Xanthine oxidase is involved in exercise-induced oxidative stress in chronic obstructive pulmonary disease

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Heunks, Leo M. A., Jose Viña, Cees L. A. Van Herwaarden, Hans T. M. Folgering, Amparo Gimeno, and P. N. Richard Dekhuijzen. Xanthine oxidase is involved in exercise-induced oxidative stress in chronic obstructive pulmonary disease. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1697–R1704, 1999.—In the present study, we hypothesized that exhaustive exercise in patients with chronic obstructive pulmonary disease (COPD) results in glutathione oxidation and lipid peroxidation and that xanthine oxidase (XO) contributes to free radical generation during exercise. COPD patients performed incremental cycle ergometry until exhaustion with (n = 8) or without (n = 8) prior treatment with allopurinol, an XO inhibitor. Reduced (GSH) and oxidized glutathione (GSSG) and lipid peroxides [malondialdehyde (MDA)] were measured in arterial blood. In nontreated COPD patients, maximal exercise (~75 W) resulted in a significant increase in the GSSG-to-GSH ratio (4.6 ± 0.9% at rest vs. 9.3 ± 1.7% after exercise). In nontreated patients, MDA increased from 0.68 ± 0.08 nmol/ml at rest up to 1.32 ± 0.13 nmol/ml 60 min after cessation of exercise. In contrast, in patients treated with allopurinol, GSSG-to-GSH ratio did not increase in response to exercise (5.0 ± 1.2% preexercise vs. 4.6 ± 1.1% after exercise). Plasma lipid peroxide formation was also inhibited by allopurinol pretreatment (0.72 ± 0.15 nmol/ml preexercise vs. 0.64 ± 0.09 nmol/ml 60 min after exercise). We conclude that strenuous exercise in COPD patients results in blood glutathione oxidation and lipid peroxidation. This can be inhibited by treatment with allopurinol, indicating that XO is an important source for free radical generation during exercise in COPD.

glutathione; lipid peroxidation

STRENUOUS PHYSICAL EXERCISE results in increased generation of free radicals (FR), which is associated with skeletal muscle fatigue. Indeed, it has been demonstrated that heavy exercise in healthy subjects results in blood glutathione oxidation (8, 30), which is a marker of oxidative stress. Moreover, strenuous exercise is associated with lipid peroxidation (16, 33), which is considered to be a marker for FR-induced tissue damage. Reid et al. (29) showed that FR are involved in the development of limb skeletal muscle fatigue in healthy subjects. Exercise-induced blood glutathione oxidation also occurs in patients with severe chronic obstructive pulmonary disease (COPD) (36).

Healthy subjects performing a maximal incremental exercise test are limited by their cardiocirculatory system. In patients with moderate to severe COPD, the threshold of fatigue during exercise is reduced. When the forced expiratory volume in the first second (FEV1) is below ~50% of predicted value, maximal exercise is ventilatory limited. This results in an inability to eliminate adequately CO2. The ventilatory limitation is a result of the inability of the respiratory muscles to maintain a sufficient level of ventilation to assure normal alveolar ventilation. In addition to ventilatory limitation, peripheral skeletal muscle weakness also contributes to exercise limitation in COPD (9). In severe COPD, arterial hypoxemia may occur during exercise as a result of diffusion limitation at the alveolar-capillary membrane due to loss of lung tissue.

Generally, it is assumed that the mitochondria are an important source of FR, because ~2% of all oxygen reduced by the mitochondria escape the respiratory chain to yield superoxide anion (10). When the oxygen flux through mitochondria increases, as for example during exercise, it is expected that FR generation will increase. Surprisingly, the degree of blood glutathione oxidation after exhaustive physical exercise is rather similar between healthy well-trained subjects (30) and patients with severe COPD (36), whereas maximal exercise (Wmax) oxygen consumption (Vo2max) is much lower in patients with COPD compared with healthy subjects.

Therefore, we investigated whether other sources of FR are involved in exercise-induced FR generation in patients with COPD. During exhaustive physical exercise, ATP degradation occurs as indicated by the release of hypoxanthine from skeletal muscles (12). The ubiquitous enzyme xanthine oxidase (XO), which is also present in human skeletal muscles (14), can generate superoxide radical in the presence of hypoxanthine and oxygen (19). In the present studies, we tested the following hypotheses.

