Original Hypothesis

N-Acetylcysteine as a Candidate Therapeutic for Recurrent Aphthous and Aphthous-Like Ulcers

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Abstract

Introduction: Recurrent aphthous stomatitis (RAS) is a painful ulcerative oral disease with a general population prevalence exceeding 20%. The etiology of RAS remains largely unknown, however, nutritional deficiency, autoimmunity, psychological stress, and, recently, oxidative stress have been implicated. The pain associated with RAS may be very severe and disabling, hence, treatment is centered on the control of pain and acceleration of healing. N-acetylcysteine (NAC) is a potent antioxidant with anti-inflammatory, immune-modulatory, and antimicrobial properties. It is available as a safe dietary supplement, and has been successfully used as adjuvant/treatment of inflammatory conditions including ulcerative lesions. **The Hypothesis:** Using NAC as a candidate for treatment and/or prevention of RAS and aphthous-like ulcers is hypothesized here. We propose to use NAC systemically or topically in the form of powder, paste, adhesive tablets, or mouthwash to treat active RAS or for prophylaxis in cases with frequent attacks. **Evaluation of the Hypothesis:** The current hypothesis should be tested on animal models of RAS. However, because NAC is currently approved and used for other indications, the hypothesis can also be directly evaluated in well-designed, randomized clinical trials.

Key words: Antioxidant, aphthous ulcer, N-acetylcysteine, oxidative stress, recurrent aphthous stomatitis

Introduction

Recurrent aphthous stomatitis (RAS), also called recurrent aphthous ulcers or Canker sore, is the most common oral mucosal disease characterized by recurring painful ulcers of the nonkeratinized lining mucosa, that are oval or round usually with a gray-white pseudomembranous center surrounded by erythematous halo reflecting an intense inflammation.[1] Based on the size and number of the ulcers, RAS is categorized as minor, major, and herpetiform.[2] Because the episodes are short in duration, the point prevalence of RAS is low ranging from 0.9%[3] to 2.7%.[4] However, the annual and lifelong prevalence rates have been reported to be as high as 20% and 40%, respectively.[3,5] Certain subpopulations are at higher risk of developing RAS including high socioeconomic strata, females, nonsmokers, and young adults.[6]

The pain associated with RAS can be so severe that it interferes with talking, eating, swallowing, and oral health practices[7] and significantly lowers the patient's quality of life.[8-10] Many treatment modalities of RAS have been studied revealing inconsistent results; these have been comprehensively reviewed elsewhere.[11,12] In principle, a curative therapy is lacking and treatment is centered on the control of pain and acceleration of healing.

Despite the extensive research done on RAS, the etiology and pathogenesis of the disease remains poorly understood. A number of factors have been implicated in the etiology of RAS including malnutrition,[13,14] psychological stress,[15,16] viral infection, oral microbial dysbiosis,[17-20] trauma,[21] immune dysfunction,[1,22-24] genetic predisposition,[25-30] and allergy.[31] Recently, there has been growing evidence to support a strong role for oxidative stress in the etiology of RAS; this is reflected in significant upregulation of oxidant

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and/or downregulation of antioxidant markers in serum[32-37] and/or locally in saliva.[38,39] In brief, oxidative stress is characterized by an increased level of reactive oxygen species (ROS) that disrupts the intarcellular reduction–oxidation (redox) balance. While the production of ROS is an essential part of normal cellular metabolism, when increased, it creates a state of oxidative stress that may impair metabolism, causes oxidative damage, and eventually results in cell death and tissue destruction.[40] In fact, oxidative stress is implicated in the pathogenesis of many inflammatory diseases such as atherosclerosis,[41] diabetes mellitus,[42] rheumatoid arthritis, and chronic periodontitis.[43] Therefore, maintaining/restoring the redox balance is a potentially useful approach for the management of these diseases.

N-acetylcysteine (NAC) is an FDA-approved dietary supplement. It is converted in the human body by deacetylation into the amino acid cysteine. The latter is in turn metabolized by the action of two enzymes, glutamate cysteine ligase (GCL) and glutathione synthetase (GS), into glutathione, a key antioxidant.[44] It is rapidly absorbed following an oral dose with a plasma half-life of 2.5 hours. No NAC is detectable 10–12 hours after administration. The oral bioavailability of the intact NAC molecule was estimated to be approximately 10%. Renal clearance constitutes approximately 30% of total body clearance of NAC, with only 3% excreted in feces after oral administration. The almost complete absorption leaves a large amount of NAC for in-vivo metabolism, cellular uptake, deacetylation to cysteine, and synthesis of glutathione or other sulphur compounds.[45]

