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RESEARCH ARTICLE

Up-regulation of blood arachidonate (20:4) levels in patients with chronic obstructive pulmonary disease

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Abstract

Context and objective: Plasma arachidonate (20:4) levels in patients with chronic obstructive pulmonary disease (COPD) were investigated.

Methods: Plasma was extracted and free fatty acids (FFAs) were separated using column chromatography and measured by fluorescence. Plasma 20:4 levels and its percentage relative to total FFA levels (%20:4) were measured in COPD ($n = 18$) and control ($n = 20$) subjects.

Results and conclusions: FFA levels were lower in COPD compared with normals. However, there was a significant increase in %20:4 levels in COPD patients (GOLD stage I/II 0.9 ± 0.4%; GOLD stage III/IV 1.1 ± 0.1%) compared with control subjects (0.6 ± 0.1 , $p < 0.05$). %20:4 is a potential biomarker for COPD.

Keywords: neutrophil, oxidative stress, free fatty acid, lipid mediators, inflammation

Introduction

Chronic obstructive pulmonary disease (COPD) is an inflammatory condition of the airways caused by cigarette smoke exposure which also has a major systemic component. This leads to respiratory failure and death in patients with the advanced disease. Although the precise mechanism underlying the persistent nature of the inflammatory response remains to be elucidated, cigarette smoke exposure induces neutrophilic inflammation, which is characteristic of COPD (Barnes 2000, 2010). Thus, induction of chemoattractants in the lung and subsequent migration of neutrophils to the lung is a key pathogenic process. Supporting this, previous reports showed elevated 8-isoprostane levels in urine (Praticò et al. 1998) and leukotriene B4 levels in sputum (Montuschi et al. 2003) from COPD patients. These findings suggested that the metabolites and oxidized products of arachidonate (20:4) may contribute to disease pathogenesis. In addition, various inhibitors of leukotriene B4 (Grönke et al. 2008; Hicks et al. 2010) and 5-lipoxygenase (Woodruff et al. 2011) have been used clinically as treatments for COPD. This suggests that plasma 20:4 levels may be abnormal in COPD and could potentially be a disease marker of COPD. However, systemic 20:4 levels are rarely reported in COPD patients.

The plasma levels of total free fatty acids (FFAs) were previously reported as an index of the nutritional status in COPD patients (Wada et al. 2005). In this study, further sub-analysis of each component of FFA, including 20:4,

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was determined and compared between COPD patients and the controls.

Methods

Enrollment

Eighteen COPD patients (10 patients with stage I/II disease and 8 with stage III/IV) and 20 age-matched controls were enrolled for investigating the degree of oxidative stress and systemic levels of free fatty acid (FFA) (Wada et al. 2005, 2006). All the enrolled COPD patients were diagnosed based on the smoking history, airway symptoms and spirometric changes, i.e. the ratio of forced expiratory volume in 1 s ($FEV_{1)}$ to forced vital capacity (FVC) was <70%, and subjects conformed to the criteria within the guidelines defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) in 2006 (National institute of healthy, National Heart, Lung and Blood Institute, Global Initiative for Chronic Obstructive Lung Disease 2001) and the Japanese Respiratory Society in 2009 (Japanese Respiratory Society 2009). Chest computed tomogram (CT) scans of all the COPD patients also revealed either emphysematous changes or low attenuation areas. The patients gave informed consent to the analyses, and the process was reviewed by the local ethics committee.

