Free Radical Research, September 2008; 42(9): 807–814

informa
healthcare

Implication of xanthine oxidase in muscle oxidative stress in COPD patients

DELPHINE DELAMPLE[1], FABIENNE DURAND[1], ARNOLD SEVERAC[2], MONIA BELGHITH[1], EMILIE MAS[3], FRANCOISE MICHEL[3], JEAN-PAUL CRISTOL[3], MAURICE HAYOT[1,2], & CHRISTIAN PREFAUT[1,2]

[1]*INSERM ERI25 'Muscle and Pathologies', Université Montpellier I, Montpellier, France,* [2]*Department of Clinical Physiology, CHU A. de Villeneuve, Montpellier, France, and* [3]*Department of Biochemestry, CHU Lapeyronie, Montpellier, France*

Accepted by Professor F. Kelly

(Received 10 June 2008; revised 30 July 2008)

Abstract

Objective: The aim of this study was to determine the implication of xanthine oxidase (XO) in the exercise-induced muscle oxidative stress and muscle dysfunction of these patients.

Methods: A randomized, crossover and double-blind study was conducted in nine severe COPD patients, who performed a localized quadriceps endurance test after oral treatment with allopurinol, a XO inhibitor or placebo. Redox status was studied in arterial and venous femoral blood before and after the endurance test.

Results: In placebo condition, muscle exercise resulted in a significant increase in AOPP and isoprostanes, with a significant increase in the venoarterial difference (v-a) in isoprostanes after exercise as compared with before ($p < 0.05$). In contrast, allopurinol treatment prevented the elevation in AOPP levels and v-a isoprostanes after exercise. However, no significant improvement in quadriceps muscle endurance was observed, but allopurinol treatment seemed to preserve muscle strength properties.

Conclusion: This study demonstrates that XO is implicated in the exercise-induced muscle oxidative stress of COPD patients. Allopurinol administration seemed to improve only some muscle properties. Therefore other sources of muscle oxidative stress should be implicated in muscle dysfunction observed in these patients.

Keywords: *COPD, quadriceps exercise, muscle oxidative stress, xanthine oxydase*

Introduction

Oxidative stress can be defined as an imbalance between reactive oxygen species (ROS) and antioxidant systems. In chronic obstructive pulmonary disease (COPD) patients, oxidative stress has been observed in muscle of these patients after exercice [1,2]. By inducing structural, metabolic and functional alterations in muscle, this oxidative stress is associated with the peripheral muscle dysfunction observed in these patients, as several studies from this group and others have reported [1,3,4]. Moreover, it was recently shown that a high dose of an antioxidant (N-acetylcysteine) prevented exercise-induced oxidative stress and significantly improved COPD quadriceps endurance [5]. On the other hand, the mechanisms underlying oxidative stress have not been fully elucidated, although they are likely to be clinically relevant. Of the different sources of ROS

Correspondence: Delphine Delample, INSERM ERI25, 'Muscle and Pathologies', CHU A. de Villeneuve, 371 Avenue du doyen Giraud, 34295 Montpellier, France. Tel: +33 467 335 910. Fax: +33 467 335 923. Email: ddelample@yahoo.fr

ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.1080/10715760802429039

production implicated in exercise-induced oxidative stress, xanthine oxidase (XO) plays an important role. XO, when activated, catalyses the oxidation of hypoxanthine during purine metabolism and then generates superoxide radicals [6] and nitric oxide [7]. XO is also present in human skeletal muscle [8] and is activated (1) by inflammation [8,9], which is a physiopathologic characteristic of COPD, including in the muscle compartment; (2) by increased intracellular calcium concentration and ATP breakdown products, including hypoxanthine and its precursor inosine monophosphate (IMP), which are exhibited during exercise; and (3) during the ischemia-reperfusion that occurs during and after exercise [10,11]. The potential role of this enzyme in generating oxidative stress was substantiated by Pouw et al. [12], who reported elevated IMP levels in the quadriceps of COPD patients at rest compared with healthy subjects. Moreover, during exhaustive physical exercise, hypoxanthine is released from skeletal muscle as ATP degradation occurs [13]. Previously, Heunks et al. [14] demonstrated that systemic oxidative stress induced by cycling exercise can be prevented in COPD patients by pre-treating the patients with allopurinol, a potent XO inhibitor. However, this study focused on whole body exercise and therefore did not localize the activated XO at the muscle level. To investigate the role of XO in exercise-induced muscle oxidative stress, the methodology was adapted by using (1) highly localized muscle exercise and (2) the femoral venoarterial difference. The localized limb exercise has been reported to induce only moderate cardiac and ventilatory responses [15]. It is a reliable clinical method to assess peripheral muscle endurance in patients with COPD while reducing as much as possible the sources of oxidative stress that are intrinsic to whole body exercise. The femoral venoarterial difference allows the exact quantification of the oxidative damage (like lipid peroxidation) induced by muscle oxidative stress in the anterior thigh muscles.

It was hypothesized that if XO was implicated in muscle exercise-induced oxidative stress, allopurinol, as an inhibitor of XO, would diminish it and as a result improve peripheral muscle endurance. This hypothesis was tested in a randomized, double-blind and cross-over placebo study by investigating the effects of acute allopurinol supplementation on muscle oxidative damage evaluated from the femoral venoarterial difference after localized muscle exercise.