NAC has several pharmacological actions with potential therapeutic applications. For example, it has potent anti-inflammatory and immunomodulatory properties,[46-50] and possesses antimicrobial and antibiofilm activities.[51-55] In addition, it is well-known for its strong antioxidative effects; i.e., regulation of the glutamatergic system, which is the basic mechanism behind its therapeutic effects.[56] In clinical situations where glutathione deficiency and/or oxidative stress are involved, treatment with NAC has proven to be effective. For example, it is a life-saving drug for acetaminophen poisoning.[57] NAC has also been shown to scavenge oxygen free radicals that mediate cell necrosis after myocardial infarction[58] and angioplasty.[59] In addition to its use as an antioxidant, NAC has been used for pulmonary diseases as a mucolytic agent, in the management of addictive behaviors, psychiatric illnesses such as schizophrenia, and infectious diseases such as human immunodeficiency viris (HIV) and hepatitis C, and as a bioprotectant against toxicities, chronic kidney, and heart diseases, as well as some cancers.[60-62] NAC also acts as a methyl donor in the conversion of homocysteine (a risk factor for cardiovascular disorders) to methionine,[63] and has been shown to possess vasodilatory properties.[64]

NAC is available as a dietary supplement as well as a medication that is administrated orally or intravenously, or is inhaled as a mist.[62] It is a safe drug; mild gastrointestinal symptoms are the most common side effects.[65] There are, however, rare reports of renal stone formation during NAC treatment.[32] When used as a mucolytic agent, it has also been reported to cause local irritation.[66] Side effects are more noticeable at high doses (>3 g/day) and when administered intravenously.[67]

The Hypothesis

In view of the role of the oxidative stress, inflammation, immune dysregulation, microbial, and nutritional factors in the etiopathogenesis of RAS, we hereby postulate NAC as a strong candidate therapeutic for the treatment and/or prophylaxis of RAS and aphthous-like ulcers. Our hypothesis is primarily based on NAC being a potent antioxidative but also on its anti-inflammatory, immunomodulatory, antimicrobial, and nutritional properties, as elaborated below.

In RAS patients, there is a decrease in the total antioxidant status (TAS) against an increase in the total oxidative status (TOS) and oxidative status index (OSI) in RAS patients.[32,35,37,38] Specifically, the antioxidant enzymessuperoxide dismutase,[36,39] glutathione peroxidase,[33,36,39] arylesterase,[35] and catalase[36,39] are depleted, whereas there is a buildup of reactive oxygen species (ROS), namely malondialdehyde[33,34,36] and oxidized glutathione.[34] This high oxidative stress results in cellular damage and a vicious cycle of inflammation,[37] which in the case of RAS is translated into ulceration. In this context, a potent antioxidant, such as glutathione, can be used to reverse the oxidative stress and inflammation in RAS and subsequently reduce the pain and promote healing. However, glutathione, although available as dietary supplement, is destroyed by intestinal enzymes and has limited bioavailability;[68] instead, NAC is well absorbed from the intestine and is a precursor of cysteine, the main factor limiting the synthesis of reduced glutathione.[69] In addition, NAC works as a direct reactive oxygen species scavenger.[70] The antioxidant effects of NAC have in fact been demonstrated in several previous studies[49,50,71-76] and are dose-dependent.[77]

Upregulation of inflammatory mediators and dysregulation of the immune system have been reported to be involved in RAS. High levels of cytokines including IL-2, IL-10, IL-12, IL-13, IL-17, and IL-18, and INF-gamma[78-81] have been demonstrated in RAS patients. An increase in CD56+ cells,[80] autoimmunity potential,[23] and chemokine receptors such as CCR5 and CXCR3[79] have also been reported. Interestingly, NAC has been shown to reverse the dysregulation in many of these cytokines,[47-50,82-88] probably through inhibition of proinflammatory transcription factors such as activator protein-1 and NF-KB.[46-49,86,89] Therefore, the anti-inflammatory and immunomodulatory properties of NAC represent another basis for using it for managing RAS.

The possible role of oral microbiota in the etiopathogenesis of RAS is emerging although investigators believe it plays

a secondary role to other factors. A recent study using next generation sequencing (NGS) of the 16S rRNA gene demonstrated that species belonging to *Porphyromonadaceae* and *Veillonellaceae* predominated in ulcerated sites whereas *Streptococcaceae* spp. predominated in the mucosa of healthy controls.[17] Other studies also claimed a role for the changes in the oral microbial community, bacteria, and/or viruses in the pathogenesis of RAS.[18,19,90,91] NAC can potentially play a role in the management of RAS at this level as well given its antimicrobial activities. NAC, for example, has been found to be more effective on both *Streptococcus mutans* and *Enterococcus faecalis* than sodium hypochlorite and chlorhexidine, and thus has been suggested as an endodontic irrigant.[53-55] It has also been shown to decrease biofilm formation, inhibit bacterial adherence, reduce the production of extracellular polysaccharide matrix, and the cell viability of various Gram-negative and Gram-positive bacteria.[51,92] The antibacterial effect of NAC is mediated by its thiol group that breaks disulfide bonds and thus results in the irreversible damage of bacterial proteins that are essential for bacterial growth.[51,92] How NAC influences the structure and function of the oral microbiome is an area that needs to be investigated further using NGS technologies.

