The Journal of Laryngology & Otology
May 2001, Vol. **115,** pp. 363–368

Nebulized surfactant as a treatment choice for otitis media with effusion: an experimental study in the rabbit

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Abstract

Exogenous surfactant can improve eustachian tube function in experimentally induced otitis media with effusion (OME). Performing tympanometric recordings, the efficacy of inhaled nebulized surfactant, as compared with inhaled nebulized physiological saline was investigated, for the treatment of OME experimentally induced in the rabbit by intrabullar inoculation of heat-killed *Streptococcus pneumoniae*. In addition, the histological changes in middle ears after the treatment were investigated in order to establish whether the pathological findings correlated with the results.

Middle-ear pressure values before, and after, treatment were analyzed by the Wilcoxon statistical method, and the Mann-Whitney *U* test was used to compare the post-treatment values between groups. In all ears with OME in the affected animals, which were treated with nebulized surfactant inhalation, a positively significant ($p<0.05$) increase of pressure more than 20 daPa was recorded. In the control group, after inhalation of nebulized physiological saline, there was no positive increase in the affected middle-ear pressures; on the contrary, more negative pressure changes were recorded. In the histological evaluation, middle-ear epithelia and sub-epithelial space were normal in surfactant-treated ears with OME, whereas mucosal thickening with an oedematous sub-epithelial space containing occasional inflammatory cells and increases in connective tissue and vascularity, and effusions on the epithelial surface were present in the ears with OME in the control group. The significant improvement in the negative middle-ear pressure after nebulized surfactant treatment and the histological findings shown in our study can support the theory that surface-active agents are of importance in eustachian tube function even under pathologic conditions, such as OME.

Key words: Otitis Media with Effusion; Surface-active Agents; Therapy; Inhalation Exposure; Rabbits

Introduction

Middle-ear effusions are referred to in the literature by a variety of synonyms, including serous otitis media, secretory otitis media, glue ear, chronic non-suppurative otitis media, chronic otitis media with effusion, and simply, otitis media with effusion (OME).[1,2] Acute otitis media and OME are very common among children.[3] Eighty-five per cent of children experience at least one episode of otitis media[4] and about 50 per cent of all children between two and five years of age were found to have had at least one episode of OME. During such episodes, not only is the child's hearing impaired, but there is also an increased risk of contracting acute otitis media.[5] We can say that OME is one of the most important, unresolved clinical problems in otolaryngology.[4] The costs of treatment modalities of OME, both medical and surgical, are very high in some countries.[3] Approximately 25 per cent of all children with OME are treated surgically, ventilating tubes being inserted through the tympanic membrane.[5,6] Such surgery requires general anaesthesia, and is thus costly to the community as well as distressing both for the children and their parents.[5] Effective pharmacological treatment could reduce such problems.

The cause of OME is multifactorial, but most often it is associated with infection and/or tubal dysfunction. While one aetiological factor may initiate the disease, another causes it to persist, and yet another aggravates it.[7] Abnormal function of the eustachian tube appears to be the most important factor in the pathogenesis of middle-era disease in all age groups.[8]

The eustachian tube serves an important function in equalizing pressure between the middle ear and the nasopharynx.[9] The eustachian tube in the normal state is closed, or collapsed and is opened only intermittently. Peritubal pressure is one of several factors that keep the tube closed, including the elasticity of the tubal cartilage, the condition of the mucous membranes and the surface tension and viscosity of the mucous film in the eustachian tube. It

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This paper has been presented, in part, at the 2nd Seminar on Otology and Neuro-Otology, Stara-Zagora, Bulgaria, May 19–20, 2000.
Accepted for publication: 24 November 2000.

is opened and middle-ear pressure normally regulated actively by means of swallowing, yawning or other movements of the jaw involving muscular activity. Middle-ear pressure can also be changed passively through a pressure-induced opening of the eustachian tube, without any muscular activity.[3]

Reduced opening function of the eustachian tube has long been suggested to be a predisposing factor in the development and maintenance of OME.[5] The force needed to open the eustachian tube must overcome the elasticity of the peritubal tissues and adhesive forces of the mucus in the tube.[10] It seems logical that the passage of air through the eustachian tube would be facilitated if the surface tension of the secretions is reduced.[3]