1) Exhaustive Physical Exercise in Patients with Severe COPD Results in Plasma Lipid Peroxidation

Previous studies have revealed that strenuous exercise in COPD results in blood glutathione oxidation (36), indicating exercise-induced oxidative stress. It is unknown whether this is accompanied by tissue damage. To establish FR-induced tissue damage, we measured plasma concentrations of lipid peroxides prior to and after an incremental cycle ergometer test in eight patients with COPD.

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2) XO is an Important Source of Exercise-Induced FR Generation in Patients with Severe COPD

To study the contribution of XO, we treated patients with allopurinol, a clinically used inhibitor of XO, prior to incremental cycle ergometry in another group of eight COPD patients.

METHODS

Subjects

Sixteen patients with COPD were recruited from our outpatient clinic. General characteristics and pulmonary function data are shown in Table 1. All patients had severe pulmonary obstruction with little reversibility in FEV1 after β2-agonist inhalation (<15% of predicted value). Pulmonary hyperinflation was present, as indicated by increased total lung capacity (TLC). Furthermore, carbon monoxide diffusion capacity (DLCO) was severely reduced. The severity of COPD did not differ between the group receiving no allopurinol (n = 8) and the group with pretreatment with allopurinol (n = 8).

Patients using supplemental antioxidants or suffering from exercise-limiting diseases besides COPD were excluded. The study was conducted according to the Declaration of Helsinki and was approved by the ethical committee of our hospital. Informed written consent was obtained from all patients.

Procedures

Patients were alternately (in order of entrance in the study) divided into control or allopurinol-treated groups. Patients in the treated group received two tablets of 300 mg allopurinol (Zyloprim, Genfarma, The Netherlands). The first tablet was taken 24 h prior to cycle ergometry, and the second was taken 1 h before. We administered allopurinol for a very short period of time, because treatment during several days results in gradual accumulation of oxyipurinol (24); high concentrations of oxyipurinol may result in direct FR scavenging independent of XO inhibition (see DISCUSSION). Patients took the second tablet 1 h before ergometry, because peak plasma concentration of allopurinol occurs 1 h after oral ingestion (24). Plasma, for determination of allopurinol and its metabolite oxyipurinol concentration, was collected before ergometry to verify patient compliance. All treated patients took the tablets as indicated by adequate plasma concentration of allopurinol and oxyipurinol (13.0 ± 2.8 and 60.0 ± 4.4 μmol/l, respectively; means ± SE). The physician supervising the exercise test (HTMF) was blinded for treatment groups. The laboratories performing the biochemical measurements were blinded for treatment group and the order in which samples were collected.

A cannula was inserted into the brachial artery under local anesthesia to obtain blood, which was collected 1 min prior to the start of cycle ergometry ("preexercise"), at Wmax ("maximum exercise"), and 3 min after maximum load (postexercise). Recovery samples were obtained 60 min after discontinuation of exercise (recovery).

Pulmonary Function Testing and Cycle Ergometry

Spirometry and DLCO were performed prior to cycle ergometry. The patients cycled on an electrically braked cycle ergometer (Lode, Groningen, The Netherlands) at a pedaling rate of 60 rpm breathing room air. The work rate was increased each minute by 10% of estimated Wmax until exhaustion (4). Minute ventilation, oxygen consumption (VO2), and carbon dioxide production was measured every 30 s by a breath-by-breath ergospirometry unit (Vmax Sensormedics, Biltoven, The Netherlands). Electrocardiography was conducted throughout the test. After the cycle ergometry, patients recovered for 60 min. During this period, patients were allowed to drink water.

Biochemistry

Blood for determination of hemoglobin, hematocrit, and leukocytes was collected in vacutainers containing EDTA and analyzed immediately according to standard laboratory assays. To determine creatine kinase (CK), uric acid, and glucose, blood was collected in dry vacutainers and analyzed immediately in serum according to standard laboratory assays. For the measurement of lactate and pyruvate, 2.0 ml blood were collected in tubes containing 2 ml perchloric acid. The tubes were immediately centrifuged, and the clear supernatant was stored at −80°C until analysis (which, in all cases, was performed within 1 wk). Measurements were routine enzymatic laboratory assays. Blood for blood gas was collected into heparinized syringes and immediately analyzed.

Reduced glutathione (GSH) was measured according to Brigelius et al. (2). Briefly, 0.5 ml blood was immediately added to 0.5 ml chilled 30% trichloroacetic acid solution containing 2 mM EDTA. The samples were immediately centrifuged, and the clear supernatant was stored at −80°C until later analysis. Oxidized glutathione (GSSG) was determined according to Viña et al. (35). Blood (0.5 ml) was immediately added to 0.5 ml 12% perchloric acid containing 40 mM N-ethylmaleimide and 0.2 M bathophenanthroline sulfonic acid. Samples were immediately centrifuged, and the clear supernatant was stored at −80°C for subsequent analysis.