The severe forms of RAS have been found to be associated with malnutrition,[13,14,93] and correction of such malnutrition have been shown to result in dramatic clinical improvement.[93] The most commonly implicated nutrients are vitamin B_{12}, foliate, and iron.[14,94] However, these deficiencies seem to contribute to RAS again by evoking a state of oxidative stress. For example, vitamin B_{12} deficiency has been found to cause a profound reduction of plasma glutathione and total antioxidant capacity (TAC).[95] Intriguingly, treatment with glutathione precursor (e.g., NAC) has been shown to reverse the oxidative stress induced by this deficiency.[96] In addition, supplementation with NAC has been demonstrated to increase available glutathione by up to 510% in a malnourished population.[97] In mice with protein malnutrition, NAC supplementation results in accelerated wound healing and restored early inflammatory responses.[98]

The use of NAC for the treatment and/or prevention of RAS is thus proposed based on its antioxidant, anti-inflammatory, immune-modulatory, nutritional, and anti-microbial properties.

Evaluation of the Hypothesis

Drug discovery usually passes through the following stages set by the US Food and Drug Administration (FAD): discovery and development; preclinical research (*in-vitro* and experimental animals); clinical research (four phases of clinical trials); and review and post-market safety monitoring. NAC is already FDA approved – although for non-RAS indications – and has broad margins of safety.[40-42] Therefore, the current hypothesis can be evaluated in animal models as well as in humans.

An animal model of oral ulceration has been described and used to test the therapeutic effects of many drugs.[99-102] The ulcers are chemically-induced using disks impregnated with acetic acid. This model can be employed to demonstrate both the therapeutic and prophylactic properties of NAC. Topical application of NAC, in the form of powder or adhesive paste, on the induced ulcer can be compared with other drugs in common use, such as amlexanox and triamcinolone, whereas systemic whereas systemic administration can be compared with systemic corticosteroids such as prednisolone or with systemic colchicine. Experimental outcomes should comprise the severity of ulceration and time required to develop the ulcers (prophylactic effects) and ulcer healing time and levels of inflammatory markers (therapeutic effects). These experiments will help identify the right form, route of administration, and dosage of NAC.

In humans, the therapeutic potential of NAC can be evaluated in well-designed, parallel-arm randomized clinical trials (RCTs). Guided by results from the animal studies, the right dose of NAC can be administered topically (powder, paste, adhesive tablets, chewing tablets, lozenges, or mouthwash) or systemically to treat active RAS, and compared with topical or systemic steroids, as described for the animal studies above. The main outcomes to be measured can include pain intensity, healing time, and levels of inflammatory markers. The prophylactic potential can on the other hand be assessed in crossover RCTs. A group of patients with frequent attacks of RAS can be followed for a period of time to establish recurrence rate; NAC will then be given systemically and the group will be followed up to reassess recurrence rate and compare it with the baseline rate.

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Conflict of interest

There are no conflicts of interest.

References

1. Slebioda Z, Szponar E, Kowalska A. Etiopathogenesis of recurrent aphthous stomatitis and the role of immunologic aspects: Literature review. Arch Immunol Ther Exp 2014;62:205-15.
2. Gurkan A, Ozlu SG, Altiaylik-Ozer P, Kurtul BE, Karacan CD, Senel S. Recurrent Aphthous Stomatitis in Childhood and Adolescence: A Single-Center Experience. Pediatr Dermatol 2015;32:476-80.
3. Cicek Y, Canakci V, Ozgoz M, Ertas U, Canakci E. Prevalence and handedness correlates of recurrent aphthous stomatitis in the Turkish population. J Public Health Dent 2004;64:151-6.
4. Rivera-Hidalgo F, Shulman JD, Beach MM. The association of tobacco and other factors with recurrent aphthous stomatitis in an US adult population. Oral Dis 2004;10:335-45.