Materials and methods

Subjects

This study included nine ex-smokers with stable severe COPD as defined by the Global Initiative for Chronic Obstructive Lung Disease guidelines [16] (Table I). Exclusion criteria were unstable COPD (i.e. exacerbation in the last 2 months), long-term supplemental oxygen, allopurinol treatment, neuromuscular, cardiac, renal and metabolic disease and psychiatric and addictive pathologies. None of these patients had taken systemic oral steroid medication in the 6 months before the study and all medication regimens remained unchanged during the study period. During an individual interview, patients were questioned on their dietary habits to exclude supplementation with antioxidants, vitamins or anti-aggregates. Informed written consent was obtained from all patients and the research protocol was approved by the institutional ethics committee of Montpellier and conducted in accordance with the Helsinki declaration and the European guidelines for 'good clinical practice'.

Table I. Population characteristics.

Sex (f/m)	1/8
Age, years	59.9 ± 2.8
Weight, kg	68.4 ± 5.1
BMI, kg/m^2	24.1 ± 1.3
FEV_1, L	1.3 ± 0.2
FEV_1, % predicted	46.4 ± 6
FEV_1/FVC, %	52.4 ± 4.1
PaO_2 (mmHg)	76 ± 2.6

Study protocol

Pulmonary function test. All subjects underwent spirometry including measurements of FVC and FEV_1. The FEV_1/FVC ratio was also calculated. The results of pulmonary function testing were related to the normal values of Quanjer et al. [17].

Localized muscle exercise. The maximal voluntary contraction (MVC) and endurance of the quadriceps were assessed on an exercise bench (Banc de Koch, Genin Medical, France) according to the technique of Andersen et al. [18], as modified by Serres et al. [19] and recently described by Couillard et al. [20]. Briefly, the exercises were performed with all patients in a seated position at 90° knee and hip flexion, with arms crossed in front of the chest. The dominant leg of each patient was determined as the leg that developed the highest value of MVC. Then, quadriceps endurance of the dominant leg was assessed by having the patients repeat maximal knee extensions against weights corresponding to 30% of MVC until exhaustion. The duration of the endurance test was called the endurance limit time (TLim) (expressed in seconds). Two minutes after the end of the endurance test, a second maximal voluntary contraction test [MVC(2)] was performed to measure quadriceps fatigue. The sensation of muscle fatigue was also measured at rest and immediately after exercise on a 10 cm visual analogic scale. The same investigator supervised the endurance tests and gave standardized verbal encouragement to all participants. Neither this

investigator nor the patients knew the test condition (allopurinol or placebo).

Allopurinol supplementation. Each patient was randomly and blindly assigned to allopurinol-supplemented (Allop) and placebo exercise trials separated by at least 7 days. The supplementation consisted of two capsules of 300 mg Allop or placebo (lactose, magnesium stearate, amidon). The first capsule was taken 24 h prior to the localized muscle exercise and the second was taken 1 h before. The Allop and placebo doses were prepared and conditioned in blind capsules (Clinical packaging, Pharmacy, Lapeyronie Hospital, Montpellier, France) in accordance with French regulations for clinical trials. This was a crossover study as each patient underwent the two trials (Allop and placebo) serving as a self-control to eliminate biological variability in the response to the treatment.

Catheter placement. After the groin of the dominant leg was disinfected and anaesthetized with Xilocaine (Xylocaine® 2%, AstraZeneca, Monts, France), the catheter (Central venous catheterization set, model CV-04701, ARROW®, USA) was inserted into the femoral vein 2 cm below the external femoral artery. Another catheter (Arterial leader cath, model 5115.098, Vygon®, Ecouen, France) was then placed in a radial artery after local anaesthesia with cream (EMLA 5%, lidocaine-prilocaine cream, AstraZeneca, Sodertalje, Sweden).

Blood analysis

Arterial and venous blood was sampled in standard, sterile, heparinized, ethylenediaminetetraacetic acid-containing and clot-activator tubes. Immediately after sampling, serum and plasma were removed by centrifugation (2500 rpm for 10 min at 4°C), put into 500 µl aliquots and stored at −80°C until analysis.

Markers of oxidative stress. Plasma-free and esterified isoprostanes (F_2-IsoP) as markers of lipid peroxidation were determined. Lipids were first extracted from samples (1 ml of plasma containing [$^2H_4$]-15-F_{2t}-IsoP as internal standard) with Folch solution (chloroform/methanol 2:1; v/v) and then subjected to alkaline hydrolysis. The pH of the extract was adjusted to 2.2 with HCl and diluted in water to 10 ml. Samples were then treated with two extraction procedures using successively an inverse-phase and an aminopropyl-phase cartridge (Sep-Pak Vac RC C_{18} and Vac RC NH_2, Waters S.A., Guyancourt, France) as previously described [21,22]. After the last step of purification, samples were derivatized as pentafluorobenzylester and trimethylsilyl ethers. The derivatized samples were analysed by gas chromatography-negative ion chemical ionization mass spectrometry. Quantification was achieved by relating the peak area of the F_2-IsoP to the internal standard.