Plasma lipid peroxides were determined by measuring malondialdehyde (MDA) formation according to Wong et al. (37). Two milliliters of blood were collected in EDTA vacutainers and immediately centrifuged, and the supernatant was stored at −80°C until later analysis. Samples were thawed and subsequently hydrolyzed by boiling in diluted phosphoric acid. MDA, one of the hydrolysis products, was reacted with thiobarbituric acid (TBA) to form MDA-TBA adduct. Plasma proteins were precipitated with methanol and removed from the reaction mixture by centrifugation. The protein-free extract was fractionated by HPLC on a column of octadecyl silica gel to separate the MDA-TBA adduct from interfering chromogens. The adduct was eluted from the column with methanol-phosphate buffer and quantified spectrophotometrically at 532 nm.

Blood for analysis of allopurinol and its active metabolite oxyipurinol was collected in heparinized vacutainers, immedi-

Table 1. Patient characteristics and pulmonary function in control and allopurinol-treated COPD patients

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<tr>
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<th>Control</th>
<th>Allopurinol</th>
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<tr>
<td>n</td>
<td>8</td>
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<tr>
<td>Male</td>
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<tr>
<td>Age, yr</td>
<td>64 ± 3</td>
<td>63 ± 4</td>
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<tr>
<td>FEV1, l</td>
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<td>1.16 ± 0.13</td>
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<tr>
<td>FEV1,%pred</td>
<td>36 ± 3</td>
<td>35 ± 4</td>
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<tr>
<td>FEV1/VC,%pred</td>
<td>46 ± 5</td>
<td>48 ± 6</td>
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<tr>
<td>TLC,%pred</td>
<td>115 ± 7</td>
<td>111 ± 8</td>
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<tr>
<td>DLCO,%pred</td>
<td>34 ± 7</td>
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Values represent means ± SE. COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume in first second; VC, vital capacity; TLC, total lung capacity; DLCO, diffusion capacity for carbon monoxide; pred, predicted value.
Allopurinol Effects on Resting Free Radical Generation in Patients with Severe COPD

Glutathione oxidation and lipid peroxidation. Blood GSH and GSSG concentrations are summarized in Fig. 1. Due to a technical problem, the glutathione samples of one allopurinol-treated patient were lost. Thus the samples of eight control COPD and seven allopurinol-treated COPD patients were analyzed. No significant differences were observed in blood GSH and GSSG status between control and allopurinol-treated patients at rest. Allopurinol treatment did not affect the GSSG-to-GSH ratio at rest (4.6 ± 0.9% in control patients vs. 5.0 ± 1.2% in allopurinol-treated COPD patients). In addition, no differences were observed in plasma MDA between control and allopurinol-treated patients (n = 8 in each group; Fig. 2A).

Allopurinol Effects on Exercise-Induced Oxidative Stress in Patients with Severe COPD

Glutathione oxidation. Figure 1 shows that incremental cycle ergometry until W_max decreased blood GSH concentration immediately after exercise in control COPD patients. In contrast, in allopurinol-treated patients, GSH was not affected by exercise. In control patients, blood GSSG significantly increased up to ~170% of preexercise value immediately after maximum exercise. GSSG approached preexercise concentration during the recovery period. In contrast, in allopurinol-treated patients, GSSG did not increase after incremental exercise. The degree of oxidative stress is frequently expressed as the GSSG-to-GSH ratio. Allopurinol treatment prevented the elevation in blood GSSG-to-GSH ratio that occurred immediately after exercise in control patients (4.6 ± 0.9% preexercise vs. 9.3 ± 1.7% postexercise in control patients (P < 0.05) and 5.0 ± 1.2% preexercise vs. 4.6 ± 1.1 postexercise in allopurinol-treated patients).

Lipid peroxidation. Figure 2 shows that exhaustive exercise in patients with severe COPD was accompanied by increased lipid peroxidation in plasma. In control patients, MDA significantly increased immediately after exercise (0.68 ± 0.08 μmol/l up to 1.08 ± 0.12 μmol/l, P < 0.05). Lipid peroxides further increased until 1.32 ± 0.13 μmol/l 60 min after exercise (P < 0.05). In allopurinol-treated patients, however, exercise was not accompanied by an elevation of plasma lipid peroxides. The increase in MDA (Fig. 2B) and the effect of allopurinol treatment on inhibition of exercise-induced lipid peroxidation (Fig. 2C) were very consistent.