5. Shulman JD. An exploration of point, annual, and lifetime prevalence in characterizing recurrent aphthous stomatitis in USA children and youths. J Oral Pathol Med 2004;33:558-66.
6. Koybasi S, Parlak AH, Serin E, Yilmaz F, Serin D. Recurrent aphthous stomatitis: Investigation of possible etiologic factors. Am J Otolaryngol 2006;27:229-32.
7. McCullough MJ, Abdel-Hafeth S, Scully C. Recurrent aphthous stomatitis revisited; clinical features, associations, and new association with infant feeding practices? J Oral Pathol Med 2007;36:615-20.
8. Al-Omiri MK, Karasneh J, Alhijawi MM, Zwiri AM, Scully C, Lynch E. Recurrent aphthous stomatitis (RAS): A preliminary within-subject study of quality of life, oral health impacts and personality profiles. J Oral Pathol Med 2015;44:278-83.
9. Hapa A, Aksoy B, Polat M, Aslan U, Atakan N. Does recurrent aphthous stomatitis affect quality of life? A prospective study with 128 patients evaluating different treatment modalities. J Dermatolog Treat 2011;22:215-20.
10. Krisdapong S, Sheiham A, Tsakos G. Impacts of recurrent aphthous stomatitis on quality of life of 12- and 15-year-old Thai children. Qual Life Res 2012;21:71-6.
11. Tarakji B, Gazal G, Al-Maweri SA, Azzeghaiby SN, Alaizari N. Guideline for the diagnosis and treatment of recurrent aphthous stomatitis for dental practitioners. J Int Oral Health 2015;7:74-80.
12. Belenguer-Guallar I, Jimenez-Soriano Y, Claramunt-Lozano A. Treatment of recurrent aphthous stomatitis. A literature review. J Clin Exp Dent 2014;6:e168-74.
13. Kozlak ST, Walsh SJ, Lalla RV. Reduced dietary intake of vitamin B12 and folate in patients with recurrent aphthous stomatitis. J Oral Pathol Med 2010;39:420-3.
14. Sun A, Chen HM, Cheng SJ, Wang YP, Chang JY, Wu YC, *et al.* Significant association of deficiencies of hemoglobin, iron, vitamin B12, and folic acid and high homocysteine level with recurrent aphthous stomatitis. J Oral Pathol Med 2015;44:300-5.
15. Alshahrani S, Baccaglini L. Psychological screening test results for stress, depression, and anxiety are variably associated with clinical severity of recurrent aphthous stomatitis and oral lichen planus. J Evid Based Dent Pract 2014;14:206-8.
16. Huling LB, Baccaglini L, Choquette L, Feinn RS, Lalla RV. Effect of stressful life events on the onset and duration of recurrent aphthous stomatitis. J Oral Pathol Med 2012;41:149-52.
17. Hijazi K, Lowe T, Meharg C, Berry SH, Foley J, Hold GL. Mucosal microbiome in patients with recurrent aphthous stomatitis. J Dent Res 2015;94(3 Suppl):87S-94S.
18. Ghodratnama F, Riggio MP, Wray D. Search for human herpesvirus 6, human cytomegalovirus and varicella zoster virus DNA in recurrent aphthous stomatitis tissue. J Oral Pathol Med 1997;26:192-7.
19. Seoudi N, Bergmeier LA, Hagi-Pavli E, Bibby D, Fortune F. The seroprevalence and salivary shedding of herpesviruses in Behcet's syndrome and recurrent aphthous stomatitis. J Oral Microbiol 2015;7:27156.
20. Gomes CC, Gomez RS, Zina LG, Amaral FR. Recurrent aphthous stomatitis and *Helicobacter pylori*. Med Oral Patol Oral Cir Bucal 2016;21:e187-91.
21. Aminabadi NA. Recurrent aphthous stomatitis may be initiated by traumatic epithelial implantation and sustained by localized pathergic status. Med Hypotheses 2008;70:522-4.
22. Lewkowicz N, Lewkowicz P, Dzitko K, Kur B, Tarkowski M, Kurnatowska A, *et al.* Dysfunction of CD4+CD25 high T regulatory cells in patients with recurrent aphthous stomatitis. J Oral Pathol Med 2008;37:454-61.
23. Ozdemir IY, Calka O, Karadag AS, Akdeniz N, Ozturk M. Thyroid autoimmunity associated with recurrent aphthous stomatitis. J Eur Acad Dermatol Venereol 2012;26:226-30.
24. Scully C, Yap PL, Boyle P. IgE and IgD concentrations in patients with recurrent aphthous stomatitis. Arch Dermatol 1983;119:31-4.
25. Manchanda A, Iyengar AR, Patil S. Association between serotonin transporter gene polymorphism and recurrent aphthous stomatitis. Dent Res J 2016;13:206-10.