Levels of protein oxidation were determined in plasma by assessment of advanced oxidation protein product (AOPP) using a semi-automated method previously described by Witko-Sarsat et al. [23]. Briefly, AOPP were measured in duplicate by spectrophotometry on a microplate reader (Model MR 500, Dynatec, Paris, France) and were calibrated with chloramine-T solutions (Sigma, St Louis, France), which absorb at 340 nm in the presence of potassium iodide. The chloramine-T absorbance at 340 nm is linear within the range of 0–100 µmol/l. AOPP were expressed as micromoles per litre of chloramine-T equivalents.

Markers of the antioxidant level. Plasmatic vitamin E was measured by high-performance liquid chromatography according to Cachia et al. [24]. Plasma triglycerides and cholesterol, as strongly correlated with plasma vitamin E, were determined using commercially available test kits (Konelab™, Thermo Electron Corporation) and a Coulter CPA analyser (Coultonics, France SA). Total antioxidant status (TAS) was measured in plasma. The TAS assay (Randox) is based on the reaction between metmyoglobin and hydrogen peroxide, which generates fenylmyoglobine, which in turn reacts with a chromogen to form a stable colour radical cation. This kit can measure concentrations at 600 nm on automated analysers. Plasma uric acid was assessed with the enzymatic colour test on Olympus analysers. Plasma GPx activity was measured using a Pentra 400 autoanalyser (Pentra 400, ABX, France) with reagents from Randox. Results were expressed per litre of plasma [25].

Study design

Subjects were instructed to abstain from strenuous physical activity during the study. During the first morning visit, they underwent spirometry and performed localized muscle exercise (MVC, quadriceps endurance test and MVC(2)) in order to familiarize themselves with the endurance test procedure. Six days later, the investigator visited the patients to give them the capsules corresponding to their first randomly assigned trial. The day after Allop or placebo supplementation, the patients returned to the laboratory without breakfasting at ~8 am and the last single dose of 300 mg was then taken. Catheters were placed and 1 h later resting arterial and venous blood samples were collected (T0). Then, localized muscle exercise (i.e. MVC, quadriceps endurance test and MVC(2)) was performed with the dominant leg. Arterial and venous blood samples were collected 1 h 30 min after the end of quadriceps endurance (T1). For ethical reasons, the arterial and venous catheters

were removed following this sample. A last systemic venous sample was however collected 6 h after the quadriceps endurance test (T6). After an interval of at least 7 days, the patients received the capsules corresponding to the second randomly assigned trial and then returned to the laboratory for a third visit. The quadriceps endurance test was performed at the same absolute intensity of 30% of the MVC determined during the first morning visit. The protocol was strictly identical to that of the initial trial.

Statistical analysis

Data were analysed with Statistica 6.0 (Statsoft, France, 2003). Values are reported as mean ± SEM. A one-way repeated measures analysis of variance (or a one-way repeated measures analysis on ranks when normality test failed) was used to test the effects of the supplementation on the exercise parameters. A two-way analysis of variance followed by Tukey's pairwise multiple comparison procedure determined the effects of the treatment and the localized muscle exercise on the biological markers. Significance was set at the 0.05 level.

Results

Redox status in severe COPD patients

The data at rest in placebo condition are summarized in Table II. After exercise, redox status was modified. Indeed, in the venous compartment in placebo condition, a significant increase was found in venous AOPP levels at T6 as compared with T0 (29.6 ± 1.7 vs 13 ± 0.1 μm/L of chloramine-T equivalents, respectively; $p < 0.05$) (Figure 1) and a significant increase in venous F_2-IsoP levels at T1 compared with T0 (491.7 ± 56.2 pg/mL vs 394.5 ± 33 pg/mL, respectively; $p < 0.05$) (Figure 2).

No increase in arterial F_2-IsoP levels was observed after exercise in placebo condition (385.9 ± 33 pg/ml vs 354.5 ± 46.8 pg/ml, respectively). Then, a significant increase in venoarterial (v-a) F_2-IsoP was observed after exercise in placebo condition ((v-a) F_2-IsoP at T1 = 142.4 ± 34.8 pg/mL; (v-a) F_2-IsoP at T0 = 2 ± 28.6 pg/mL; $p < 0.05$) (Figure 3). For the antioxidant system, no modification in venous vitamin E was observed after exercise and GPx activity significantly increased at T6 as compared with T0 (694.2 ± 28.1 U/L vs 624 ± 39.6 U/L, respectively; $p < 0.05$).

Allopurinol effects on redox status in severe COPD patients

At rest, venous blood AOPP levels were not significantly modified by Allop treatment (Figure 1). In arterial and venous blood, we observed no change in F_2-IsoP levels between placebo and Allop conditions. Allop significantly decreased venous uric acid at rest as compared with placebo condition ($p < 0.05$). TAS also decreased in Allop condition as compared with placebo condition (1.74 ± 0.03 mmol/l vs 1.86 ± 0.05 mmol/l, respectively; $p < 0.05$). We did not observe any change in plasma vitamin E levels or GPx activity.