The pulmonary alveoli are lined with a surface-active material, pulmonary surfactant, that stabilizes the alveoli by lowering surface tension on expiration, thus preventing alveolar collapse.[11] Surface-tension-lowering substances, surfactants, have been reported to be present also in the eustachian tube and middle ear, both in animals[12–14] and in humans,[10,15] since the possible involvement of surfactant in eustachian tube function was first suggested by Filsberg *et al.*[16]

It has been reported in some studies[17–19] that surfactant-precursors are synthesized in cells from the lower part of the tubal wall and in the tubal glands. However, it was not clear where the surface-tension-lowering substances found in the eustachian tube were secreted. In their morphological study on the eustachian tubes of mice, Karchev *et al.* have found surfactant-producing cells, which are morphologically similar to type II pneumocytes, in the cartilaginous roof tubothelium.[20] Recently, surfactant protein gene expression in the eustachian tube was demonstrated by Paananen *et al.*[21]

It has been proposed that the surfactant present in the middle ear and the eustachian tube prevents collapse of the eustachian tube during normal functioning.[12,15] Therefore, surfactant may play an important part in the normal eustachian tube physiology by facilitating the tubal opening to allow for aeration of the middle ear and adequate drainage.[9] An alteration in the amount or composition of surfactant may affect eustachian tube function.[22] Some investigators[15,16] have documented that relative deficiencies in surface-active phospholipids are seen in some effusions of OME. These deficiencies may be secondary to altered surface active substance, production, or degradation of surfactant secondary to the action of enzymes liberated during infection.[9]

Recent experimental studies have demonstrated a measurable decrease in opening force by invasively placing exogenous surfactant into the middle ear or eustachian tube. White *et al.*[23] have shown that exogenous surfactant derived from pig lungs clearly reduced the pressure required to force the eustachian tube open in rats with acute otitis media. In their experimental study in gerbils, Fornadley and Burns[24] have found that surfactant can lower the eustachian tube opening pressure, even in the presence of changes related to OME. They reported the manoeuvres to instill surfactant or to increase natural surfactant could be beneficial in controlling OME. In a study conducted with approximately 400 children and adults with OME, treatment with ambroxol, which is claimed to increase the synthesis and the secretion of surfactant in the lungs, improved some of the parameters studied, as compared with place.[25]

For the first time, Nemechek *et al.*[26] have evaluated the efficacy of delivering nebulized surfactant to improve eustachian tube function in experimentally induced OME. They have suggested that inhaled nebulized surfactant could be efficacious in treating eustachian tube dysfunction. However, this suggestion seems to need further investigation. Because in the study there was no control group, which should have been delivered nebulized physiological saline, in order to investigate whether it was the surfactant or the nebulization method, or both that improved the eustachian tube function.

In our study, through a clinical aspect of OME (performing oto-microscopic examination and tympanometric recordings), the efficacy of inhaled nebulized surfactant was investigated, as compared with inhaled nebulized physiological saline, in the treatment of OME experimentally induced in the rabbit, in which important anatomical structures (tympanic membrane, eustachian tube and eustachian tube muscles) were preserved. In addition, the histological changes in middle ears after the treatment were investigated, in order to establish whether the pathologic findings correlated with the results.

Materials and methods

Healthy, young, white New Zealand rabbits having a mean weight of 600 g were used. After anaesthesia (ketamine 50 mg/kg i.m. + xylazine 10mg/kg i.m.), otoscopic examination was performed with an operating microscope. When the appearance of the tympanic membrane was normal and the middle-ear space was verified as free of disease, tympanometric measurement was performed by using the impedance audiometer model AZ7-Interacoustics instrument. To decrease the hysteresis effect, all tympanometric measurements were performed from positive to negative. The peak of the tympanogram (the pressure at the highest point of the compliance) is an indication of the pressure status in the tympanic cavity.[27] Tympanometry was performed at least three times in each ear before the examination in order to observe whether the recordings were repeatable (accepted as, pressure changes between peak pressures of pre-experimental recordings in a single ear were less than 5 daPa) or not.