26. Bidoki AZ, Harsini S, Sadr M, Soltani S, Mohammadzadeh M, Najafi S, *et al.* NLRP3 gene polymorphisms in Iranian patients with recurrent aphthous stomatitis. J Oral Pathol Med 2016;45:136-40.
27. Karasneh J, Bani-Hani M, Alkhateeb A, Hassan A, Alzoubi F, Thornhill M. TLR2, TLR4 and CD86 gene polymorphisms in recurrent aphthous stomatitis. J Oral Pathol Med 2015;44:857-63.
28. Karasneh JA, Bani-Hani ME, Alkhateeb AM, Hassan AF, Thornhill MH. Association of MMP but not TIMP-1 gene polymorphisms with recurrent aphthous stomatitis. Oral Dis 2014;20:693-9.
29. Kalkan G, Yigit S, Karakus N, Bas Y, Seckin HY. Association between interleukin 4 gene intron 3 VNTR polymorphism and recurrent aphthous stomatitis in a cohort of Turkish patients. Gene 2013;527:207-10.
30. Kalkan G, Yigit S, Karakus N, Bas Y, Pancar GS, Balta I. Association between MEFV gene mutations and recurrent aphthous stomatitis in a cohort of Turkish patients. J Dermatol 2013;40:516-21.
31. Wardhana XX, Datau EA. Recurrent aphthous stomatitis caused by food allergy. Acta Med Indones 2010;42:236-40.
32. Akoglu G, Metin A, Kilinc F, Pektas SD, Isikoglu S, Akbas A, *et al.* Total serum oxidant/antioxidant status and arylesterase activity in recurrent aphthous stomatitis. Ann Dermatol 2013;25:273-7.
33. Arikan S, Durusoy C, Akalin N, Haberal A, Seckin D. Oxidant/antioxidant status in recurrent aphthous stomatitis. Oral Dis 2009;15:512-5.
34. Bagan J, Saez G, Tormos C, Gavalda C, Sanchis JM, Bagan L, *et al.* Oxidative stress and recurrent aphthous stomatitis. Clin Oral Investig 2014;18:1919-23.
35. Bilgili SG, Ozkol H, Takci Z, Ozkol HU, Karadag AS, Aslan M. Assessment of the serum paraoxonase activity and oxidant/antioxidant status in patients with recurrent aphthous stomatitis. Int J Dermatol 2013;52:1259-64.
36. Ozturk P, Belge Kurutas E, Ataseven A. Copper/zinc and copper/selenium ratios, and oxidative stress as biochemical markers in recurrent aphthous stomatitis. J Trace Elem Med Biol 2013;27:312-6.
37. Tugrul S, Kocyigit A, Dogan R, Eren SB, Senturk E, Ozturan O, *et al.* Total antioxidant status and oxidative stress in recurrent aphthous stomatitis. Int J Dermatol 2016;55:e130-5.
38. Caglayan F, Miloglu O, Altun O, Erel O, Yilmaz AB. Oxidative stress and myeloperoxidase levels in saliva of patients with recurrent aphthous stomatitis. Oral Dis 2008;14:700-4.
39. Karincaoglu Y, Batcioglu K, Erdem T, Esrefoglu M, Genc M. The levels of plasma and salivary antioxidants in the patient with recurrent aphthous stomatitis. J Oral Pathol Med 2005;34:7-12.
40. Kranzer K, Elamin WF, Cox H, Seddon JA, Ford N, Drobniewski F. A systematic review and meta-analysis of the efficacy and safety of N-acetylcysteine in preventing aminoglycoside-induced ototoxicity: Implications for the treatment of multidrug-resistant TB. Thorax 2015;70:1070-7.
41. Greene SC, Noonan PK, Sanabria C, Peacock WF. Effervescent N-Acetylcysteine Tablets versus Oral Solution N-Acetylcysteine in Fasting Healthy Adults: An Open-Label, Randomized, Single-Dose, Crossover, Relative Bioavailability Study. Curr Ther Res Clin Exp 2016;83:1-7.
42. Dauletbaev N, Fischer P, Aulbach B, Gross J, Kusche W, Thyroff-Friesinger U, *et al.* A phase II study on safety and efficacy of high-dose N-acetylcysteine in patients with cystic fibrosis. Eur J Med Res 2009;14:352-8.
43. Fernandes Teixeira FM, Figueiredo Pereira M, Gomes Ferreira NL, Miranda GM, Andrade Aguiar JL. Spongy film of cellulosic polysaccharide as a dressing for aphthous stomatitis treatment in rabbits. Acta Cir Bras 2014;29:231-6.
44. Rushworth GF, Megson IL. Existing and potential therapeutic uses for N-acetylcysteine: The need for conversion to intracellular glutathione for antioxidant benefits. Pharmacol Ther 2014;141:150-9.
45. Pendyala L, Creaven PJ. Pharmacokinetic and pharmacodynamic studies of N-acetylcysteine, a potential chemopreventive agent