After exercise, Allop treatment prevented the elevation in venous AOPP levels at T6 (Figure 1) and in venous F_2-IsoP levels at T1 (Figure 2). Indeed, Allop treatment decreased the increase in venous F_2-IsoP levels observed at T1 in placebo condition by 31.5% ($p < 0.05$) (Figure 2). No increase in arterial F_2-IsoP levels was observed after exercise in Allop condition (367.4 ± 30.5 pg/ml vs 344.7 ± 38 pg/ml, respectively). Thus, Allop treatment prevented the elevation in (v-a) F_2-IsoP after exercise observed in placebo condition (Figure 3). No modification in venous vitamin E was observed. GPx activity significantly increased in Allop condition at T6 as compared with T0 (688.8 ± 41.9 U/L vs 647.6 ± 35.5 U/L, respectively; $p < 0.05$). We also observed a significant difference between GPx activity at T6 in placebo and Allop condition, as the increase in GPx activity is less important in Allop condition ($p < 0.05$).

Physiological responses during exercise

We did not observe any difference between MVC performed on the first morning (i.e. without catheter) and on the day corresponding to placebo condition (i.e. with catheter) (23.1 ± 2.7 kg vs 22.7 ± 2.8 kg, respectively).

In both conditions, the endurance test significantly increased perceived muscle fatigue (placebo condition: 0.3 ± 0.2 vs 4.4 ± 0.6, $p < 0.05$; Allop condition: 0.4 ± 0.2 vs 4.9 ± 0.5, $p < 0.05$, before and after exercise, respectively). Despite the absence of increase

Table II. Redox status at rest.

Oxidant system	AOPP (μm/L of chloramine-T equivalents)		13.02 ± 0.99
	F_2-IsoP (pg/mL)	Venous level	394.5 ± 29.1
		Arterial level	385.9 ± 33
		(v-a) F_2-IsoP	2 ± 28.6
Antioxidant system	Vit E/(Chol + TG)		5.4 ± 0.4
	TAS (mmol/L)		1.86 ± 0.05
	Uric acid (μmol/L)		338.8 ± 26.3
	GPx activity (U/L)		624 ± 39.6

Vit E/(Chol + TG) = ratio plasmatic vitamin E/(cholesterol + triglycerides); (v-a)F_2-IsoP = venoarterial difference in F_2-IsoP.

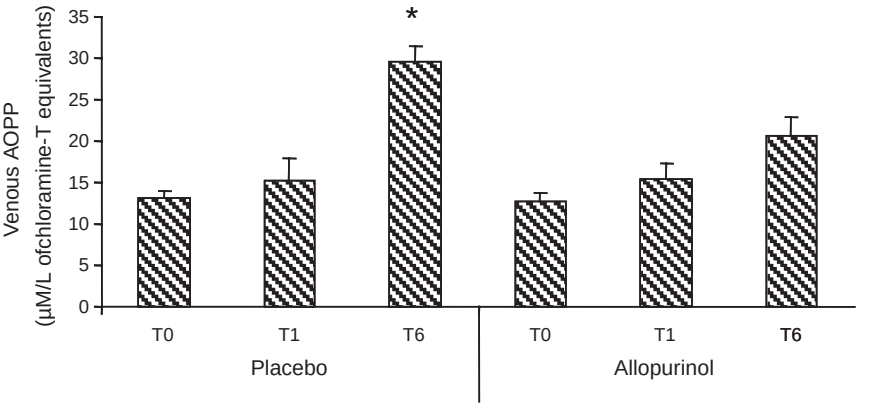

Figure 1. Effects of exercise on venous AOPP at rest (T0), 1 h 30 min after exercise (T1) and 6 h after exercise (T6) in placebo and Allop condition. *$p < 0.05$: T6 vs T0.

in (v-a) F_2-IsoP in Allop condition, peripheral muscle function was not enhanced. Indeed, we did not observe any significant improvement in MVC or Tlim in Allop condition. The endurance test induced a clinically and statistically significant decrease in MVC(2) in both placebo and Allop conditions (Table III) ($p < 0.05$). Moreover, the decrease in MVC(2) is more important in placebo condition as compared with Allop condition ($p < 0.05$).

Discussion

These experiments demonstrate that localized intense exercise-induced oxidative stress in patients with severe COPD can be prevented by XO inhibition. Moreover, given the venoarterial difference and our use of localized muscle exercise, we can assert that XO inhibition with allopurinol led to a decrease in oxidative damage arising from the anterior thigh muscles. This decrease in muscle oxidative damage resulted in a partial improvement in peripheral muscle function.

Methodological considerations

XO-pathway inhibition. Allopurinol [1H-pyrazolo(3,4-d)pyrimidin-4-ol] is an oxypurine base widely used in clinical medicine for the treatment of hyperuricemia. The major pharmacological action of allopurinol is mediated by its metabolite, oxypurinol. Both are structural analogues of the purine bases hypoxanthine and xanthine and competitively bind to XO. Therefore, they inhibit the XO-mediated conversion of hypoxanthine to xanthine and xanthine to uric acid and thus the generation of free radicals [6,7]. Our treatment consisted of 300 mg of allopurinol twice prior to exercise. This dosage was used in a previous study [14]. In both cases, the treatment was well tolerated: none of the patients complained of any side effects. Both in Heunks et al.'s [14] paper and in our case this dosage successfully inhibited the XO-pathway, as we observed a significant decrease in venous uric acid concentrations.