Tympanometry was chosen as the investigation method for this work because it provides a rapid, non-invasive and objective assessment of middle-ear status that provides insight into eustachian tube function.[27] For example, when a negative pressure is recorded, the state is usually indicative of a resolving or forming middle-ear effusion.[28] In addition, tympanometry is widely used for the clinical diagnosis and follow-up of OME, and it can also be recorded in the rabbit with clinical instruments.[29]

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The only probe tone in the instrument we used was 220 Hz. However, it has been reported in a multi-frequency tympanometric study on acoustic middle-ear reflexes in non-anaesthetized rabbits that probe tones around 1000 Hz are most suitable for use with rabbits[29] because the resonant frequency of the middle ear is higher than it is in man[30] and low-amplitude tympanograms, which can sometimes make determination of maximal amplitudes uncertain, can be obtained with the 220 Hz probe.[29,31] When non-defined, double-peaked, or non-repeatable tympanograms were observed before the experiment, the animals with such unacceptable recordings were excluded. If an acceptable tympanogram was obtained, and if recorded middle-ear pressure in both ears was between ± 50 daPa, the rabbit was marked and included into the study.

The operation was performed to each ear of 18 rabbits, that were included into the study. After deepening anaesthesia with additional ketamine and xylazine dosages, the bulla was exposed by dissection under sterile conditions. Under the visualization of the operating microscope, a thin hole (as wide as the plastic canal of a 24 no. intracut) was drilled through the bulla without destroying the middle-ear structures. Through this hole, a heat-killed pneumococci suspension (0.1 ml) was administered into both ears of seven rabbits and into one ear of 10 rabbits. Into the other ears of these 10 rabbits and into both ears of one rabbit equal amounts (0.1 ml) of sterile phosphate buffer solution (PBS) were administered. The hole on the bulla was closed by application of dental cement at the outer (periost) surface, and the skin was closed suturing with 4/0 silk.

Preparation of killed bacteria: *Streptococcus pneumoniae* (strain ATCC 6305) wsa subcultured in brain-heart infusion broth. Stationary phase bacteria were washed and suspended in PBS to a concentration of 10^8 colony-forming units per ml. These were kept in a water bath at 20°C for 45 minutes, the heat-killed bacteria were obtained and divided into aliquots (0.1 ml). The sterility of the aliquots was confirmed with subsequent repeat culture on nutrient agar. The aliquots that would be used in the experiments were kept at –20°C.

On the post-operative fifth day, the rabbits' ears were examined microscopically and tympanometric measurements were recorded by the first examiner, who did not know in which ear killed streptococci were given. The rabbits, in which effusion was seen in at least one ear, or abnormal otoscopic signs such as retraction or an erythematous tympanic membrane, (in addition to an increase in negative pressure of more than 20 daPa by tympanometry), were considered to be affected subjects and the ears with these abnormal findings were considered to have OME. The preceding experimental model of OME is a modification of the method described by Lowell *et al.*,[32] injecting heat-killed *Streptococcus pneumoniae* by the transtympanic route. This is an effective model of experimental OME, which closely parallels human disease.[24] The three non-affected animals, which had no OME in any ear, were killed with an overdose of intravenous pentobarbital.

The affected animals were organized into two groups. Nebulized surfactant was administered to each animal in the first group (surfactant-treated group) and nebulized physiological saline was administered to each animal in the other (control group). Commercially available surfactant (Survanta, Abbott) obtained from bovine lung was used in the experiment. It has the composition, which consists of 21.25 to 28.75 mg/ml total phospholipids (re-inforced with three synthetically obtained lipids, dipalmitoilphosphatidylcholine, palmithic acid and tripalmithine, to optimize the surface activity), 1.4 to 3.5 mg/ml free fatty acids, 0.5 to 1.75 mg/ml triglycerides and 0.1 to 1.0 mg/ml proteins related to surfactant, suspended in physiological saline in 8 ml vial (Survanta package insert, Abbott Laboratories, North Chicago, IL, USA). A total dosage of 4 ml suspension containing 1.3 ml survanta and 2.7 ml physiological saline was placed in the nebulizer for administration to each animal in the surfactant-treated group, and a dosage of 4 ml physiological saline was placed in the nebulizer for administration to each animal in the control group. Administration lasted until all of the dosage was finished. This procedure was repeated at 12 hour intervals for four days. On the fifth day, tympanometry was performed and the pressure values of all ears were recorded. After recordings, animals were re-examined microscopically and were then killed with an overdose of intravenous pentobarbital. Their temporal bones were excised for histological evaluation, and cultures of middle-ear cavities were taken.

