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Effect of cold and dry environment on the expression of matrix metalloproteinase 1 mRNA in bone tissues of different pathogenic factors induced models*☆

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Abstract

BACKGROUND: The studies have showed that the dry and cold environment of Xinjiang area can affect many diseases, but the specific mechanism is unknown, which is not conducive to carry out the geographical and personalized treatment for the diseases.

OBJECTIVE: To reveal the effect of cold and dry environment composite different diseases predisposing factor on the expression of matrix metalloproteinase 1 mRNA in rat bone tissue.

METHODS: The models were established by smoking or dripping pancreatic elastase in trachea combined with smoking, and stimulated with cold and dry environment, the fluorescence quantitative PCR was used to determine the expression of matrix metalloproteinase 1 mRNA in bone tissue.

RESULTS AND CONCLUSION: The expression of matrix metalloproteinase 1 mRNA in bone tissue of smoking composite dry and cold environment group was higher than that of the smoking group ($P < 0.05$); the expression of matrix metalloproteinase 1 mRNA in bone tissue of smoking+pancreatic elastase composite dry and cold environment group was higher than that of the smoking+pancreatic elastase group ($P < 0.01$). It shows that dry and cold environment can exacerbate the damage of bone when compound with different diseases predisposing factors, such as smoking, smoking and pancreatic elastase.

INTRODUCTION

With the development of society, the effect of environment on disease and illness is increasingly attracted the attention of the medical profession. Xinjiang is located in the northwest of China and has a dry and cold climate, which has varying degrees of impact on the development of a variety of diseases. However, the research on the effects is rare, which has affected the development of the geographical and personalized treatment for the diseases. Based on the previous studies, the authors believe that the cold dry environment can increase the risk of chronic obstructive pulmonary disease (COPD) secondary osteoporosis[1], and the preliminary studies found that cold and dry environment can influence the expression of matrix metalloproteinases (MMP) and the components of extracellular matrix in rats simply treated with smoking[2]. But the effects on the bone tissue, especially on different stressors caused bone tissue metabolic status are not system revealed. In the process of establishing animal model of COPD, simulate Xinjiang cold and dry environment as a stressor to treat rats, and to analyze the effect on the expression of MMP1 during the model establishing process as an independent pathogenic factor, in order to provide reference for the prevention of extrapulmonary symptoms of COPD in the Xinjiang dry and cold environment.

MATERIALS AND METHODS

Design

A randomized controlled animal experiment.

Time and setting

The experiment was completed in Animal Laboratory of Xinjiang Medical University and Respiratory Physiology and Pathology Laboratory, Xinjiang•National Clinical Research Base of Traditional Chinese Medicine from October 2011 to January 2012.

Materials

The experimental animals were 35 male

Wistar rats in 4 weeks with the body mass of (100±20) g, which provided by Experimental Animal Center of Xinjiang Medical University without free, License No. SCXK(Xin)2003-001.

Reagents and instruments are as follows:

Reagent and instrument	Source
FLI-2000H artificial climate chamber	Japan EYELA Company, No. 10908061
BS-1105 type electronic balance	Beijing Saiduokesi balance Co., Ltd.
Xuelian cigarettes	Xinjiang Cigarette Factory, Tar 12 mg, nicotine 1.0 mg, CO 13 mg
M-MlV reverse transcription kit	Invitrogen, Lot number: C28025-032
platinum SYBR Green qPCR SuperMix-UDG	Invitrogen, Lot number: C11733-038
Diethyl pyrocarbonate (DEPC)	Sangon Biotech (Shanghai) Co., Ltd., Lot number: 0214S11
Elastase (Trypsin)	Shanghai Huayi Biotechnology Co., Ltd.