during a phase I trial. Cancer Epidemiol Biomarkers Prev 1995;4: 245-51.
46. Bemeur C, Vaquero J, Desjardins P, Butterworth RF. N-acetylcysteine attenuates cerebral complications of non-acetaminophen-induced acute liver failure in mice: Antioxidant and anti-inflammatory mechanisms. Metab Brain Dis 2010;25:241-9.
47. Lasram MM, Lamine AJ, Dhouib IB, Bouzid K, Annabi A, Belhadjhmida N, *et al.* Antioxidant and anti-inflammatory effects of N-acetylcysteine against malathion-induced liver damages and immunotoxicity in rats. Life Sci 2014;107:50-8.
48. Liu Y, Yao W, Xu J, Qiu Y, Cao F, Li S, *et al.* The anti-inflammatory effects of acetaminophen and N-acetylcysteine through suppression of the NLRP3 inflammasome pathway in LPS-challenged piglet mononuclear phagocytes. Innate Immun 2015;21:587-97.
49. Uraz S, Tahan G, Aytekin H, Tahan V. N-acetylcysteine expresses powerful anti-inflammatory and antioxidant activities resulting in complete improvement of acetic acid-induced colitis in rats. Scand J Clin Lab Invest 2013;73:61-6.
50. Dhouib IB, Lasram MM, Abdeladhim M, Gharbi N, Ahmed MB, El-Fazaa S. Immunosuppression and oxidative stress induced by subchronic exposure to carbosulfan in rat spleen: Immunomodulatory and antioxidant role of N-acetylcysteine. Toxicol Mech Methods 2014;24:417-27.
51. Dinicola S, De Grazia S, Carlomagno G, Pintucci JP. N-acetylcysteine as powerful molecule to destroy bacterial biofilms. A systematic review. Eur Rev Med Pharmacol Sci 2014;18:2942-8.
52. May ER, Conklin KA, Bemis DA. Antibacterial effect of N-acetylcysteine on common canine otitis externa isolates. Vet Dermatol 2016;27:188-e147.
53. Moon JH, Choi YS, Lee HW, Heo JS, Chang SW, Lee JY. Antibacterial effects of N-acetylcysteine against endodontic pathogens. J Microbiol 2016;54:322-9.
54. Quah SY, Wu S, Lui JN, Sum CP, Tan KS. N-acetylcysteine inhibits growth and eradicates biofilm of *Enterococcus faecalis*. J Endod 2012;38:81-5.
55. Ulusoy AT, Kalyoncuoglu E, Reis A, Cehreli ZC. Antibacterial effect of N-acetylcysteine and taurolidine on planktonic and biofilm forms of *Enterococcus faecalis*. Dent Traumatol 2016;32:212-8.
56. Sansone RA, Sansone LA. Getting a Knack for NAC: N-Acetyl-Cysteine. Innov Clin Neurosci 2011;8:10-4.
57. Heard K, Rumack BH, Green JL, Bucher-Bartelson B, Heard S, Bronstein AC, *et al.* A single-arm clinical trial of a 48-hour intravenous N-acetylcysteine protocol for treatment of acetaminophen poisoning. Clin Toxicol 2014;52:512-8.
58. Arstall MA, Yang J, Stafford I, Betts WH, Horowitz JD. N-acetylcysteine in combination with nitroglycerin and streptokinase for the treatment of evolving acute myocardial infarction. Safety and biochemical effects. Circulation 1995;92:2855-62.
59. Pollman MJ, Hall JL, Gibbons GH. Determinants of vascular smooth muscle cell apoptosis after balloon angioplasty injury. Influence of redox state and cell phenotype. Circ Res 1999;84:113-21.
60. McCullough PA, Wolyn R, Rocher LL, Levin RN, O'Neill WW. Acute renal failure after coronary intervention: Incidence, risk factors, and relationship to mortality. Am J Med 1997;103:368-75.
61. Tepel M, van der Giet M, Schwarzfeld C, Laufer U, Liermann D, Zidek W. Prevention of radiographic-contrast-agent-induced reductions in renal function by acetylcysteine. N Engl J Med 2000;343:180-4.
62. Dodd S, Dean O, Copolov DL, Malhi GS, Berk M. N-acetylcysteine for antioxidant therapy: Pharmacology and clinical utility. Expert Opin Biol Ther 2008;8:1955-62.
63. Trabetti E. Homocysteine, MTHFR gene polymorphisms, and cardio-cerebrovascular risk. J Appl Genet 2008;49:267-82.
64. Zhang H, Spapen H, Nguyen DN, Rogiers P, Bakker J, Vincent JL. Effects of N-acetyl-L-cysteine on regional blood flow during endotoxic shock. Eur Surg Res 1995;27:292-300.