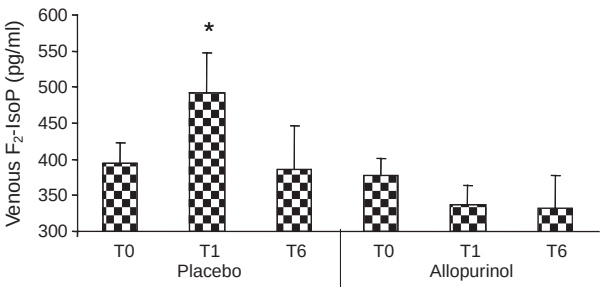

Figure 2. Effects of exercise on venous F_2-IsoP at rest (T0), 1 h 30 min after exercise (T1) and 6 h after exercise (T6) in placebo and Allop condition. *$p < 0.05$: T1 vs T0.

Localized muscle exercise and venoarterial difference. Several studies have demonstrated that this exercise protocol is highly specific to the quadriceps muscle, as it greatly minimizes the cardiac and respiratory responses to exercise [5,15,19]. In our study, we also determined the venoarterial difference in the biological markers of oxidative damage. Thus, we can assume that the presence or absence of these biological

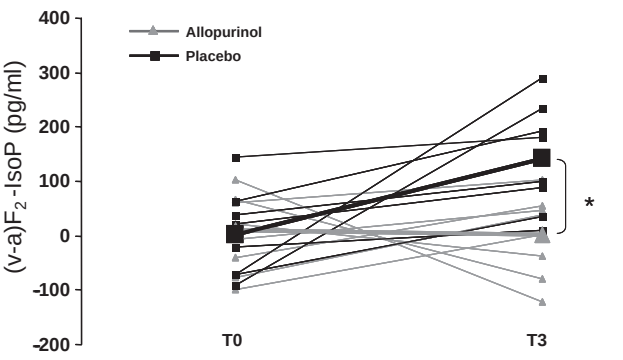

Figure 3. Effects of exercise on venoarterial (v-a) F_2-IsoP difference in placebo and Allop conditions. Bold lines represent the mean in placebo (black squares) and allopurinol (grey triangles) conditions. *$p < 0.05$: Allopurinol vs Placebo.

Table III. Localized muscle exercise in placebo and Allopurinol conditions.

Localized muscle exercise	Placebo	Allop
MVC, kg	22.7 ± 2.8	23.9 ± 2.5
Tlim, second	431.3 ± 48.1	404.8 ± 45.5
MVC(2), kg	17.5 ± 1.9,**	20 ± 1.3*
% of decrease between MVC and MVC(2)	22.8**	16.5
Muscle fatigue before exercise	0.3 ± 0.2	0.4 ± 0.2
Muscle fatigue after exercise	4.4 ± 0.6*	4.9 ± 0.5*

*$p < 0.05$: after vs before exercise; **$p < 0.05$: Allop vs placebo.

markers of oxidative damage depended for the most part on the redox status of the quadriceps muscle.

Biological markers of oxidative stress. Isoprostanes are generated *in vivo* by peroxidation of membrane phospholipids by free radicals and reactive oxygen species [26]. These prostaglandin-like compounds are the most reliable markers of oxidative damage *in vivo* [27] and F_2-IsoP is the most studied class of isoprostanes due to its stability [28]. This marker, for the first time used in plasma in COPD disease, is reproducible, as we observed no change in the arterial and venous blood levels of F_2-IsoP at rest. Moreover, this marker confirms that oxidative stress appeared in COPD patients at rest. In the literature, the range of isoprostane levels in healthy subjects is between 239 ± 21 pg/ml and 310 ± 13 pg/ml [29–31]. In our group, the isoprostane level observed in 60 healthy elderly volunteers (age = 67.8 ± 3.3 years, IMC = 25.4 ± 3 kg/m^2) was 312.3 ± 12.6 pg/ml (unpublished data). In this study, the isoprostane level at rest under placebo condition was significantly higher (394.5 ± 29.1 pg/ml) ($p < 0.05$) than in the healthy elderly subjects. This new marker in plasma of COPD patients is relevant to determine oxidative stress.

The detection of oxidized amino acids in biological systems also provides a fingerprint of oxidative damage. To assess oxidant-mediated protein damage, we measured advanced oxidation protein product (AOPP). Numerous studies have used AOPP as a sensitive marker to assess oxidant-mediated protein damage after oxidative stress [32,33], including in COPD patients [5].