Measurements

Plasma was extracted from the blood obtained from all individuals. Plasma levels of each component of FFA were determined according to the method previously reported (Hara et al. 1999; Wada et al. 2005). In brief, plasma aliquots (50 μL) were mixed with 200 μL of methanol containing 12.5 μM margaric acid (internal standard) and then centrifuged at 12,000 rpm for 3 min. A sample of the supernatant (50 μL) was dried under a stream of N_2 and the residue was mixed with 1 μL of diethyl phosphorocyanidate and 50 μL of *N*,*N*-dimethyl formamide containing monodansylcadaverine (2 mg/mL). After standing at room temperature in the dark for 20 min, 5 μL was injected onto an octadecylsilyl column (3 μm, 3.3 cm × 4.6 mm i.d., Supelco, Kyoto, Japan) and a pKb-100 column (5 μm, 25 cm × 4.6 mm i.d., Supelco) connected in series. FFA components were measured by fluorescence (Model-821 FP, Japan Spectroscopic, Tokyo, Japan) with excitation selected at 320 nm and emission measured at 520 nm. The mobile phase consisted of acetonitrile/methanol/water (17.5/65.0/17.5, v/v/v) delivered at a flow rate of 1.5 mL/min with analytical columns maintained at 40°C.

Statistical analysis

Data were expressed as the mean ± standard deviation (SD). The statistical analysis was performed using the Prism 3.02 (Graph Pad Software, Inc., San Diego, USA). Comparison between three groups was initially analyzed by non-parametric Kruskal–Wallis ANOVA with Dunn's post-test analysis, and individual comparisons made using the Mann–Whitney non-parametric *t*-test. Significance is defined as a p value < 0.05

Results

Table 1 describes the clinical features of the three groups of age-matched controls, COPD patients (GOLD I/II) and COPD patients (GOLD III/IV). COPD patients were significantly heavier smokers ($p < 0.01$) and suffered more deteriorations in lung physiology including forced vital capacity (FVC) ($p < 0.01$) and FEV_1 % predicted ($p < 0.001$). As the disease severity increased the FFA levels were significantly lower ($p < 0.001$) confirming earlier data (Wada et al. 2005).

In parallel to the total FFA levels, each component, including plasma 20:4 levels, was significantly reduced in COPD patients ($p < 0.05$ or <0.01 versus control) (Table 2). Thus, the percentages of the components of the total FFAs were generally the same between the control groups and COPD patients. However, the percentage of 20:4 compared to total FFA (%20:4) was significantly higher in COPD patients (GOLD stage I/II, 0.9 ± 0.4%; GOLD stage III/IV, 1.1 ± 0.6%) compared to those in age-matched controls (0.6 ± 0.1, $p < 0.05$) (Figure 1).

We further assessed the regulation of 20:4 in association with poly-unsaturated fatty acid (PUFA). The ratio of %20:4 to PUFA levels (%20:4/PUFA) was significantly greater in GOLD stage I/II COPD patients than in control subjects (4.9 ± 2.1 vs. 2.9 ± 1.1, $p = 0.0245$). Although the %20:4/PUFA ratio was also higher in GOLD stage III/IV COPD patients (4.7 ± 2.7), this did not reach statistical significance compared to control subjects ($p = 0.0503$).

Regression analyses on all 38 enrolled patients demonstrated a significant association between FEV_1 % predicted and %20:4 (95% CI = −0.009427 to −0.001528; $r = -0.4247$; $p = 0.0079$) (Figure 2).

Discussion

Several studies suggested that 20:4 and its oxidized and/or metabolized products may be involved in COPD

Table 1. Clinical features of enrolled individuals.

	Control	COPD (stage I/II)	COPD (stage III/IV)
n	20	8 (0/8)	10 (2/8)
Age	67.2 ± 10.1	75.5 ± 4.5	72.3 ± 8.3
Smoking	24.2 ± 25.4	67.8 ± 24.3**	73.6 ± 38.3**
BMI	21.3 ± 2.7	22.8 ± 3.0	18.7 ± 4.5*
FVC	2.48 ± 0.93	2.87 ± 0.30**	1.90 ± 0.47**
%FVC	75.8 ± 24.1	91.2 ± 9.5*	60.8 ± 12.9
FEV_1	2.14 ± 0.79	1.34 ± 0.28	0.66 ± 0.14***
$\%FEV_1$	87.1 ± 26.3	62.4 ± 16.1***	29.1 ± 7.6***
FEV_1/FVC			
Total FFA	430.1 ± 62.1	199.4 ± 62.4*	155.7 ± 29.0**

Results are presented as mean ± standard deviation (SD).
BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second; FFA, free fatty acid.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus control.

