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Malondialdehyde Levels in Middle Ear Fluid from Patients of Otitis Media with Effusion

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Otitis media with effusion (OME) is an inflammatory disease of the middle ear cleft. Oxygen free radicals have been implicated in a variety of inflammatory disorders. Oxygen free radicals may also be involved in the pathogenesis of OME. To evaluate the involvement of oxygen free radicals in the pathogenesis of OME, the level of malondialdehyde, which gives an index of lipid peroxidation by oxygen free radicals, was measured by the reaction with thiobarbituric acid. Malondialdehyde level in the middle ear fluid from the OME group was higher than that in the normal control group. Malondialdehyde level in the middle ear fluid from a mucoid subgroup was higher than that in the serous subgroup. Malondialdehyde levels in the middle ear fluid from the serous subgroup was significantly correlated with symptom duration. The Pearson correlation coefficient between malondialdehyde levels in the middle ear fluid from the serous subgroup and symptom duration was 0.842 ($P<0.05$). These results indicate that lipid peroxidation by oxygen free radicals may be involved in the pathogenesis of human OME.

Keywords: Malondialdehyde, Otitis media with effusion, Oxygen free radicals

Introduction

Oxygen free radicals can be defined as oxygen molecules or molecular fragments that have an unpaired electron (Moslen, 1994; Punchard and Kelly, 1996). They are formed in all living organisms during physiological and pathophysiological metabolism, and cause cell and tissue damages due to their high chemical reactivity (Moslen, 1994; Punchard and Kelly, 1996). They can react with macromolecules including lipid, protein, and DNA (Yagi, 1994). Peroxidation of lipids exposed to oxygen free radicals is responsible for damage to cells and tissues *in vivo*, where it may cause cancer (Ames and Shigenaga, 1993; O'Brien, 1994), inflammatory diseases (Leff, 1994), atherosclerosis (Reaven, 1994), heart disease (Ferrari, 1994), cataract (Cha and Kim, 1998), and liver disease (Mun, 1994). Identification and quantification of malondialdehyde gives an indirect index of oxidative injury which results in lipid peroxidation (Brown and Kelly, 1996). Malondialdehyde is the most abundant aldehyde arising from lipid peroxidation, and its determination, by measurement of the coloured product formed upon reaction with thiobarbituric acid, is one of the most common assays used in lipid peroxidation studies (Buege and Aust, 1978; Brown and Kelly, 1996).

We measured malondialdehyde levels, which is used as a marker of free radical-induced tissue damage, from the middle ear fluid in patients with OME to determine if oxygen free radicals play a role in the pathogenesis of human OME which is one of the inflammatory diseases.

Materials and methods

Patients The middle ear fluids in patients with OME (OME group) were taken in the operating room during tympanostomy tube placement procedures. The OME group was further subdivided into two subgroups by gross findings; serous OME group with 11 patients and mucoid OME group with 11 patients. The mean age of OME patients was 18.3 ± 21.5 years and there were 12 males and 10 females. In the control group, sera from 29 healthy blood donors were collected for this study. All control donors were determined to be otologically normal through patient history taking and the otoscopic findings. The mean age of the control group was 37.6 ± 18.4 years and there were 22 males and 7 females.

Chemicals Thiobarbituric acid (TBA) and trichloroacetic acid were purchased from Sigma (St. Louis, USA). All other chemicals were of the highest commercially available purity.

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Malondialdehyde assay The amount of malondialdehyde was measured by the thiobarbituric acid assay which is based on the reaction of malondialdehyde with thiobarbituric acid to give a red species absorbing at 535nm (Buege and Aust, 1978). The sample was mixed with a TBA reagent consisting of 0.375% TBA and 15% trichloroacetic acid in 0.25 N hydrochloric acid. The reaction mixtures were placed in a boiling water bath for 15 min and centrifuged at $3000 \times g$ for 5 min, after which the absorbance of the supernatant was read at 532 nm. The malondialdehyde concentration of the sample was calculated using an extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$.

Statistics Values were expressed as mean ± S.D. Statistical evaluation of significant difference between means was performed with the Student's *t*-test. *P* values of ≤0.05 were considered significant. Correlation between several parameters including symptom duration and malondialdehyde level was examined.

Results and Discussion

Otitis media with effusion, one of the most common pediatric health problems, is an infectious, inflammatory condition of the middle ear associated with middle ear effusion behind intact tympanic membrane. Despite numerous studies, the definite cause of OME is unknown. Infection, eustachian tube dysfunction, allergy, immunodeficiency, as well as several inflammatory mediators are known as etiologies of OME (Blustone and Klein, 1990; Gates, 1998). Oxygen free radicals have been implicated in a variety of inflammatory disorders including animal ear tissues from animal model of OME (Parks *et al.*, 1995). Oxygen free radicals may also be involved in the pathogenesis of human OME. To evaluate the involvement of oxygen free radicals in the pathogenesis of OME, the level of malondialdehyde, which gives an index of lipid peroxidation by oxygen free radicals, was measured by the reaction with thiobarbituric acid in middle ear fluid from patients with OME.