Methods

Grouping and modeling

Thirty-five Wistar rats were adaptive fed for 2 days and then randomly divided into five groups according to the random number table: smoking group, cold and dry+ smoking group, smoking+pancreatic elastase group, cold and dry+ smoking+pancreatic elastase group and control group, 7 rats in each group. Rats in the smoking group, cold and dry+ smoking group, smoking+ pancreatic elastase group and cold and dry+ smoking+pancreatic elastase group were put in the homemade Acrylic exposure box (60 cm×70 cm× 100 cm) with the volume of 420 L every day from the first day to the 90th day, there was a vent on the top of the box with the diameter of 2.0 cm and a CPU fan was hanged in the middle of the box to make the smoke evenly distributed. Before smoking, 250 g silica gel desiccant were placed in the box and the exposure box was connected with smoking devices. The smoking was injected into the exposure box after aspirated with tee and 100 mL feeder, and ready to add the smoking with the frequency of 15 times per minute, in order to maintain the concentration of smoke relatively stable. The smoking was lasted for 1 hour every time and performed one time each morning and afternoon with an interval of 4 hours. During smoking, drove the animals appropriately in order to prevent the animal get together and then affect smoking. In cold and dry+ smoking+pancreatic elastase group and smoking+pancreatic elastase group, 20 U elastase were added into 8 mL normal saline and then intratracheal instillation was performed at 30 days according to the body weight per kilogram[3]. In cold and dry+smoking group and cold and dry+smoking+ pancreatic elastase group, rats were put in the artificial climate chamber every night at 60 days in the temperature of 6 ℃ and relative humidity (25%–32.8%), 8 to 10 hours each day. Rats in the control group were fed in the environment of room temperature (25±3) ℃ and relative humidity of 60%–80%. At 30 days, rats in the control group were treated with intratracheal instillation of 8 mL normal saline.

Fluorescence quantitative PCR detection

At 90 days, rats were sacrificed by the inferior vena cava blood after modeling, and then three right hind leg bones were selected from each group randomly. After that, the MMP-1 mRNA in the bone tissue was detected with fluorescence quantitative PCR.

Main outcome measures

The expression of MMP-1 mRNA in the bone tissue.

Statistical analysis

The experimental data was analyzed with SPSS 11.5 and represented with mean±standard deviation, comparison between groups was tested with independent sample *t*-test, $P < 0.05$ was considered as significant, $P < 0.01$ was considered as statistically significant.

RESULTS

Analysis of the number of experimental animals

At the beginning of the experiment, there were seven rats in each group. During the experiment, one rat dead in cold and dry+pancreatic elastase group and cold and dry+smoking+pancreatic elastase group, and finally, 33 rats were left. Three rat hind leg bones were selected randomly from each group, and then restored in –80 ℃.

Effect of cold and dry composite smoking on the expression of MMP-1 mRNA

The expression of MMP-1 mRNA in the cold and dry+ smoking group was higher than that in the smoking group and control group ($P < 0.05$) (Table 1).

Table 1 Effect of cold and dry composite smoking on the expression of matrix metalloproteinase 1 (MMP-1) mRNA in rat bone tissue

Group	*n*	MMP-1 mRNA ($\bar{x}$±*s*)	95% confidence interval
Smoking	3	0.97±0.66	[0.80,1.13]
Cold and dry+smoking	3	1.26±0.18^{ab}	[0.82,1.70]
Control	3	1.00±0.00	[1.00,1.00]

$^{a}P < 0.05$, *vs.* control group; $^{b}P < 0.05$, *vs.* smoking group

Effect of cold and dry composite smoking and pancreatic elastase on the expression of MMP-1 mRNA

The expression of MMP-1 mRNA in the smoking+ pancreatic elastase group and cold and dry+smoking+

pancreatic elastase group was higher than that in the control group ($P < 0.05$, $P < 0.01$); and the expression of MMP-1 mRNA in the cold and dry+smoking+pancreatic elastase group was higher than that in the smoking+ pancreatic elastase group ($P < 0.01$) (Table 2).