Table 2. Plasma levels of each composition of FFA.

	Control		COPD (stage I/II)		COPD (stage III/IV)	
n	20		8 (0/8)		10 (2/8)	
FEV_1 (%pred)	87.1 ± 26.3		62.4 ± 16.1***		29.1 ± 7.6***	
	Levels (μM)	(% of total FFA)	Levels (μM)	(% of total FFA)	Levels (μM)	(% of total FFA)
Total FFA	430.1 ± 62.1		199.4 ± 62.4*		155.7 ± 29.0**	
SaFA	162.8 ± 21.7		87.6 ± 24.6*		64.1 ± 10.6**	
PUFA	91.2 ± 12.4	22.5 ± 1.0	41.2 ± 12.6*	21.4 ± 0.7	35.1 ± 6.9**	22.0 ± 1.1
MUFA	176.1 ± 28.9	37.7 ± 1.6	70.6 ± 25.9*	32.3 ± 2.0	56.5 ± 12.2**	33.4 ± 2.4
C18:3	7.2 ± 1.1	1.7 ± 0.1	2.8 ± 0.9**	1.5 ± 0.1	2.5 ± 0.3**	1.6 ± 0.3
C14:0	9.9 ± 1.4	2.5 ± 0.1	6.6 ± 2.3	3.2 ± 0.4	4.0 ± 0.9**	2.7 ± 0.3
C16:1	18.9 ± 3.9	3.9 ± 0.4	7.7 ± 3.0*	3.5 ± 0.4	5.0 ± 1.4**	2.8 ± 0.4
C22:6	14.6 ± 2.4	3.6 ± 0.3	7.9 ± 1.5*	5.2 ± 1.1	4.2 ± 0.6**	2.9 ± 0.3
C20:4	2.4 ± 0.3	0.6 ± 0.1	1.6 ± 0.3*	1.1 ± 0.2	1.2 ± 0.1**	1.0 ± 0.1*
C18:2	67.0 ± 9.3	16.5 ± 0.7	29.0 ± 10.3**	13.6 ± 0.9	27.3 ± 5.9**	16.5 ± 1.2
C16:0	114.5 ± 16.0	27.1 ± 0.4	58.8 ± 16.3*	30.9 ± 1.2	42.5 ± 7.6**	28.5 ± 1.5
C18:1	157.2 ± 25.5	33.8 ± 1.4	63.0 ± 23.0*	28.8 ± 1.7	51.5 ± 11.0**	30.7 ± 2.1
C18:0	38.4 ± 4.5	10.2 ± 0.7	22.2 ± 6.3*	12.3 ± 1.0	17.7 ± 2.3**	13.4 ± 1.6

Results are presented as mean ± standard deviation (SD).
PUFA, poly-unsaturated fatty acid; MUFA, mono-unsaturated fatty acid, SaFA, saturated fatty acid.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus control.

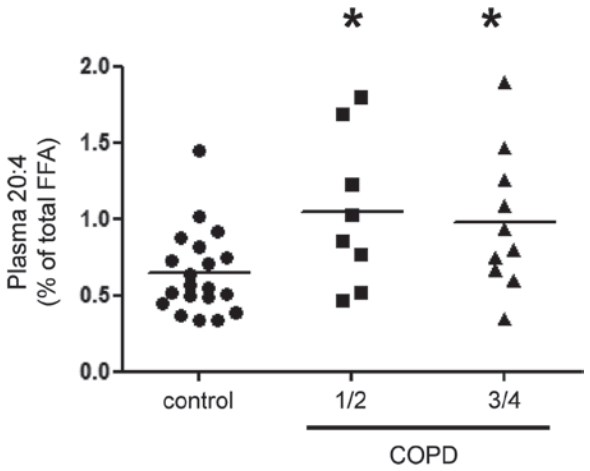

Figure 1. Plasma arachidonic acid (20:4) as a percentage of total free fatty acids (FFAs) in normal (n = 20) and chronic obstructive pulmonary disease (COPD) patients separated according to GOLD stage (I/II, n = 8 and III/IV, n = 10). *$p < 0.05$ compared to control levels.