Histological evaluation After fixation in 10 per cent formalin for 24 hours, excision materials of temporal bones were decalcified in 10 per cent of formic acid. After routine tissue follow-up, 4μ cuts through the middle ear were stained with haematoxylin and eosin for subsequent histological evaluation. Slides prepared in this way were examined with a light microscope by a pathologist, who did not know in which ear the findings of OME were expected.

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS 9.0 for Windows, SPSS Inc., Chicago, Ill.) software (License no: 105192, Trakya University Faculty of Medicine, Edirne, Turkey). Middle-ear pressure values before, and after, treatment were analyzed by the Wilcoxon statistical method, and the Mann-Whitney *U* test was usd to compare the post-treatment values between groups. A level of $p<0.05$ was accepted as statistically significant.

The Ethics Committee of Trakya University, Faculty of Medicine, approved the animal use protocol for this study in compliance with the guidelines of the Declaration of Helsinki.

Results and analysis

Two rabbits, which were given in one ear heat-killed bacteria and in the other ear PBS, showed pain-

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indicating behaviour during the early post-operative hours. Intramuscular morphine (5cc/kg) was administered as an analgesic drug. In the first post-operative day, one of these two rabbits died. Euthanasia was performed by an overdose of intravenous pentobarbital to the other one on the post-operative third day because a wound infection developed. Another three animals, that had OME findings in none of their ears, were also excluded from the study.

OME developed in 14 ears of the remaining 13 rabbits (one had bilateral and 12 had unilateral OME). There was no effusion in any of the BPS injected ears nor was a negative pressure change more than 20 daPa recorded (Table I). Our successful inoculation rate of 63.6 per cent (14 of 22 ears injected with killed bacteria by the intrabullar route) compared favourably with that of other investigators[24,26] who used the same method, but by the transtympanic route.

The surfactant-treated group consisted of eight affected ears of seven rabbits and the control group consisted of six affected ears of six rabbits. In all affected ears treated with nebulized surfactant inhalation, a positive increase of pressure more than 20 daPa was recorded, but none of the values (the last tympanometric recordings) was higher than 50 daPa (Table I). The positive pressure change in the surfactant-treated group was found to be significant (Table II). In the control group, after inhalation of nebulized physiological saline, there was no positive increase in the affected middle-ear pressures; on the contrary, more ngative pressure changes were recorded. However, these changes were not statisticlly significant (TableII). When the post-treatment pressure values were compared between groups, the positive increase of pressure in the surfactant-treated group was found to be significant ($p = 0.038$).

At the end of the study, in the surfactant-treated ears with OME and in ears instilled with PBS, no effusion was seen in the microscopic examination, and the middle-ear epithelia and sub-epithelial space were normal by histological evaluation (Figure 1). Mucosal thickening with an oedematous sub-epithelial space containing occasional inflammatory cells,

TABLE I

MIDDLE EAR PRESSURE VALUES BEFORE INTRABULLAR KILLED-BACTERIA OR PBS[†] INJECTION, FIVE DAYS AFTER INJECTION AND AFTER FOUR-DAYS NEBULIZATION TREATMENT