65. Grandjean EM, Berthet P, Ruffmann R, Leuenberger P. Efficacy of oral long-term N-acetylcysteine in chronic bronchopulmonary disease: A meta-analysis of published double-blind, placebo-controlled clinical trials. Clin Ther 2000;22:209-21.
66. Dippy JE, Davis SS. Rheological assessment of mucolytic agents on sputum of chronic bronchitics. Thorax 1969;24:707-13.
67. Mant TG, Tempowski JH, Volans GN, Talbot JC. Adverse reactions to acetylcysteine and effects of overdose. Br Med J 1984;289:217-9.
68. Atkuri KR, Mantovani JJ, Herzenberg LA. N-Acetylcysteine a safe antidote for cysteine/glutathione deficiency. Curr Opin Pharmacol 2007;7:355-9.
69. Zhang H, Forman HJ, Choi J. Gamma-glutamyl transpeptidase in glutathione biosynthesis. Methods Enzymol 2005;401:468-83.
70. Elbini Dhouib I, Jallouli M, Annabi A, Gharbi N, Elfazaa S, Lasram MM. A minireview on N-acetylcysteine: An old drug with new approaches. Life Sci 2016;151:359-63.
71. Amrouche-Mekkioui I, Djerdjouri B. N-acetylcysteine improves redox status, mitochondrial dysfunction, mucin-depleted crypts and epithelial hyperplasia in dextran sulfate sodium-induced oxidative colitis in mice. Eur J Pharmacol 2012;691:209-17.
72. Koksel O, Cinel I, Tamer L, Cinel L, Ozdulger A, Kanik A, *et al.* N-acetylcysteine inhibits peroxynitrite-mediated damage in oleic acid-induced lung injury. Pulm Pharmacol Ther 2004;17:263-70.
73. Koksel O, Ozdulger A, Ercil M, Tamer L, Ercan B, Atik U, *et al.* Effects of N-acetylcysteine on oxidant-antioxidant balance in oleic acid-induced lung injury. Exp Lung Res 2004;30:431-46.
74. Ocal K, Avlan D, Cinel I, Unlu A, Ozturk C, Yaylak F, *et al.* The effect of N-acetylcysteine on oxidative stress in intestine and bacterial translocation after thermal injury. Burns 2004;30: 778-84.
75. Ozdemir R, Yurttutan S, Sari FN, Uysal B, Unverdi HG, Canpolat FE, *et al.* Antioxidant effects of N-acetylcysteine in a neonatal rat model of necrotizing enterocolitis. J Pediatr Surg 2012;47: 1652-7.
76. Wang Q, Hou Y, Yi D, Wang L, Ding B, Chen X, *et al.*: Protective effects of N-acetylcysteine on acetic acid-induced colitis in a porcine model. BMC Gastroenterol 2013;13:133.
77. Briguori C, Colombo A, Violante A, Balestrieri P, Manganelli F, Paolo Elia P, *et al.* Standard vs double dose of N-acetylcysteine to prevent contrast agent associated nephrotoxicity. Eur Heart J 2004;25:206-11.
78. Albanidou-Farmaki E, Markopoulos AK, Kalogerakou F, Antoniades DZ. Detection, enumeration and characterization of T helper cells secreting type 1 and type 2 cytokines in patients with recurrent aphthous stomatitis. Tohoku J Exp Med 2007;212:101-5.
79. Dalghous AM, Freysdottir J, Fortune F. Expression of cytokines, chemokines, and chemokine receptors in oral ulcers of patients with Behcet's disease (BD) and recurrent aphthous stomatitis is Th1-associated, although Th2-association is also observed in patients with BD. Scand J Rheumatol 2006;35:472-5.
80. Freysdottir J, Lau S, Fortune F. Gamma delta T cells in Behcet's disease (BD) and recurrent aphthous stomatitis (RAS). Clin Exp Immunol 1999;118:451-7.
81. Ozyurt K, Celik A, Sayarlioglu M, Colgecen E, Inci R, Karakas T, *et al.* Serum Th1, Th2 and Th17 cytokine profiles and alpha-enolase levels in recurrent aphthous stomatitis. J Oral Pathol Med 2014;43: 691-5.
82. Oka S, Kamata H, Kamata K, Yagisawa H, Hirata H. N-acetylcysteine suppresses TNF-induced NF-kappaB activation through inhibition of IkappaB kinases. FEBS Lett 2000;472:196-202.
83. Nascimento MM, Suliman ME, Silva M, Chinaglia T, Marchioro J, Hayashi SY, *et al.* Effect of oral N-acetylcysteine treatment on plasma inflammatory and oxidative stress markers in peritoneal dialysis patients: A placebo-controlled study. Perit Dial Int 2010;30:336-42.
84. Omara FO, Fournier M, Vincent R, Blakley BR. Suppression of rat and mouse lymphocyte function by urban air particulates (Ottawa dust) is reversed by N-acetylcysteine. J Toxicol Environ Health A 2000;59:67-85.