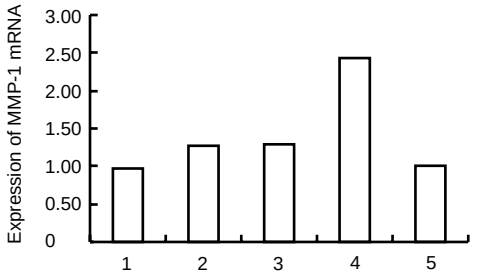
Table 2 Effect of cold and dry composite smoking and pancreatic elastase on the expression of matrix metalloproteinase 1 (MMP-1) mRNA in rat bone tissue

Group	*n*	MMP-1 mRNA ($\bar{x}\pm s$)	95% confidence interval
Smoking+pancreatic elastase	3	1.29±0.09[a]	[1.08,1.51]
Cold and dry+ smoking+pancreatic elastase	3	2.40±0.18[bc]	[1.98,2.89]
Control	3	1.00±0.00	1.00-1.00]

[a]$P < 0.05$, [b]$P < 0.05$, *vs.* control group; [c]$P < 0.05$, *vs.* smoking+pancreatic elastase group

Comparison of the MMP-1 mRNA expression in the rat bone tissue of each group

Conclusion from figure 1 that cold and dry environment superimposed a causative factor had greater effect on the expression of MMP-1 mRNA than single factor, and the difference was significant.



1: Smoking group;
2: Cold and dry+smoking group;
3: Smoking+pancreatic elastase group;
4: Cold and dry+smoking+pancreatic elastase group;
5: Control group

Figure 1 Expression of matrix metalloproteinase 1 (MMP-1) mRNA in bone tissue of five groups

DISCUSSION

Patients with COPD often have malnutrition and weight loss, and these patients may be at high risk of osteoporotic fractures state[4], Bolton *et al* [5] found that the bone mineral density of the patients with is lower than that in the control group, it performance with the increasing of bone resorption and inhibition of bone formation, which result in the loss of bone mass and thus causing osteoporosis. For the patients with severe COPD patients, osteoporosis or fracture is a common complication, and the literatures have reported that 35%–72% COPD patients have osteomalacia and 36%–60% COPD patients have osteoporosis[6-8]. At present, there is a consistent view that COPD is an independent risk factor for secondary osteoporosis[9]. Smoking is a major predisposing factor for COPD, as smoking can induce the chronic inflammation in the modeling process of COPD. While airway instillation of elastase can formed bulla which is one of the primary means to establish the model of COPD. Whether cold and dry environment can aggravate the effect of COPD predisposing factor smoking and airway instillation of elastase on extrapulmonary bone tissue, the experiment in this paper was designed to identify the influence.

The preliminary research has found that the cold and dry environment can lead to the model immune dysfunction[10], hypothalamic-pituitary-adrenal axis dysfunction[11], and then resulting in bleeding symptoms[12], while the immune function, hypothalamic-pituitary-adrenal axis equilibrium and the formation of stasis are the main reason for onset and the symptom of deterioration. Stasis formation can not only lost the effect on the bone, but also can block in the body for a long time which can seriously affect the normal operation of the blood and the organs function, so that it can lead to the slow bone tissue blood metaplasia and affect the normal functional activities of bone tissue. Eventually, lead to poor blood and bone marrow dystrophy, then has the clinical manifestations of systemic or lower back pain, and ultimately cause osteoporosis[13]. Therefore, we considered that cold and dry environment can increase the risk of COPD secondary osteoporosis. Similarly, with the in-depth research, the modern medicine thinks that COPD is not only a simple airway disease, but also the pulmonary vascular disease, is the disease that involving the whole lung. Meanwhile, it is a systemic disease[14]. The changes of coagulation status may be one of the systemic pathological changes of COPD, and closely related with the secondary osteoporosis.