Allop treatment and muscular oxidative stress during exercise

In this study, localized quadriceps endurance induced a clinically and statistically significant increase in muscle fatigue. Indeed, we observed a decrease of at least 15% in MVC after exercise as compared with before exercise [4], associated with an increase in muscle fatigue sensation measured on a visual analogic scale. In placebo condition, the substantial increase in venous levels of F_2-IsoP and AOPP after exercise indicated that exercise induced oxidative damage. The kinetic of these markers is different as we observed the increase in F_2-IsoP venous levels at T1 and the increase in AOPP venous levels at T6. This result is in accordance with many other studies that have reported systemic oxidative stress induced by exercise in patients with COPD [5,20,34]. Moreover, the muscular origin of exercise-induced oxidative stress in these patients was supported by the rise in (v-a) F_2-IsoP after exercise. Unfortunately, on account of the absence of arterial sample at T6, venoarterial difference in AOPP was not achieved. The muscular origin of oxidative stress in COPD patients has been previously described [1,35]. However, the interesting result was in the Allop condition: after exercise, we did not observe any rise in (v-a) F_2-IsoP. This suggests that exercise-induced muscle oxidative stress was prevented by XO inhibition. And it strongly supports the hypothesis that XO is involved in this exercise-induced muscle oxidative stress. Prolonged exhaustive exercise subjects the active muscle to a marked level of metabolic stress and local ischemia/reperfusion phases may occur. A recent study [36] using near-infrared spectroscopy (NIRS) showed that a period of re-oxygenation occurs during the immediate recovery phase after exercise in COPD patients. In 1981, Granger et al. [37] proposed an ischemia-reperfusion injury hypothesis: the ischemic phase causes a burst of reactive oxygen species that are formed in the XO-catalysed reaction. Moreover, Viña et al. [38] found that oxygen supplementation attenuates exercise-induced oxidative stress in patients with COPD by decreasing metabolic stress to tissue. It thus seems that ischemia-reperfusion may play an important role in XO-mediated muscle exercise-induced oxidative stress. We speculate that Allopurinol, by inhibiting the XO-pathway, could then avoid ROS formation during the reperfusion phase which occurs during recovery. After oral administration, allopurinol is well absorbed from the gastrointestinal tract. Peak plasma concentrations of allopurinol and its metabolite oxypurinol are reached after ~1 and 3 h, respectively [39]. The half-life times after oral administration of allopurinol and oxypurinol are >1 and ~20 h, respectively. Allopurinol and oxypurinol can act as scavengers of both the hydroxyl radical and hypochlorous acid [40]. One and a half hours after local exercise, muscle oxidative stress was nearly avoided in Allop condition. This could be explained by (1) the scavenging power of oxypurinol or (2) the role of ischemia-reperfusion in XO-mediated oxidative stress. Indeed, the isoprostane half-life is 16 min [41]. Therefore, 1 h 30 min after exercise, we observed oxidative damage induced only by reperfusion and, in a wider sense, by XO-mediated oxidative stress. Concerning the antioxidant compartment, we observed an increase in GPx activity after exercise. This increase is however less important in Allop condition as compared with Placebo condition,

suggesting that Allop, by preventing muscle oxidative burden, mitigate the response of the antioxidant defenses.

Redox status and peripheral muscle function of patients with COPD

Allop treatment did not modify exercise endurance. In COPD patients, a study has shown that 4 days of NAC treatment improved exercise endurance time by preventing exercise-induced oxidative stress [5]. NAC is able to restore intracellular GSH levels [42], which is a major antioxidant especially present in muscle fibre consumed in the first place during exercise [43]. Our treatment did not explore the same pathway of cellular protection as allopurinol is above all use to prevent ROS production. So, its antioxidative power is not comparable to GSH. Nevertheless, many studies report that long-term allopurinol administration improves cardiac structure and function [44–48] and Ca^{2+} responsiveness of myofilaments, thereby improving contractility. In our study, we found that in Allop condition, the decrease in MVC(2) is less important than in placebo condition. This may be suggesting some effects of allopurinol in preserving contractile strength after an exhaustive endurance exercise. Further studies are thus now needed to determine whether long-term allopurinol administration could improve muscle function

Conclusions

It is widely acknowledged that exercise induces muscle oxidative stress in COPD patients, which is implicated in the physiopathology of the disease. However, no study has elucidated the sources of this oxidative stress within the muscle. This study demonstrates that xanthine oxidase is an important source of muscle oxidative stress induced by exercise in these patients. Short-term allopurinol administration nevertheless did not improve exercise endurance even if it seemed to play a role in preserving muscle strength properties, suggesting that (1) the effects of long-term allopurinol administration should be studied in COPD patients and (2) other mechanisms and other sources of ROS within the muscle may explain the muscle dysfunction observed in these patients.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Couillard A, Maltais F, Saey D, Debigare R, Michaud A, Koechlin C, LeBlanc P, Prefaut C. Exercise-induced quadriceps oxidative stress and peripheral muscle dysfunction in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2003;167:1664–1669.

[2] van Helvoort HA, Heijdra YF, de Boer RC, Swinkels A, Thijs HM, Dekhuijzen PN. Six-minute walking-induced systemic inflammation and oxidative stress in muscle-wasted COPD patients. Chest 2007;131:439–445.

[3] Mador MJ, Deniz O, Aggarwal A, Kufel TJ. Quadriceps fatigability after single muscle exercise in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2003;168:102–108.

[4] Saey D, Debigare R, LeBlanc P, Mador MJ, Cote CH, Jobin J, Maltais F. Contractile leg fatigue after cycle exercise: a factor limiting exercise in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2003;168:425–430.