				Peak pressure in tympanometry (daPa)			
Rabbit	No.	Ears R/L*	Intrabullar injection	Before injection	5 days after injection	After treatment	Nebulized material
1.	5	R	Streptococci	5	0	–5	Survanta
		L	Streptococci	–5	–45	–5	
2.	6	***R***	Streptococci	–25	–100	–10	Survanta
		L	Streptococci	50	–15	40	
3.	7	***R***	Streptococci	15	–45	15	Survanta
		L	Streptococci	–25	0	35	
4.	9	R	Streptococci	–25	–35	–25	Survanta
		L	Streptococci	50	–25	15	
5.	10	***R***	Streptococci	25	–65	35	Survanta
		L	PBS	–30	–20	5	
6.	11	***R***	Streptococci	10	–50	10	Survanta
		L	PBS	–10	–10	–5	
7.	13	R	PBS	–50	–45	–45	Survanta
		L	Streptococci	20	–55	15	
8.	1	R	PBS	50	50	50	PS[‡]
		L	Streptococci	50	5	–175	
9.	2	R	PBS	40	45	20	PS
		L	Streptococci	50	–35	–60	
10.	3	***R***	Streptococci	5	–20	–80	PS
		L	PBS	–15	–25	–25	
11.	4	***R***	Streptococci	25	–15	–40	PS
		L	PBS	–25	–25	–25	
12.	8	R	Streptococci	–10	–5	5	PS
		L	Streptococci	–10	–35	–50	
13.	12	***R***	Streptococci	15	–20	–20	PS
		L	Streptococci	25	–25	–25	
14.	14	R	PBS	–25	–25	–	–
		L	PBS	–25	–25		
15.	15	R	Streptococci	0	–10	–	–
		L	Streptococci	5	15		
16.	16	R	Streptococci	0	0	–	–
		L	PBS	–40	–35		
17.	17	R	PBS	–15	Wound	–	Euthanasia
		L	Streptococci	–15	Infection		
18.	18	R	Streptococci	–10	Exitus	–	–
		L	PBS	–35			

*Bold, italic capitals indicate the ears with the findings of otitis media with effusion.
[†]PBS: Phosphate buffered saline.
[‡]PS: Physiological saline.

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TABLE II
MEAN PRESSURE CHANGES IN EARS WITH OME AFTER TREATMENT (N = NUMBER OF EARS)

Treatment group	Mean pressure change	*p* value (Wilcoxon)
Nebulized surfactant (n = 8)	+64.4 daPa	0.012
Nebulized saline (n = 6)	−50.8 daPa	0.249

and increases in connective tissue and vascularity, and effusions on the epithelial surface were present in the ears with OME in the control group (Figure 2). These pathological findings were similar to the previous experimental model of OME developed by Lowell *et al.*[32] Cultures from the middle-ear cavity taken at temporal bone excision from all animals were sterile.

Discussion and conclusion

In our study, the significant improvement in the negative middle ear pressure after nebulized surfactant treatment, and the histological findings support the theory[23,24] that surface active agents are of importance in eustachian tube function even under pathological conditions, such as OME. Showing that inhalation of nebulized physiological saline had no effect on experimental OME in the rabbit, also made the nebulized surfactant efficacy for eustachian tube function[26] more reliable. As we know, surfactant has not been used for the treatment of OME in humans. There is no synthetic surfactant preparation that can be administered practically. Our experimental study indicates pre-clinical criteria for its administration, and assesses objectively the use of surfactant for the treatment of OME.

The aetiology of OME is a combination of micro-organisms and negative pressure in the middle ear.[6,7] An ideal drug for the treatment of OME should be able to deal with the infection and improve the eustachian tube dysfunction that causes negative pressure in the middle ear. Antibiotic therapy is the only non-surgical treatment shown to be effective for patients with OME.[33] Following a course of antibiotics, if the middle-ear effusion persists, surgical options include insertion of ventilation tubes, adenoidectomy, or adenoidectomy plus myringotomy or tubes. But these tubes can cause complications and sequelae, and do not always cure patients over the long-term. One