Recent years, some scholars have proposed the assumption that COPD is the autoimmune diseases[15-17], studies also found that COPD has the pulmonary and systemic immune deficiency[18-19]. And some evidence have showed that adaptive immunity plays an important role in the occurrence and development of COPD, and thought that COPD airway inflammation is the chronic inflammation that involving the interaction of a variety of inflammatory cells. Long-term chronic inflammatory stimulation can lead to the changes of immune function of the patients and results in the cytokines abnormally elevated, network response disorders[20] and lower cellular immune function. The typical autoimmune responses often

exists continuously[21-23]; meanwhile, the autoimmune disease often involves multi-organ and multi-system, thus many clinical characteristics of COPD may also be the results of autoimmune responses. The series of immunologically reactive substances released by lymphocytes and monocytes have strongly effect in promoting bone resorption; studies have showed that T cells may play a role in postmenopausal bone loss, and there exists bone regulation relationships between the estrogen and the immune system[24]. Based on that, the author can speculate that the disorder of the immune function in cold and dry environment may be one of the mechanisms of COPD secondary osteoporosis.

The study found that the expression of MMP-1 mRNA in the bone tissue of cold and dry+smoking group was higher than that of the smoking group ($P < 0.05$), and higher than the control group ($P < 0.05$). The expression of MMP-1 mRNA in the bone tissue of smoking+pancreatic elastase group and cold and dry+smoking+pancreatic elastase group was higher than that of the control group ($P < 0.05$，$P < 0.01$); the expression of MMP-1 mRNA in the bone tissue of cold and dry+smoking+pancreatic elastase group was higher than that of the smoking+pancreatic elastase group ($P < 0.01$). It means that no matter cold and dry environment composite smoking or composite smoking and pancreatic elastase, cold and dry environment superimposed a causative factor had greater effect on the expression of MMP-1 mRNA than single factor, and the difference was significant. That indicates that cold and dry environment composite smoking can promote the expression of MMP-1 mRNA. Recent studies have found that the MMP plays an important role in the degradation process of the bone matrix[25]. Studies have indicated that when the expression of MMP-1 mRNA increased, the number of osteoclasts that can express MMP-1mRNA was increased. It indicates that the increasing of the osteoclast activity can promote the bone matrix degradation and decrease the synthesis of collagen[26]. When the osteoblast simulated with the bone-resorption stimulating factor, it can secret MMP-1 which can degenerate the type Ⅰ collagen, and thereby activate the osteoclasts. MMP-1 is the key factor for starting the bone resorption and bone matrix degeneration. So, the research on MMP has great significance in understanding the resorption and degradation matrix of bone matrix, understanding the effect of cold and dry environment and smoking on the quality of bone and finding the prevention and control targets. Therefore, the study thought that cold and dry environment can promote the expression of MMP-1 mRNA and affect the metabolic balance of bone tissue.

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寒燥对不同致病因素诱发模型骨组织基质金属蛋白酶 1 mRNA 表达的影响*☆

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文章亮点：首次揭示了寒燥分别复合烟熏、烟熏加气道滴注弹性蛋白酶等不良诱发因素对模型骨组织基质金属蛋白酶 1 mRNA 表达的影响。探讨寒燥、抽烟等复合因素对以新疆为代表的西北地区人群骨质的影响并寻找其防治靶点，具有重要的现实意义。

关键词：寒燥；熏烟；胰蛋白酶；骨组织；基质金属蛋白酶 1 mRNA

摘要

背景：研究发现新疆干燥寒冷的环境对当地多种疾病的发生发展产生影响，但目前具体机制不明，不利于疾病地域化、个性化治疗的开展。

目的：揭示寒燥环境复合不同的疾病诱发因素对大鼠骨组织基质金属蛋白酶 1mRNA 表达的影响。

方法：利用寒燥环境分别与熏烟、熏烟加气道滴注胰蛋白酶复合作用于大鼠建立模型，利用荧光定量 PCR 法检测骨组织中基质金属蛋白酶 1 mRNA 的表达。

结果与结论：寒燥+熏烟组骨组织中基质金属蛋白酶 1 mRNA 表达高于熏烟组($P < 0.05$)；寒燥+熏烟+胰酶组骨组织中基质金属蛋白酶 1 mRNA 表达高于寒燥+胰酶组($P < 0.01$)。提示寒燥可以加剧熏烟、熏烟加胰酶等不同疾病诱发因素对骨组织的损伤程度。

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