[5] Koechlin C, Couillard A, Simar D, Cristol JP, Bellet H, Hayot M, Prefaut C. Does oxidative stress alter quadriceps endurance in chronic obstructive pulmonary disease? Am J Respir Crit Care Med 2004;169:1022–1027.

[6] McCord JM, Roy RS, Schaffer SW. Free radicals and myocardial ischemia. The role of xanthine oxidase. Adv Myocardiol 1985;5:183–189.

[7] Li H, Samouilov A, Liu X, Zweier JL. Characterization of the magnitude and kinetics of xanthine oxidase-catalyzed nitrate reduction: evaluation of its role in nitrite and nitric oxide generation in anoxic tissues. Biochemistry 2003;42: 1150–1159.

[8] Hellsten Y, Frandsen U, Orthenblad N, Sjodin B, Richter EA. Xanthine oxidase in human skeletal muscle following eccentric exercise: a role in inflammation. J Physiol 1997;498: 239–248.

[9] Devrim E, Avci A, Erguder IB, Karagenc N, Kulah B, Durak I. Activities of xanthine oxidase and superoxide dismutase enzymes in rat intestinal tissues in sepsis. J Trauma 2008;64: 733–735.

[10] O'Brien JD, Ferguson JH, Howlett SE. Effects of ischemia and reperfusion on isolated ventricular myocytes from young adult and aged Fischer 344 rat hearts. Am J Physiol Heart Circ Physiol 2008;294:H2174–H2183.

[11] Van Wylen DG. Effect of ischemic preconditioning on interstitial purine metabolite and lactate accumulation during myocardial ischemia. Circulation 1994;89:2283–2289.

[12] Pouw EM, Schols AM, van der Vusse GJ, Wouters EF. Elevated inosine monophosphate levels in resting muscle of patients with stable chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1998;157:453–457.

[13] Hellsten Y, Sjodin B, Richter EA, Bangsbo J. Urate uptake and lowered ATP levels in human muscle after high-intensity intermittent exercise. Am J Physiol 1998;274:E600–E606.

[14] Heunks LM, Vina J, van Herwaarden CL, Folgering HT, Gimeno A, Dekhuijzen PN. Xanthine oxidase is involved in exercise-induced oxidative stress in chronic obstructive pulmonary disease. Am J Physiol 1999;277:R1697–R1704.

[15] Coronell C, Orozco-Levi M, Mendez R, Ramirez-Sarmiento A, Galdiz JB, Gea J. Relevance of assessing quadriceps endurance in patients with COPD. Eur Respir J 2004;24: 129–136.

[16] Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, Zielinski J. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2007; 176:532–555.

[17] Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault J. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993;16:5–40.

