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Effects of Inhalation of Thermal Water on Exhaled Breath Condensate in Chronic Obstructive Pulmonary Disease

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Key Words
Lung function · Normal saline · Aerosol therapy · Leukotriene B_4 · Pulmonary inflammation

Abstract
Background: Inhalation of thermal water (TW) is traditionally used as part of the treatment of chronic obstructive pulmonary disease (COPD), but its benefit and mechanisms are controversial. We previously observed a reduced proportion of neutrophils in induced sputum after treatment with TW. ***Objectives:*** The aim of this study was to determine whether inhalation of TW in COPD patients is associated with biochemical changes of airway lining fluid, including a reduction in the neutrophil chemoattractant leukotriene B_4 (LTB_4). ***Methods:*** Thirteen COPD patients were randomly assigned to receive a 2-week course of TW and normal saline inhalation in a cross-over, single-blind study design. Exhaled breath condensate (EBC) was collected before and after treatments. LTB_4 concentrations in EBC were determined by ELISA, and EBC pH was measured before and after argon deaeration. ***Results:*** No significant differences in LTB_4 concentrations in EBC were detected with either treatment. A significant decrease in pH of non-deaerated EBC was observed after a standard course of TW (median 7.45, interquartile range 6.93–7.66, vs. median 6.99, interquartile range 6.57–7.19; $p = 0.05$), which disappeared after argon deaeration. ***Conclusions:*** There is no evidence that TW treatment affects LTB_4 concentration in EBC. The results of EBC pH measurements suggest that TW inhalation induces an imbalance of volatile components of the buffer system in airway lining fluid.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by pulmonary inflammation, progressive airflow limitation not completely reversible, and is often associated with symptoms of chronic bronchitis [1]. Management of COPD requires the integration of several different components: minimizing risk factors, educational programs, improving symptoms with a stepwise treatment approach, preventing exacerbations, rehabilitation, oxygen therapy and eventually surgical treatments. Inhalation of thermal water (TW) is traditionally used as part of the treatment of COPD and chronic bronchitis, but is not included among management options by the most recent guidelines [1], since its benefit and physiopathological mechanisms are not yet well clarified. While TW is shown to have some anti-inflammatory properties in patients with rhinitis [2], few investigations were performed in the lower respiratory tract [3]. We previously observed a reduced proportion of neutrophils in induced

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Table 1. Physical/chemical characteristics of TW and normal saline

Parameters	TW	Normal saline
pH	7.1	4.5–7.0
Na^+, g/l	1.24	3.54
Cl^-, g/l	2.18	5.46
K^+, g/l	0.088	absent
Ca_2^+, g/l	0.080	absent
Mg_2^+, g/l	0.366	absent
NH_4^+, g/l	0.0027	absent
SO_4^{2-}, g/l	0.980	absent
HCO_3^-, g/l	0.169	absent
Br^-, mg/l	13.6	absent
I^-, mg/l	0.82	absent
H_2S, mg/l	1.67	absent
Osmotic pressure, atm	3.10	7.83

sputum after TW treatment, suggesting that TW may have a mild anti-inflammatory effect on the airways [4]. The measurement of markers in exhaled breath condensate (EBC) has been suggested to be a useful and relatively inexpensive method for assessing and monitoring airway inflammation [5]. Compared with bronchoalveolar lavage, EBC is noninvasive and does not require instillation of saline into the lung. Furthermore, it does not influence the percentages of airway inflammatory cells, observed in some instances after inhalation of hypertonic saline solution for sputum induction [6].

The aim of this study was to determine whether inhaled salt-bromide-iodine TW could modify airway lining fluid in COPD patients. For this purpose, we analyzed EBC pH and concentrations of the neutrophil chemoattractant leukotriene B_4 (LTB_4) before and after a 2-week course of TW inhalations using normal saline treatment as a control, in a cross-over, single-blind study design.

