Molecular and Cellular Endocrinology 392 (2014) 136–143

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Tissue concentrations of estrogens and aromatase immunolocalization in interstitial pneumonia of human lung

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ARTICLE INFO

Article history:
Received 9 December 2013
Received in revised form 18 April 2014
Accepted 16 May 2014
Available online 23 May 2014

Keywords:
Aromatase
Estrogen
Interstitial pneumonia
Lung

ABSTRACT

Interstitial pneumonia (IP) is characterized by various degrees of pulmonary fibrosis and inflammation. Estrogens have been demonstrated to play important roles in physiological and pathological conditions of human lung, but significance of estrogens has remained unknown in human IP. Therefore, we measured estrogen concentrations and immunolocalized aromatase and estrogen receptor β (ERβ) in IP tissues. Estradiol concentration was significantly (2.8-fold) higher in IP than normal lung tissues, and aromatase activity evaluated by estradiol/testosterone ratio was also significantly (7.2-fold) elevated in IP tissues. Aromatase immunoreactivity in alveolar epithelial cells was significantly frequent in IP than normal lung or inflammatory lung disease other than IP, and it was positively associated with ERβ immunoreactivity in these cells of IP. These results suggest that estradiol concentration is locally increased in human IP tissue by aromatase, and increased estrogens may play an important role in the development of IP through ERβ in the alveolar epithelial cells.

1. Introduction

Interstitial lung disease, or diffuse parenchymal lung disease, is a heterogeneous group of a pulmonary disorder which shares similar clinical, radiological or pathological features with miscellaneous etiologies, such as drug toxicity, collagen vascular diseases, occupational/environmental exposures and other unknown causes (American Thoracic Society and European Respiratory Society, 2001; King, 2005; Ryu et al., 2007). A great majority of interstitial lung disease is associated with some features of interstitial pneumonia (IP), which is characterized by various degrees of pulmonary fibrosis and inflammation (American Thoracic Society and European Respiratory Society, 2001; King, 2005; Ryu et al., 2007). The mechanisms of pulmonary fibrosis have been gradually clarified in recent years (Selman and Pardo, 2006; Willis et al., 2006; Strieter and Mehrad, 2009), but several subtypes of IP, including the idiopathic pulmonary fibrosis, are still associated with poor clinical outcome and high mortality rate despite the advent of therapy (Raghu et al., 2011). Therefore, it has become very important to examine the details of biological features of IP and to develop the targeted therapies aimed at the specific factors involved in its biological behaviors.

Estrogens have been recently demonstrated to be involved in various physiological and/or pathological functions in many human organs other than their classical target tissues, through binding to estrogen receptor (ER) (Gustafsson, 1999; Morani et al., 2008; Carey et al., 2007). ER consists of ERα and ERβ in human. Human lung tissue expresses both ERα and ERβ and show biological responses to estrogen (Stabile et al., 2002), and its predominant subtype is considered ERβ (Couse et al., 1997; Taylor and Al-Azzawi, 2000; Omoto et al., 2001). Estrogens have been also

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http://dx.doi.org/10.1016/j.mce.2014.05.016
0303-7207/