[18] Andersen P, Adams RP, Sjogaard G, Thorboe A, Saltin B. Dynamic knee extension as model for study of isolated exercising muscle in humans. J Appl Physiol 1985;59: 1647–1653.
[19] Serres I, Gautier V, Varray A, Prefaut C. Impaired skeletal muscle endurance related to physical inactivity and altered lung function in COPD patients. Chest 1998;113:900–905.
[20] Couillard A, Koechlin C, Cristol JP, Varray A, Prefaut C. Evidence of local exercise-induced systemic oxidative stress in chronic obstructive pulmonary disease patients. Eur Respir J 2002;20:1123–1129.
[21] Milne GL, Morrow JD. Isoprostanes and related compounds: update 2006. Antioxid Redox Signal 2006;8:1379–1384.
[22] Nourooz-Zadeh J, Gopaul NK, Barrow S, Mallet AI, Anggard EE. Analysis of F2-isoprostanes as indicators of non-enzymatic lipid peroxidation *in vivo* by gas chromatography-mass spectrometry: development of a solid-phase extraction procedure. J Chromatogr B Biomed Appl 1995;667:199–208.
[23] Witko-Sarsat V, Nguyen Khoa T, Jungers P, Drueke T, Descamps-Latscha B. Advanced oxidation protein products: oxidative stress markers and mediators of inflammation in uremia. Adv Nephrol Necker Hosp 1998;28:321–341.
[24] Cachia O, Leger CL, Boulot P, Vernet MH, Michel F, Crastes de Paulet A, Descomps B. Red blood cell vitamin E concentrations in fetuses are related to but lower than those in mothers during gestation. A possible association with maternal lipoprotein (a) plasma levels. Am J Obstet Gynecol 1995;173:42–51.
[25] Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158–169.
[26] Morrow JD, Awad JA, Wu A, Zackert WE, Daniel VC, Roberts LJ, 2nd. Nonenzymatic free radical-catalyzed generation of thromboxane-like compounds (isothromboxanes) *in vivo*. J Biol Chem 1996;271:23185–23190.
[27] Morrow JD, Roberts LJ, 2nd. Mass spectrometric quantification of F2-isoprostanes in biological fluids and tissues as measure of oxidant stress. Methods Enzymol 1999;300:3–12.
[28] Awad JA, Morrow JD, Takahashi K, Roberts LJ, 2nd. Identification of non-cyclooxygenase-derived prostanoid (F2-isoprostane) metabolites in human urine and plasma. J Biol Chem 1993;268:4161–4169.
[29] Lee CY, Jenner AM, Halliwell B. Rapid preparation of human urine and plasma samples for analysis of F2-isoprostanes by gas chromatography-mass spectrometry. Biochem Biophys Res Commun 2004;320:696–702.
[30] Mori TA, Croft KD, Puddey IB, Beilin LJ. An improved method for the measurement of urinary and plasma F2-isoprostanes using gas chromatography-mass spectrometry. Anal Biochem 1999;268:117–125.
[31] Walter MF, Blumberg JB, Dolnikowski GG, Handelman GJ. Streamlined F2-isoprostane analysis in plasma and urine with high-performance liquid chromatography and gas chromatography/mass spectroscopy. Anal Biochem 2000;280:73–79.
[32] Kaneda H, Taguchi J, Ogasawara K, Aizawa T, Ohno M. Increased level of advanced oxidation protein products in patients with coronary artery disease. Atherosclerosis 2002; 162:221–225.
[33] Morena M, Martin-Mateo M, Cristol JP, Canaud B. [Oxidative stress, hemo-incompatibility and complications of long-term dialysis]. Nephrologie 2002;23:201–208.
[34] Van Helvoort HA, Heijdra YF, Thijs HM, Vina J, Wanten GJ, Dekhuijzen PN. Exercise-induced systemic effects in muscle-wasted patients with COPD. Med Sci Sports Exerc 2006;38: 1543–1552.
[35] Koechlin C, Maltais F, Saey D, Michaud A, LeBlanc P, Hayot M, Prefaut C. Hypoxaemia enhances peripheral muscle oxidative stress in chronic obstructive pulmonary disease. Thorax 2005;60:834–841.
[36] Okamoto T, Kanazawa H, Hirata K, Yoshikawa J. Evaluation of oxygen uptake kinetics and oxygen kinetics of peripheral skeletal muscle during recovery from exercise in patients with chronic obstructive pulmonary disease. Clin Physiol Funct Imaging 2003;23:257–262.
[37] Granger DN, Rutili G, McCord JM. Superoxide radicals in feline intestinal ischemia. Gastroenterology 1981;81:22–29.
[38] Vina J, Servera E, Asensi M, Sastre J, Pallardo FV, Ferrero JA, Garcia-De-La-Asuncion J, Anton V, Marin J. Exercise causes blood glutathione oxidation in chronic obstructive pulmonary disease: prevention by O2 therapy. J Appl Physiol 1996;81: 2198–2202.
[39] Murrell GA, Rapeport WG. Clinical pharmacokinetics of allopurinol. Clin Pharmacokinet 1986;11:343–353.
[40] Moorhouse PC, Grootveld M, Halliwell B, Quinlan JG, Gutteridge JM. Allopurinol and oxypurinol are hydroxyl radical scavengers. FEBS Lett 1987;213:23–28.
[41] Griffiths HR, Moller L, Bartosz G, Bast A, Bertoni-Freddari C, Collins A, Cooke M, Coolen S, Haenen G, Hoberg AM, Loft S, Lunec J, Olinski R, Parry J, Pompella A, Poulsen H, Verhagen H, Astley SB. Biomarkers. Mol Aspects Med 2002; 23:101–208.
[42] Ziment I. Acetylcysteine: a drug that is much more than a mucokinetic. Biomed Pharmacother 1988;42:513–519.
[43] Bachur JA, Garcia SB, Vannucchi H, Jordao AA, Chiarello PG, Zucoloto S. Anti-oxidative systems in rat skeletal muscle after acute physical exercise. Appl Physiol Nutr Metab 2007; 32:190–196.
[44] Cappola TP, Kass DA, Nelson GS, Berger RD, Rosas GO, Kobeissi ZA, Marban E, Hare JM. Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. Circulation 2001;104:2407–2411.
[45] Duncan JG, Ravi R, Stull LB, Murphy AM. Chronic xanthine oxidase inhibition prevents myofibrillar protein oxidation and preserves cardiac function in a transgenic mouse model of cardiomyopathy. Am J Physiol Heart Circ Physiol 2005;289:H1512–H1518.
[46] Engberding N, Spiekermann S, Schaefer A, Heineke A, Wiencke A, Muller M, Fuchs M, Hilfiker-Kleiner D, Hornig B, Drexler H, Landmesser U. Allopurinol attenuates left ventricular remodeling and dysfunction after experimental myocardial infarction: a new action for an old drug? Circulation 2004;110:2175–2179.
[47] Minhas KM, Saraiva RM, Schuleri KH, Lehrke S, Zheng M, Saliaris AP, Berry CE, Barouch LA, Vandegaer KM, Li D, Hare JM. Xanthine oxidoreductase inhibition causes reverse remodeling in rats with dilated cardiomyopathy. Circ Res 2006;98:271–279.
[48] Stull LB, Leppo MK, Szweda L, Gao WD, Marban E. Chronic treatment with allopurinol boosts survival and cardiac contractility in murine postischemic cardiomyopathy. Circ Res 2004;95:1005–1011.

This paper was first published online on iFirst on 24 September 2008.