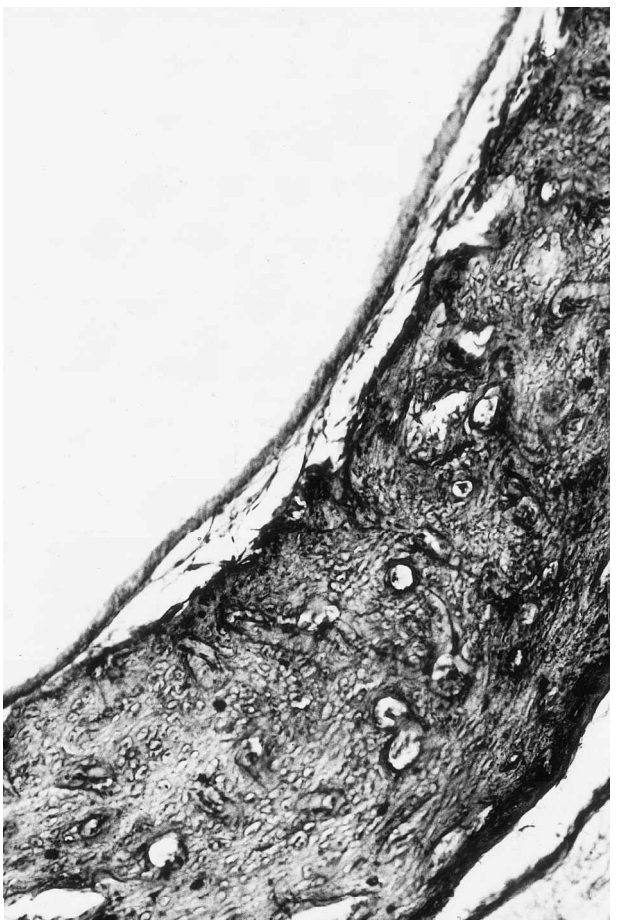

FIG. 1
Histological evaluation of middle-ear mucosa in a ear with OME after the nebulized surfactant treatment showing normal findings (H & E; ×100).

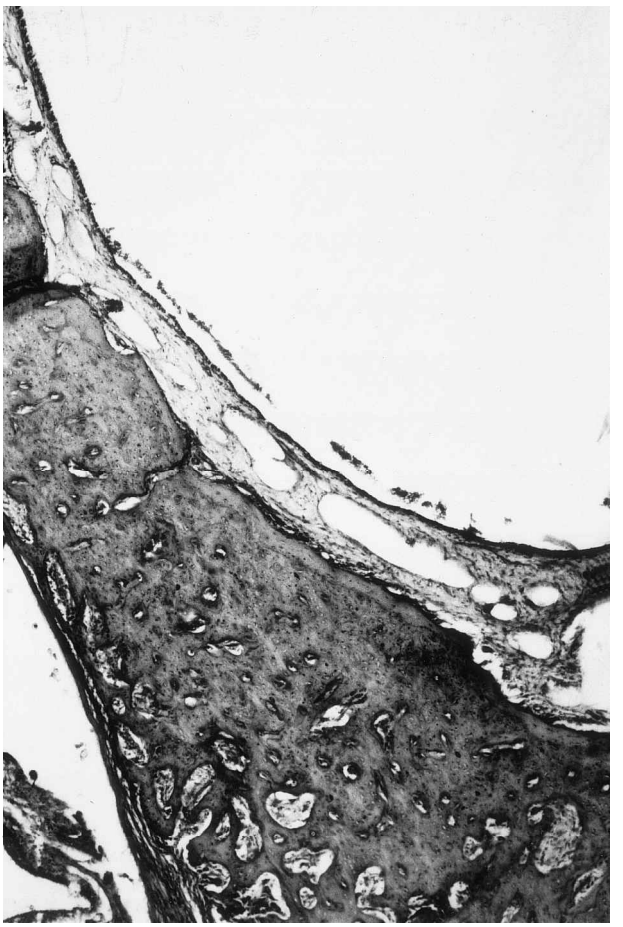

FIG. 2
Histological evaluation of middle-ear mucosa in a ear with OME after nebulized physiological saline administration showing mucosal thickening with increased intercellular space and dilated vessels. Sub-epithelial space contains inflammatory cells, and middle-ear effusion is visible overlying the epithelium (H & E; ×100).

https://doi.org/10.1258/0022215011907848 Published online by Cambridge University Press

reason for this may be the biochemical events associated with the inflammatory process and the relative hyperoxia in the middle ear caused by the insertion of ventilation tube.[6,34] Surfactant may play a role in the treatment of otitis media both by improving eustachian tube opening function[23,24] and by its proteins enhancing the host resistance against middle-ear infections.[21] With the evolution of synthetic surfactant drugs, which may be more effective and cheaper, and with supporting clinical studies, nebulized surfacfant inhalation may become a practical and non-invasive treatment choice for OME.

Acknowledgements

The authors would like to thank Dr Ilter Alpözen from Abbott Laboratories, Istanbul, Turkey and Recep Tuncel, biologist, Eczacibasi Medicine, Turkey for their help in the study.

This work was supported by the Research Foundation of Trakya University (TÜAF-208).

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Dr C. Uzun takes responsibility for the integrity of the content of the paper. This work was supported by the Research Foundation of Trakya University (TÜAF-208).
Competing interests: None declared