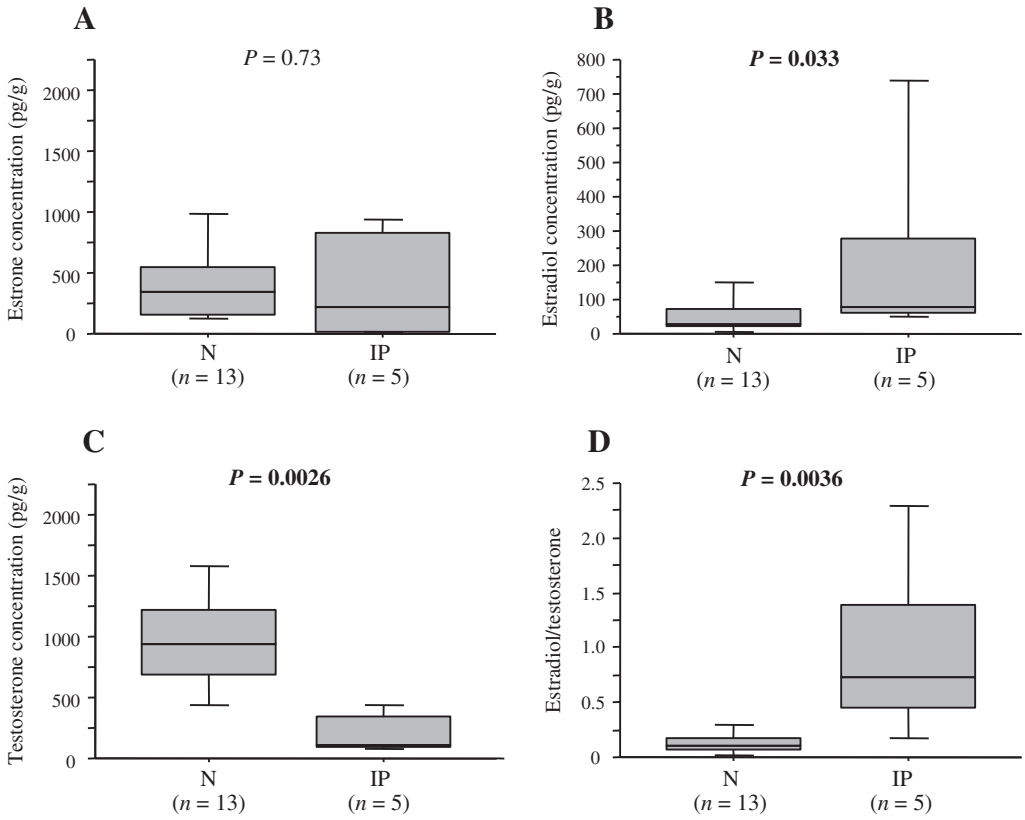

Fig. 1. Tissue concentrations of estrone (A), estradiol (B), testosterone (C) and estradiol/testosterone ratio (D) in each specimens of IP using LC–MS/MS analysis. Data are represented as box and whisker plots. The median value was represented by a horizontal line in the box pot, and gray box denoted the 75th (upper margin) and 25th percentiles of the values (lower margin), respectively. The upper and lower bars indicated the 90th and 10th percentiles, respectively. The statistical analysis was performed using a Wilcoxon test. *P*-value less than 0.05 was considered significant, and described as boldface. N: normal lung tissue with no significant pathological abnormalities, and IP: interstitial pneumonia.

well demonstrated to be locally produced from circulating inactive steroids by aromatase, a rate limiting key enzyme in estrogen biosynthesis converting androgens to estrogens. Increased local estrogen actions through aromatase and ER are closely associated with a variety of pathological conditions, such as breast carcinoma (Miki et al., 2007), hepatocellular carcinoma (Castagnetta et al., 2003; Vizoso et al., 2007), atherosclerosis (Murakami et al., 2001; Nakamura et al., 2003), diabetic nephropathy (Prabhu et al., 2010), Alzheimer disease (Ishunina et al., 2007), rheumatoid arthritis (Ishizuka et al., 2004; Schmidt et al., 2005), and skin wound healing (Ashcroft et al., 1999; Mills et al., 2005; Gilliver et al., 2007; Merlo et al., 2008).

Estrogens have been demonstrated to influence pulmonary development and physiology (Carey et al., 2007), and ERβ contributes to the maintenance of normal lung tissue functions (Patrone et al., 2003). In addition, biologically active estrogen, estradiol, significantly increased cell proliferation of ERβ-positive lung carcinoma cells, and tissue concentrations of estradiol were also reported to be elevated in lung carcinoma tissues (Niikawa et al., 2008). The intratumoral estradiol concentration of lung carcinoma was reported to be positively associated with intratumoral aromatase expression (Niikawa et al., 2008), and aromatase immunoreactivity was associated with worse prognosis in women with lung carcinoma (Mah et al., 2007). Therefore, estrogens and/or aromatase become possible new therapeutic targets of human lung cancer patients. Also, expression of other sex hormone progesterone receptor (PR) has been reported in lung carcinoma (Ishibashi et al., 2005).

Effects of sex steroids upon IP have been examined mostly in rodent models of the disease (Gharaee-Kermani et al., 2005; Voltz et al., 2008; Brass et al., 2010), and profibrotic effects of estrogens were clearly demonstrated in these models (Gharaee-Kermani et al., 2005). However, to the best of our knowledge, estrogen concentration and aromatase expression have not been previously reported in actual human IP tissues, and therefore significance of estrogens remains largely unknown in human IP. Therefore, in this study, we examined the concentration of sex steroids and immunoreactivity of aromatase and ERβ in human IP tissues, and compared the results with those in non-pathological lung tissue in order to evaluate a possible involvement of estrogenic actions and *in situ* production of estrogens in human IP.

2. Materials and methods

2.1. Patients and tissues

Snap-frozen specimens of 5 IP lesions of the human lung were retrieved from autopsy files at Tohoku University Hospital, Sendai, Japan, to measure tissue concentrations of sex hormones. These samples were obtained from five men who died of IP [median age 76 (range 61–80) yrs]. As for the controls, we also used 13 snap-frozen specimens of non-pathologic male lung tissue which were obtained from 10 patients [median age 67 (range 45–82) yrs] who underwent lung lobectomy due to primary lung carcinoma in the Department of Thoracic Surgery, Tohoku University Hospital and 3 autopsy cases at Tohoku University Hospital, Sendai, Japan. Their age and cause of death were as follows: 65 yrs (pancreatic cancer), 67 yrs (cardiac failure) and 87 yrs (prostatic cancer).