Methods

Subjects

Thirteen patients (10 men and 3 women, aged 47–83 years) with COPD, diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) [1], were recruited from a local general practice. Eligible patients were current or former smokers with at least a 5-pack-years smoking history. According to the GOLD guideline, postbronchodilator forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) <70% defines the airflow limitation of COPD, and reversibility after inhaled salbutamol (400 μg) must be <12% and 200 ml of initial FEV_1. Exclusion criteria were atopy and asthma history, other clinically significant diseases, exacerbation of COPD or respiratory infection within the last 4 weeks, and treatment with inhaled or systemic corticosteroids in the previous month. Short- and long-acting β-agonist bronchodilators were permitted during the study. All patients gave written informed consent. The study was approved by the ethics committee of the University Hospital of Padova and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Design of the Study

The subjects were randomly assigned to receive a 2-week course of TW or normal saline inhalation in a cross-over, single-blind study design. The wash-out period between treatments was at least 4 weeks. TW originated from hot springs (approximately at 80°C) in the Terme Euganee area (Abano Terme-Montegrotto, Veneto, Italy). The main characteristics of TW compared with normal saline are shown in table 1. TW and normal saline solution were kept at a temperature of approximately 37°C and nebulized with an output of 50 ml/min. The aerosols were administered once a day for 20 min. Each subject was examined before and after each treatment (TW and normal saline), for a total of 4 visits. Each visit included the evaluation of chronic bronchitis symptoms and dyspnea using the Communauté Européenne du charbon et de l'Acier (CECA) questionnaire, pulmonary function tests and EBC collection. Dyspnea was graded from 0 to 4.

To evaluate the acute effect of aerosol inhalation, EBC was collected before and after a single 20-min inhalation of normal saline and TW on 2 separated days prior to the 2-week treatments.

Pulmonary Function Tests

FEV_1 and FVC before and 15 min after inhalation of 200 μg of salbutamol were performed with a pneumotacograph (SpiroAnalyzer ST-150, Fukuda Sangyo, Japan). The predicted normal values were those from CECA [7].

EBC Collection and Analysis

EBC was collected during oral tidal breathing using a commercial condenser (Turbo Deccs 04, Italchil, Parma, Italy). The subjects were not allowed to eat or drink for at least 1 h before EBC collection. They breathed normally through a mouthpiece for 15 min and a 2-way non-rebreathing valve that also served as a saliva trap. If the subjects felt saliva in their mouth, they were instructed to swallow it. Condensate, at least 1 ml, was collected at –10°C and transferred to 3 Eppendorf tubes. Then, all samples were stored at –80°C.

pH was measured using a calibrated pH meter (model pH300, Hanna Instruments, Padova, Italy) with a flat membrane electrode (5207, Crison Instruments SA, Alella, Spain) and an accuracy of ±0.01. In the ATS/ERS task force, Horváth et al. [8] argues that although many investigators believe that the measurements of EBC pH after deaeration is the most validated technique, others consider that gas standardization is unnecessary. For the actual divergence of views, we consider of interest to perform the EBC measurements prior to and following argon gas to achieve gas standardization for 3 min, as reported previously [9]. To rule out contamination of EBC by saliva, amylase concentration in the samples was measured using an enzymatic colorimetric test (IFCC, Roche Diagnostic Modular, lower limit of detection 3 U/l). Amylase was undetectable in all of the samples tested.

LTB_4 concentrations were measured with specific enzyme immunoassay (Assay Design Inc., Ann Arbor, Mich., USA) with a sensitivity of 5.63 pg/ml. To prevent adherence of fatty acid derivates (such as leukotrienes), all polypropylene tubes were coated with Tween 20 [10].

Statistical Analysis

The values of EBC LTB_4 and pH after a single inhalation of TW and normal saline were regarded as the baseline for the 2-week treatment. Data are expressed as the means ± standard error (SE) or the median and interquartile ranges (IQR). The Mann-Whitney U test was used for comparison between groups, and the Wilcoxon rank sum test was used to compare the data before and after treatment. The significance was accepted at the 5% level.