Physiological Responses and Routine Biochemical Parameters During and After Exercise in COPD Patients

Physiological responses to exercise are shown in Table 2. Exercise endurance was not significantly different between control and allopurinol-treated patients (6.0 ± 0.6 vs. 5.6 ± 0.5 min, respectively). W_max was 74 ± 16 and 79 ± 17 W, respectively. There was no cardiovascular limitation because the patients did not reach their predicted maximum heart rate. A small but significant (P < 0.05) difference in baseline VO_2 was
observed between control and allopurinol-treated patients, but \( \text{VO}_{2\text{max}} \) did not differ significantly between these two groups.

Table 3 shows arterial blood gases at rest and at maximum exercise in both groups of patients. In both groups, exhaustive exercise significantly lowered pH. However, arterial oxygen tension significantly decreased and arterial carbon dioxide tension significantly increased only in allopurinol-treated patients (\( P < 0.05 \)).

Effects of Exercise on ATP Degradation Products

In the subsequent study, patient characteristics and exercise physiological data did not differ significantly from the patients in the study described above (data not shown). Figure 3 shows the plasma levels of hypoxanthine and xanthine. Elevated preexercise level of xanthine in allopurinol-treated patients compared with control COPD patients indicates that XO was effectively inhibited by allopurinol. Furthermore, in control COPD patients, exercise results in elevated levels of hypoxanthine and xanthine, although only the latter reached statistical significance (\( P < 0.05 \)). This indicates that ATP degradation occurred as a result of exercise. Significant elevation of hypoxanthine and xanthine was also observed in allopurinol-treated COPD patients (\( P < 0.05 \)).
It has been demonstrated in rats that glutathione levels of GSSG or increased GSSG-to-GSH ratio in tissues such as skeletal muscle (26), and increased blood reflects the glutathione status of less-accessible intracellularly, cells will export GSSG (32). Therefore, glutathione peroxidase (21). When GSSG accumulates of GSH into GSSG by the selenium-containing enzyme can be reduced by GSH, mainly through the conversion of GSH into GSSG by the selenium-containing enzyme.

Oxidative Stress During Exercise

Exercise-induced oxidative stress can be prevented by XO inhibition. Treatment with 300 mg allopurinol twice prior to incremental cycle ergometry prevented both blood glutathione oxidation and plasma lipid peroxidation. These findings indicate that patients with severe COPD performing strenuous exercise are exposed to oxidative stress resulting in tissue damage, which can be inhibited by blocking XO. Finally, this paper reports data showing that ATP degradation occurs in patients with COPD performing strenuous exercise.

Oxidative Stress During Exercise

GSH is an ubiquitous endogenous antioxidant. FR can be reduced by GSH, mainly through the conversion of GSH into GSSG by the selenium-containing enzyme glutathione peroxidase (21). When GSSG accumulates intracellularly, cells will export GSSG (32). Therefore, blood reflects the glutathione status of less-accessible tissues such as skeletal muscle (26), and increased levels of GSSG or increased GSSG-to-GSH ratio in blood can be considered as signs of oxidative stress (15). It has been demonstrated in rats that glutathione oxidation occurs in contracting skeletal muscles (34). Therefore, exercise-induced elevation in blood GSSG may be, at least partially, a result of export of GSSG from contracting skeletal muscles.

Blood GSH and GSSG concentrations at rest in the present study are in agreement with observations in healthy subjects (30) and previous studies in patients with COPD (36). Also, GSSG and GSSG-to-GSH ratio immediately after exercise in our study resembles data from previous studies in healthy subjects and patients with COPD (30, 36). In the present study, a small but significant GSH depletion occurred immediately after discontinuation of exercise in control COPD patients. GSH depletion after exercise might be due to inhibition of GSH synthesis, for instance, as a result of adrenergic stimulation (7). Significant blood GSH depletion after exercise has been reported previously in healthy subjects, although in this latter study, the depletion was more severe and persisted up to 60 min after discontinuation of exercise. As mentioned before, exercise-induced oxidative stress decreases skeletal muscle glutathione concentration in animals (31). Under these circumstances, the liver releases GSH into the blood to provide contracting muscles with GSH (31). From our study, it appears that the liver in patients with COPD can fulfill glutathione demands of peripheral tissues more adequately than in healthy subjects, because the degree of blood GSH depletion after exercise was less severe compared with healthy subjects. This may be due to the duration of oxidative stress. In the study by Sastre et al. (30) using healthy subjects, exercise duration was 12 min, whereas in our study, exercise lasted only 6.4 ± 0.6 min. Furthermore, limb skeletal muscle mass of patients with COPD is reduced compared with healthy subjects (1). Therefore, it may be argued that the GSH demand during exercise was lower in comparison to healthy subjects.