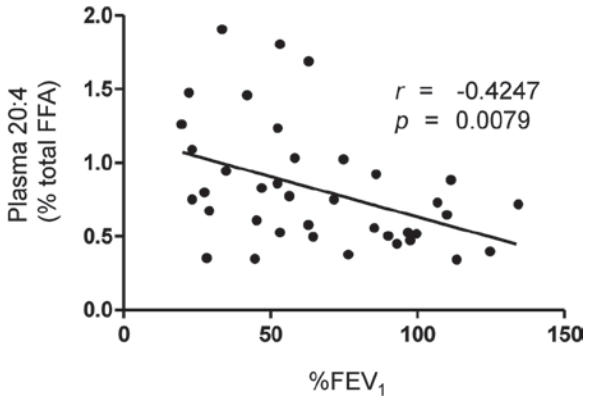

Figure 2. Correlation between plasma arachidonic acid (20:4) as a percentage of total free fatty acids (FFAs) in normal (n = 20) and chronic obstructive pulmonary disease (COPD) patients (n = 18) and forced expiratory volume in 1 second (FEV_1).

pathogenesis but, to the best of our knowledge, this is the first report to show up-regulation of the relative proportion of systemic 20:4 in the plasma of COPD patients. This up-regulation of 20:4 in COPD patients may have been missed previously as malnutrition which is seen in many COPD patients severely down-regulates plasma 20:4 levels (Wada et al. 2005). By comprehensively determining the major plasma FFA components, our study successfully detected the up-regulation of 20:4 by defining the %20:4, the percentage of 20:4 compared to total FFA. Increased %20:4 was also confirmed by determination of the %20:4/PUFA ratio. This indicated that the up-regulation of the %20:4 was not a result of generalized oxidization of PUFA for example but was specific for 20:4 production.

Arachidonic acid (20:4) can be produced in response to oxidative stress via activation of the tyrosine phosphorylation pathway, as demonstrated in the pulmonary epithelial cell line A549 (Pawliczak et al. 2002) and in the embryonic kidney-derived cell line HEK-293 (Asai et al. 2003). COPD is associated with high levels of oxidative stress either derived directly from cigarette smoke or from lung sources (Barnes 2010) and our *in vivo* analysis supports these *in vitro* studies and show that 20:4 levels are up-regulated in the systemic circulation of COPD patients. Therefore, these results suggest that the elevated %20:4 in COPD plasma reflects systemic oxidative stress and is thus a potential biomarker linking oxidative stress and neutrophilic inflammation in these patients.

The possible clinical role of elevated systemic 20:4 in COPD pathogenesis is unknown. Increased levels may reflect spill over from the production of 20:4 in the lung or alternatively, as 20:4 is also released from various cell types in other organs, the up-regulation of 20:4 may indicate enhancement of systemic inflammatory reactions and/or oxidative stress. Our study shows, however, that there is a good correlation between %20:4 and $\%FEV_1$

(Figure 2), suggesting that %20:4 is more likely to reflect pulmonary events.

The limitation of our manuscript is the relatively low number of patient samples analysed. Although the results show statistical significance by both non-parametric Kruskal–Wallis ANOVA including post-test analysis and by the non-parametric Mann–Whitney *t*-test, a larger study is required to confirm these results. Further studies investigating %20:4 and %20:4/PUFA ratios in COPD patients and disease controls will reveal the validity of %20:4 as a biomarker for COPD.

Conclusions

Collectively, our analysis demonstrated that plasma %20:4 levels were up-regulated in COPD patients. Although the absolute levels were lower probably as a result of malnutrition in the COPD patients (Wada 2005), the phenomenon was detected only through comprehensive measurement of all FFA levels. Since oxidized products and metabolites of 20:4 enhance neutrophilic migration, %20:4 may be a good disease marker by linking oxidative stress and neutrophilic inflammation, which are key to the pathogenesis of COPD.

Declaration of interest

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