Results

The characteristics of the subjects are given in table 2. No COPD exacerbation occurred during the course of the study and inhaled treatments were well tolerated. One subject withdrew his consent for personal reasons and did not complete the study.

Median baseline EBC pH was 7.09 (IQR 5.93–7.43) and 7.29 (IQR 6.27–7.57) before and after argon deaeration ($p < 0.01$), respectively.

LTB_4 concentration was measurable in the EBC of all subjects. We did not detect any changes in EBC LTB_4 concentrations after a single inhalation of either treatments (table 3). Similarly, no acute variations were demonstrated in non-deaerated EBC pH. In contrast, after a single inhalation of normal saline, deaerated EBC pH increased compared with corresponding baseline values ($p = 0.01$; table 3).

Figure 1 shows non-deaerated and deaerated EBC pH before and after a 2-week treatment with normal saline and TW. No significant effects on non-deaerated and deaerated EBC pH were observed after normal saline (7.23, IQR 6.66–7.54, vs. 7.30, IQR 6.98–7.49, and 7.47, IQR 7.29–7.79, vs. 7.45, IQR 7.34–7.87, respectively) (fig. 1a, c). Non-deaerated EBC pH after 2-week TW inhalations was significantly decreased (7.45, IQR 6.93–7.66, vs. 6.99, IQR 6.57–7.19; $p = 0.05$) (fig. 1b). No significant changes were observed in deaerated EBC pH after TW (before treatment 7.58, IQR 7.26–7.71, vs. after treatment 7.24, IQR 6.98–7.75) (fig. 1d).

EBC LTB_4 concentration did not significantly change after both treatments. Similarly, no significant differences were detected in lung function and dyspnea score (table 4).

Discussion

In this study, we showed that a conventional course of inhaled salt-bromide-iodine TW is associated with biochemical changes of airway lining fluid in COPD pa-

Table 2. Characteristics of the study subjects

Subjects	13
Males/females	10/3
Age, years	69.0 ± 3.0
BMI	27.6 ± 1.0
Smoking history, former/current	8/5
Pack-years	33.8 ± 4.3
FEV_1, % predicted	66.6 ± 3.5
Postbronchodilator FEV_1/VC, %	65.7 ± 2.3
Stage I (mild), %	6 (46)
Stage II (moderate), %	6 (46)
Stage III (severe), %	1 (8)
Stage IV (very severe), %	0 (0)
Chronic bronchitis, %	85

Data are expressed as the mean ± SE. Number in parentheses are percentages. BMI = Body mass index.

Table 3. Acute effects of a single 20-min inhalation of normal saline and TW on non-deaerated and deaerated EBC pH and LTB_4 concentrations

Biomarkers	Normal saline		TW	
	before treatment	after treatment	before treatment	after treatment
EBC LTB_4, pg/ml	28.0 (14.6–44.6)	16.5 (4.3–28.1)	15.0 (2.0–38.1)	14.0 (1.0–25.5)
Non-deaerated EBC pH	7.09 (5.93–7.43)	7.23 (6.66–7.54)	6.98 (6.74–7.45)	7.45 (6.93–7.66)
Deaerated EBC pH	7.29 (6.27–7.57)	7.47 (7.29–7.79)*	7.42 (7.02–7.78)	7.58 (7.26–7.71)

Data are expressed as the median, with interquartile ranges in parentheses. * $p = 0.01$ versus before treatment.