Malondialdehyde level in the middle ear fluid from the OME group was 6.08 ± 4.49 nmol per ml. Its level in the middle ear fluid from the OME group was 3.2 times higher than that in the normal control group (Fig. 1). This result indicates that lipid peroxidation by oxygen free radicals may be involved in the pathogenesis of human OME.

The malondialdehyde level in the middle ear fluid from the mucoid subgroup was 8.54 ± 5.19 nmol per ml. Its level in the mucoid subgroup was 2.4 times higher than that in middle ear fluid from the serous subgroup (Fig. 2). Serous fluid is a sterile, pale yellow-colored transudate with low viscosity, resembling serum, and this type of fluid is found in patients with a history of illness of short duration. Mucoid fluid is a cloudy exudate resulting from cell secretion, and the duration of infection history is longer than in serous OME (Blustone and Klein, 1990; Paparella *et al.*, 1991). Our results indicate that damages by oxygen free radicals are more severe in mucoid OME

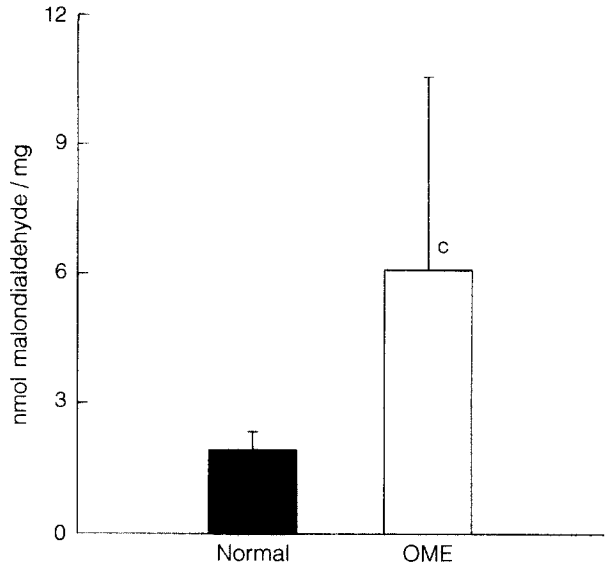

Fig. 1. Malondialdehyde levels in the middle ear fluid from patients of otitis media with effusion (OME). This was significantly different from normal control value (c; $P<0.001$).

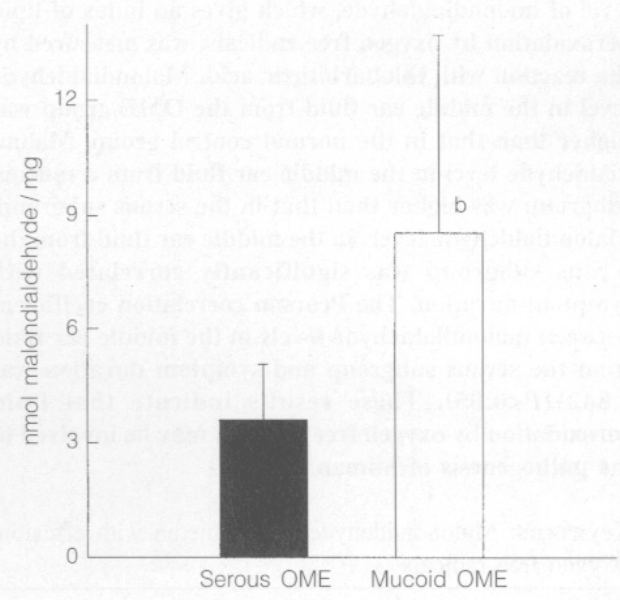

Fig. 2. Malondialdehyde levels in the middle ear fluid from patients of serous and mucoid otitis media with effusion (OME). This was significantly different from serous OME (b; $P<0.01$).

than in serous OME due to more severe inflammatory changes.

The malondialdehyde level in the middle ear fluid from patients with serous OME was significantly correlated as the only symptom for the duration among the several factors including ages (Table 1). The Pearson correlation coefficient between the malondialdehyde level in the middle ear fluid from patients with serous OME and

Table 1. Correlations between malondialdehyde level and several parameters.

Parameters	Pearson correlation coefficients
Air bone gap	0.300
Symptom duration in otitis media with effusion	0.137
Symptom duration in serous otitis media with effusion	0.842*
Symptom duration in mucoid otitis media with effusion	–0.305
Male patients of otitis media with effusion	0.128
Female patients of otitis media with effusion	0.257

*; $P<0.05$

symptom duration was 0.842 ($P<0.05$). This result may support that oxygen free radicals are responsible for the pathogenesis of the OME. And as time passes, aggravation of the inflammation in serous OME results in the increase of malondialdehyde level by lipid peroxidation.

According to these results, oxygen free radicals play a role in the pathogenesis of the OME.

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