salin ML, McCord JM (1975) Free radicals and inflammation. Protection of phagocytosine leukocytes by superoxide dismutase. J Clin Invest 56: 1319-1323.
10. Southorn PA, Powis G (1988 a) Free radicals in medicine. I. Chemical nature and biologic reactions. Mayo Clin Proc 63: 381-389.
11. Southorn PA, Powis G (1988 b) Free radicals in medicine. II. Involvement in human disease. Mayo Clin Proc 63: 390-408.
12. Polat S, Ozturk O, Uneri C, Yuksel M, Haklar G, Bozkurt S, Kullu S (2004) Determination of reactive oxygen species in myringotomized membranes: effect of vitamin E treatment. Laryngoscope 114: 720-5.
13. Dogru H, Delibas N, Doner F, Tuz M, Uygur K (2001) Free radical damage in nasal polyp tissue. Otolaryngol Head Neck Surg 124: 570-572.
14. Pasto M, Serrano E, Urocoste E, Barbacanne MA, Guissani A, Didier A, et al. (2001) Nasal polyp-derived superoxide anion: dose-dependent inhibition by nitric oxide and pathophysiological implications. Am J Respir Crit Care Med 163: 145-151.
15. Boveris A, Cadenas E, Reiter R, Filipkowski M, Nakase Y, Chance B (1980) Organ chemiluminescence: noninvasive assay for oxidative radical reactions. Proc Natl Acad Sci USA 77: 347-351.
16. Dalaman G, Haklar G, Sipahiu A, Ozener C, Akoglu E, Yalcin AS (1998) Early detection of peritonitis in continuous ambulatory peritoneal dialysis patients by use of chemiluminescence: evaluation of diagnostic accuracy by receiver-operating characteristic curve analysis. Clin Chem 44: 1680-1684.
17. Davies GR, Simmond NJ, Stevens TRJ, Sheaf MT, Bnavala N, Laurensen IF, et al (1994) Helicobacter pylori stimulates antral mucosal reactive oxygen metabolite production in vivo. 35: 179-185.
18. Van Dyke K, Castranova V (Eds) (1987) Cellular Chemiluminescence. CRC Press, London, United Kingdom, pp. 1-67.
19. Jankowski R, Bouchoua F, Coffinet L, Vignaud JM (2002) Clinical factors influencing the eosinophil infiltration of nasal polyps. Rhinology 40: 173-178.
20. Bryson JM, Tasca RA, Rowe-Jones JM (2003) Local and systemic eosinophilia in patients undergoing endoscopic sinus surgery for chronic rhinosinusitis with and without polyposis. Clin Otolaryngol 28: 55-58.
21. Szucs E, Ravandi S, Goossens A, Beel M, Clement PA (2002) Eosinophilia in the ethmoid mucosa and its relationship to the severity of inflammation in chronic rhinosinusitis. Am J Rhinol 16: 131-134.
22. Wei JL, Kita H, Sherris DA, Kern EB, Weaver A, Ponikau JU (2003) The chemotactic behavior of eosinophils in patients with chronic rhinosinusitis. Laryngoscope 113: 303-306.
23. MacPherson JC, Comhair SA, Erzurum SC, Klein DF, Lipscomb MF, Kavuru MS, et al. (2001) Eosinophils are a major source of nitric oxide-derived oxidants in severe asthma: characterization of pathways available to eosinophils for generating reactive nitrogen species. J Immunol 166: 5763-5772.
24. Someya A, Nishijima K, Nunoi H, Irie S, Nagaoka I (1997) Study on the superoxide-producing enzyme of eosinophils and neutrophils—comparison of the NADPH oxidase components. Arch Biochem Biophys 345: 207-213.
25. Takoudes TG, Haddad J Jr (1999) Lipid peroxides in middle ear fluid after acute otitis media in guinea pigs. Ann Otol Rhinol Laryngol 108: 564-568.
26. Haddad J Jr (1998) Lipoperoxidation as a measure of free radical injury in otitis media. Laryngoscope 108: 524-530.
27. Halliwell B, Grootveld M (1987) The measurement of free radical reactions in humans. Some thoughts for future experimentation. FEBS Lett 213: 9-14.
28. Allen RC, Loose LD (1976) Phagocytic activation of a luminol-dependent chemiluminescence in rabbit alveolar and peritoneal macrophages. Biochem Biophys Res Commun 69: 245-252.
29. Liochev SI, Fridovich I (1998) Lucigenin as mediator of superoxide production: revisited. Free Rad Biol Med 25: 926-928.

Cüneyd Üneri, M.D.
Marmara Üniversitesi Hastanesi
Kulak Burun Bogaz ABD
Tophanelioglu cad., No: 13/15,
81190 Altunizade, Istanbul
Turkey

Tel: +90-216-327 8294
Fax: +90-216-326 9637
E-mail: ozturkmarmara@hotmail.com