85. Khan M, Sekhon B, Jatana M, Giri S, Gilg AG, Sekhon C, *et al.* Administration of N-acetylcysteine after focal cerebral ischemia protects brain and reduces inflammation in a rat model of experimental stroke. J Neurosci Res 2004;76:519-27.
86. Palacio JR, Markert UR, Martinez P. Anti-inflammatory properties of N-acetylcysteine on lipopolysaccharide-activated macrophages. Inflamm Res 2011;60:695-704. 10.
87. Purwanto B, Prasetyo DH. Effect of oral N-acetylcysteine treatment on immune system in continuous ambulatory peritoneal dialysis patients. Acta Med Indones 2012;44:140-4.
88. Wang C, Xia Y, Zheng Y, Dai W, Wang F, Chen K, *et al.* Protective effects of N-acetylcysteine in concanavalin A-induced hepatitis in mice. Mediators Inflamm 2015;2015:189785.
89. Rocksen D, Lilliehook B, Larsson R, Johansson T, Bucht A. Differential anti-inflammatory and anti-oxidative effects of dexamethasone and N-acetylcysteine in endotoxin-induced lung inflammation. Clin Exp Immunol 2000;122:249-56.
90. Ghodratnama F, Wray D, Bagg J. Detection of serum antibodies against cytomegalovirus, varicella zoster virus and human herpesvirus 6 in patients with recurrent aphthous stomatitis. J Oral Pathol Med 1999;28:12-5.
91. Seoudi N, Bergmeier LA, Drobniewski F, Paster B, Fortune F. The oral mucosal and salivary microbial community of Behcet's syndrome and recurrent aphthous stomatitis. J Oral Microbiol 2015;7:27150.
92. Olofsson AC, Hermansson M, Elwing H. N-acetyl-L-cysteine affects growth, extracellular polysaccharide production, and bacterial biofilm formation on solid surfaces. Appl Environ Microbiol 2003;69:4814-22.
93. Garcia BG, Cardoso MF, de Faria O, Gomez RS, Mesquita RA. A case report of pernicious anemia and recurrent aphthous stomatitis. J Contemp Dent Pract 2009;10:83-9.
94. Liu HL, Chiu SC. The Effectiveness of Vitamin B12 for Relieving Pain in Aphthous Ulcers: A Randomized, Double-blind, Placebo-controlled Trial. Pain Manag Nurs 2015;16:182-7.
95. Misra UK, Kalita J, Singh SK, Rahi SK. Oxidative Stress Markers in Vitamin B12 Deficiency. Mol Neurobiol 2016 [Epub ahead of print].
96. Bito T, Misaki T, Yabuta Y, Ishikawa T, Kawano T, Watanabe F. Vitamin B12 deficiency results in severe oxidative stress, leading to memory retention impairment in *Caenorhabditis elegans*. Redox Biol 2016;11:21-9.
97. Badaloo A, Reid M, Forrester T, Heird WC, Jahoor F. Cysteine supplementation improves the erythrocyte glutathione synthesis rate in children with severe edematous malnutrition. Am J Clin Nutr 2002;76:646-52.
98. Lim Y, Levy MA, Bray TM. Dietary supplementation of N-acetylcysteine enhances early inflammatory responses during cutaneous wound healing in protein malnourished mice. J Nutr Biochem 2006;17:328-36.
99. Karavana Hizarcioglu SY, Sezer B, Guneri P, Veral A, Boyacioglu H, Ertan G, *et al.* Efficacy of topical benzydamine hydrochloride gel on oral mucosal ulcers: An in vivo animal study. Int J Oral Maxillofac Surg 2011;40:973-8.
100. Karavana SY, Gokce EH, Rencber S, Ozbal S, Pekcetin C, Guneri P, *et al.* A new approach to the treatment of recurrent aphthous stomatitis with bioadhesive gels containing cyclosporine A solid lipid nanoparticles: In vivo/in vitro examinations. Int J Nanomed 2012;7:5693-704.
101. Lee DY, Kim HB, Shim IK, Kanai N, Okano T, Kwon SK. Treatment of chemically induced oral ulcer using adipose-derived mesenchymal stem cell sheet. J Oral Pathol Med [Epub ahead of print].
102. Lim YS, Kwon SK, Park JH, Cho CG, Park SW, Kim WK. Enhanced mucosal healing with curcumin in animal oral ulcer model. Laryngoscope 2016;126:E68-73.