We used plasma lipid peroxidation as a marker for FR-induced tissue damage. Free radicals can initiate lipid peroxidation resulting in free radical chain reactions (11). Therefore, lipid peroxidation is frequently used as a marker of FR-induced tissue damage, although results should be interpreted with caution, because it is technologically complicated to determine lipid peroxidation reliably (11). We used a specific, sensitive, and reproducible HPLC method that allows a good separation between the MDA-TBA and interfering substances (37). Furthermore, the effects of exercise on MDA were very consistent in control patients (Fig. 2B). Exercise-induced lipid peroxidation in plasma of healthy subjects has been demonstrated in several studies (16, 33). In addition, expired pentane, which is another marker of lipid peroxidation, was reported to be increased after cycle ergometry in healthy subjects (5).

Fig. 3. Changes in plasma hypoxanthine and xanthine concentration in control COPD and allopurinol-treated COPD patients. Preexercise xanthine was significantly higher in allopurinol-treated patients compared with control patients. Values are means ± SE. *P < 0.05 vs. preexercise; †P < 0.05 vs. control 30 min postexercise; ‡P < 0.05 vs. control preexercise.

XO Inhibition and Exercise-Induced Oxidative Stress

Exercise-induced oxidative stress was prevented by treatment with allopurinol. In our study, both exercise-induced glutathione oxidation and elevation of lipid peroxides were prevented by allopurinol treatment. This strongly suggests that XO is involved in exercise-
induced generation of FR. XO generates FR in the presence of oxygen and hypoxanthine or xanthine, which are ATP degradation products (18). The role of XO in ischemia-reperfusion injury is well recognized (18). For instance, treating isolated rat hearts with allopurinol prior to 20 min of ischemia and subsequent 40 min of reperfusion inhibited generation of H$_2$O$_2$ by the myocardium and attenuated loss in ventricular function (3). Similar results were obtained in limb skeletal muscle (20).

Elevated generation of FR by XO during exercise may be attributed to increased XO activity. Indeed, it has been shown that immunoreactivity of XO in skeletal muscles is increased after eccentric exercise (14). In this latter study, XO was mainly present in skeletal muscle endothelium. This is in line with the observation that allopurinol treatment in mice attenuated exercise-induced skeletal muscle damage, especially in the endothelial cells (6). However, it is unlikely that increased XO expression could account for the elevated generation of FR during exercise, because elevated enzyme expression is unlikely to occur within the time span of the present study. Interestingly, it has been proposed that generation of FR by XO is not enzyme but rather substrate limited (38). This implies that when ATP degradation products accumulate, XO may generate FR. This fits well with data from the present study demonstrating elevation of ATP degradation products after exercise. This was accompanied by elevation of markers of FR, which was inhibited by allopurinol treatment. Release of xanthine and hypoxanthine from skeletal muscles after exercise has been shown previously in healthy subjects (12, 17). Moreover, uric acid release from active skeletal muscles has been shown, indicating XO activity (13).

XO activity has been demonstrated in human tissues such as liver, small intestine, heart, and skeletal muscle (14, 25). With the use of in vivo models, it is impossible to identify the source of XO for increased FR generation. However, we speculate that XO in skeletal muscles may play an important role, because ATP degradation products are elevated in skeletal muscles after exercise, providing substrate for XO to generate FR. Furthermore, allopurinol administration to mice attenuated exercise-induced morphological skeletal muscle damage (6).

Our data on the effects of allopurinol on exercise-induced oxidative stress are in line with data in horses (22), showing that allopurinol treatment prevented exercise-induced blood glutathione oxidation and lipid peroxidation. Viña et al. (36) found that oxygen supplementation attenuates exercise-induced oxidative stress in patients with COPD. This fits well with our reasoning of XO as a source for FR generation during exercise, because oxygen supplementation is likely to reduce metabolic stress to tissues and, therefore, may reduce ATP degradation.