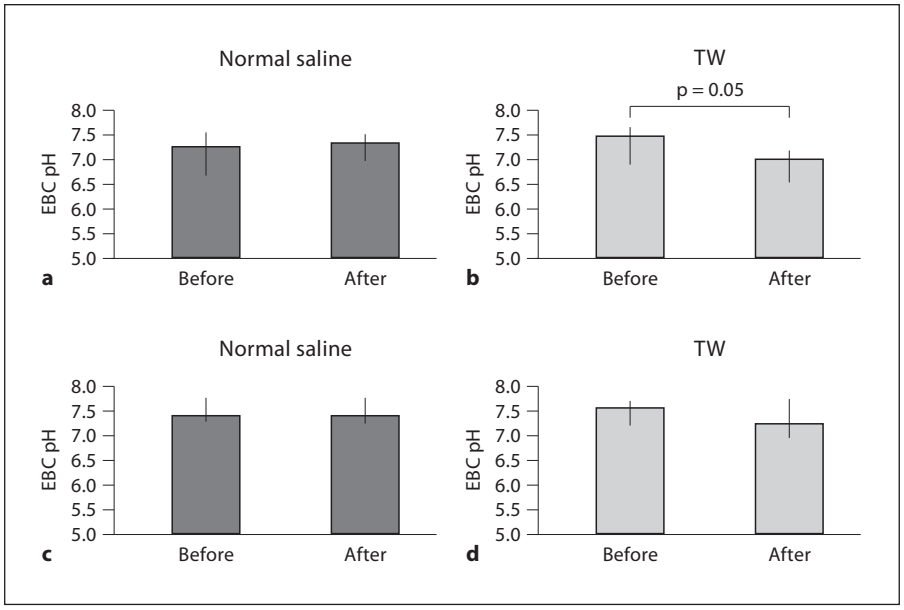

Fig. 1. Non-deaerated and deaerated EBC pH before and after 2-week treatment with normal saline and TW. **a** Median non-deaerated EBC pH before and after normal saline solution. **b** Median non-deaerated EBC pH before and after TW. **c** Median deaerated EBC pH before and after normal saline solution. **d** Median deaerated EBC pH before and after TW.

Table 4. EBC LTB_4 concentration, FEV_1 and dyspnea score before and after 2-week treatment with normal saline and TW

Parameters	Normal saline		Thermal water	
	before treatment	after treatment	before treatment	after treatment
EBC LTB_4, pg/ml	22.25 (14.75–41.0)	13.0 (4.86–48.75)	14.75 (2.04–46.50)	28.25 (12.50–46.50)
FEV_1, liters	1.80 ± 0.13	1.83 ± 0.16	1.83 ± 0.13	1.79 ± 0.14
Dyspnea score	0.77 ± 0.30	0.83 ± 0.39	0.77 ± 0.30	0.54 ± 0.14

Data are the median with interquartile ranges in parentheses, or the mean ± SE.

tients. A significant decrease was observed in non-deaerated EBC pH after inhalation of TW which disappeared after argon deaeration. No significant differences in EBC LTB_4 concentrations were detected either with TW or with normal saline treatments.

LTB_4 was measurable in EBC at concentrations above the detection limit of the immunoassay. EBC LTB_4 levels previously found in stable COPD were highly variable, ranging between 10 and 100 pg/ml [11–14]. In healthy subjects, the EBC LTB_4 concentration was higher in smokers than in nonsmokers (9.4 vs. 6.1 pg/ml) [13]. We confirmed that EBC LTB_4 concentrations are elevated in COPD. However, the results do not provide evidence that the reduced proportion of neutrophils in induced sputum after TW treatment [4] is due to decreased LTB_4 concentrations. Caution should be shown in the interpretation of LTB_4 data because LTB_4 concentrations in EBC exhibited high variability.