Allopurinol Pharmacology

Allopurinol [1H-pyrazolo[3,4-d]pyrimidin-4-ol] is an oxypurine base (mol wt 136.11) and is clinically used for treatment of hyperuricaemia. The major pharmacological actions of allopurinol are mediated by its major metabolite oxypurinol. Both are structural analogs of the purine bases hypoxanthine and xanthine and competitively bind to XO. Thereby, they inhibit the XO-mediated conversion of hypoxanthine to xanthine and xanthine to uric acid and thus generation of FR. Controversy exists regarding the role of allopurinol in a direct hydroxyl radical scavenger. Moorhouse et al. (23) reported that allopurinol and oxypurinol have direct hydroxyl scavenging properties in vitro. However, in this latter study, the concentration of both allopurinol and oxypurinol was >250 µM. Alternatively, experiments by Zimmerman et al. (39) revealed that allopurinol and oxypurinol, at concentrations of 12.0 and 12.9 µM, respectively, do not enhance FR scavenging properties of plasma. In our study, plasma concentration of allopurinol was similar to the study by Zimmerman et al. (39), although oxypurinol was somewhat higher. Therefore, it is conceivable that the effects of allopurinol treatment in our study are the result of XO inhibition and not the result of its direct antioxidant properties considering the low plasma concentrations in our patients. However, XO was blocked sufficiently as indicated by higher preexercise levels of xanthine in allopurinol-treated patients compared with untreated patients (Fig. 3).

After oral administration, allopurinol is well absorbed from the gastrointestinal tract. Peak plasma concentration of allopurinol and oxypurinol are reached after −1 and −3 h, respectively (24). Half-life time after oral administration of allopurinol and oxypurinol are >1 and >20 h, respectively. Allopurinol is usually well tolerated by patients. The most common side effects are hypersensitivity reactions. However, none of our patients reported any side effects.

Clinical Relevance

Overproduction of FR is associated with skeletal muscle fatigue. In vitro studies by Reid et al. (28) demonstrated that contracting rat diaphragm generates superoxide anion radical. Moreover, an inverse relationship exists between superoxide release and contractile function during a fatigue protocol (28). In healthy subjects, infusion with high dose N-acetylcysteine attenuated limb skeletal muscle fatigue (29). This and many other studies suggest that oxidative stress is associated with limb and respiratory skeletal muscle fatigue. However, these studies do not identify the source of FR. This may be important to effectively inhibit FR generation. An additional concern in studies using healthy subjects is how these findings can be extrapolated to patients with ventilatory exercise limitation. Unlike healthy subjects, patients with severe COPD become fatigued at low external workload; for instance, during daily life activities, which therefore may result in frequent exposure to oxidative stress. In these patients, an association exists between exercise capacity and limb and respiratory skeletal muscle force (9). Therefore, effective inhibitors of FR generation might be of clinical relevance to patients with exercise-
limiting diseases such as COPD. There are no reasons to argue that the effects of allopurinol found in the present study are specific to patients with COPD. The key issue is that the degree of exercise is severe enough to induce ATP degradation to provide substrates for XO. Because this occurs at low external workload in patients with severe COPD, these patients will be exposed to oxidative stress more frequently compared with healthy subjects.

In both groups of COPD patients, arterial PO2 decreased during exercise, whereas PCO2 increased. This phenomenon is an indication of a ventilatory limitation. Controlled studies are needed to establish the phenomenon is an indication of a ventilatory limitation. Moreover, these studies should focus on the development of agents affecting specific pathways resulting in FR formation.

An interesting question arising from the present study is whether allopurinol treatment affects physical performance. Basically, this question should be divided into two separate questions. First, does short-term allopurinol administration alter physical exercise performance? Our data indicate that allopurinol prevents accumulation of GSSG. In vitro studies revealed that intracellular accumulation of oxidants directly contributes to the loss of contractile function during fatigue (27). Thus it is possible that allopurinol reduces fatigue rate in skeletal muscles. This may be relevant to patients with COPD and to healthy athletes performing competitive exercise. Second, does long-term allopurinol administration alter physical exercise performance? Peripheral and limb skeletal muscle dysfunction frequently occurs in COPD and is associated with impaired exercise tolerance (9). Our data demonstrate that strenuous exercise results in lipid peroxidation, possibly originating from contracting skeletal muscles. Because daily life activities can be exhaustive for these patients, it is conceivable that they are exposed to lipid peroxidation frequently. This may contribute to skeletal muscle dysfunction. Therefore, long-term allopurinol administration may partly prevent such dysfunction. Controlled studies are needed to establish the effect of allopurinol administration on exercise performance.

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REFERENCES


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