EBC consists mostly of water with trapped aerosolized droplets from the airway lining fluid, as well as of water-soluble volatile compounds. It is believed that pH of EBC is determined largely by the water-soluble volatile gases and reflects, but does not precisely quantitate, that of airway lining fluid. Several investigators reported that values of deaerated EBC pH in healthy subjects range between 7.5 and 8.1 [15]. According to data distribution described by Paget-Brown et al. [16], pH values <7.4 should be considered abnormally low. We confirmed that EBC pH is lower in patients with COPD than in healthy subjects [17–19]. The causes of airway acidification in COPD have not been clarified and could reflect intrinsic airway acidification induced by altered airway pH homeostasis

as a consequence of infections and inflammatory processes [20] or the presence of hypopharyngeal gastric acid reflux, which is very common in patients with obstructive lung disease [21].

A single 20-min inhalation of aerosol tended to increase EBC pH irrespective of whether TW or normal saline is inhaled. This phenomenon was more evident with normal saline after CO_2 removal. In contrast, Carpagnano et al. [22] were unable to detect pH changes in EBC pH after saline inhalation. Probably, the longer time of inhalation and the larger output of nebulizer in our protocol can explain the discrepancy in the results. We predicted that a massive aerosol inhalation could modify the airway lining fluid pH. For this reason, the effects of inhalation treatments were evaluated after correction for the acute changes of EBC pH values after a single 20-min inhalation.

The different chemical composition of TW compared with normal saline could explain the change in EBC pH. The finding that the differences in EBC pH were more evident before argon deaeration suggests that TW inhalations may induce an imbalance of volatile components of buffer systems, NH_4^+/NH_3 and CO_2/HCO_3^-, involved in determining the EBC pH. Talking about a negative effect, a proinflammatory effect of TW is probably too strong and certainly does not correspond to what we showed in our previous study.

It has been shown that NH_4^+ is the most abundant cationic buffer in the EBC of healthy subjects and patients with COPD [23, 24]. EBC NH_4^+ derives from NH_3 gas released from the saliva especially. Bacterial degradation of urea is responsible for much of the NH_3 generated in the mouth. When the mouth is washed with acidic solutions (which tend to trap NH_3 as NH_4^+ in the saliva), EBC NH_4^+ can be reduced by 90% [25]. A decrease in ammonia by a similar washing mechanism due to massive aerosol inhalation with a consequent reduction in pH due to EBC content in CO_2 can be hypothesized. The expected effect has not occurred, but the reduction in EBC pH observed was limited, on average 0.46 and 0.34 prior and following argon deaeration, respectively. It was highlighted that between-day variability of EBC pH is high in COPD patients [9, 19]. The reason why EBC pH fluctuates more in COPD remains undetermined. There are a number of confounding factors which can affect EBC pH, including corticosteroid treatment, infections and smoking. We excluded patients on steroids, and none of the patients had exacerbation during the study. However, subclinical microorganism airway colonization cannot be excluded, and a relatively high proportion of patients (62.5%) were current smokers. It is controversial whether smoking increases EBC pH variability and decreases EBC pH. Borrill et al. [19] ruled out that being a current smoker affects EBC pH in COPD patients. Similar results were previously reported by Vaughan et al. [26]. In contrast, Do et al. [27] observed that acute smoking is associated with a low EBC pH, and asthmatic smokers exhibited more EBC pH variability [9].

This study may be underpowered to detect subtle differences in EBC pH induced by aerosol inhalation in the presence of other sources of variability. In fact, the sample size was calculated to show a difference of 2.0 pg/ml in EBC LTB_4 concentration assuming a standard deviation of 1.8.

Even though this study was not designed to show clinical and functional effects of TW, we observed some decrease in dyspnea scores after 2-week TW treatment, but the change was not statistically significant. This result is in line with our previous observations that variables related to health perception are more sensitive than functional indexes to detect effects of treatment with TW [4].

In conclusion, we have rejected the hypothesis that the reduced proportion of neutrophils in induced sputum after TW inhalations is due to decreased LTB_4. We have shown that a conventional treatment with salt-bromide-iodine TW in COPD is associated with some decrease in EBC pH. The results suggest that a chemical composition of TW induced an imbalance of volatile components of buffer systems of airway lining fluid.

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