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Age-related increase in xanthine oxidase activity in human plasma and rat tissues

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Abstract
This study assessed the role of xanthine oxidase in vascular ageing. A positive correlation between xanthine oxidase activity and age was found in human plasma. Similar results were found in rat plasma. Xanthine oxidase expression and activity in homogenates from the aortic wall were significantly higher in samples from old rats than in their young counterparts ($p < 0.01$). In rat skeletal muscle homogenates both xanthine oxidase expression and activity showed a similar age-related profile. Superoxide production by xanthine oxidase in aortic rings was higher in aged rats. Uric acid, the final product of xanthine oxidase has been proposed as a risk factor for coronary heart disease and an independent marker of worse prognosis in patients with moderate-to-severe chronic heart failure. These results give a possible explanation for this correlation and underscore the role of xanthine oxidase in ageing.

Keywords: *Oxidative stress, glutathione, xanthine oxidoreductase, superoxide anion, ageing*

Introduction

The role of free radicals in ageing was first postulated by Harman [1]. It has now been demonstrated that the mitochondrial rate of $O2^-$ production correlates negatively with maximal life span [2]. Vascular ageing is one of the most important features in human senescence. Age-related endothelial damage is primarily attributed to increased superoxide production; however its original source remains controversial. Recently, Newaz et al. [3] showed that xanthine oxidase (XO), but not NAD(P)H oxidase, was the main source of oxidative stress in old male Sprague-Dauley rats. Xanthine oxidoreductase (XOR) is transcribed from a single gene as xanthine dehydrogenase (XDH) (EC 1.1.1.204), which can be converted to XO (EC 1.1.3.22) by oxidation or proteolysis. Xanthine oxidase is involved in the pathophysiology of ischaemia-reperfusion syndrome, because it generates a burst of free radicals (mainly superoxide) when tissue reperfusion occurs, after ischaemia. Vascular endothelium is particularly rich in XO [4]. Xanthine oxidase is involved in oxidative stress in exercise [5], diabetes [6] and hypertension [7]. Circulating XO has been shown to contribute to vascular dysfunction in animal models of hypercholesterolemia [8]. Netea et al. [9] suggested that uric acid, the final product of XO, causes direct damaging effects. Zou et al. [10] showed that plasma XOR levels were higher in old rats than in young and that a restricted diet could decrease

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plasma XOR expression. However, other authors like Csiszar et al. [11] showed changes with ageing in iNOS and eNOS mRNA levels by RT-PCR in coronary arterioles but no changes in XO, COX_2, Mn-SOD or Cu/Zn-SOD. Previous studies reported that XO was involved in vascular ageing [12,13], but this is now a matter of debate as recent reports provide opposite results [14,15].

In view of these discrepancies the purpose of our work was to assess the role of XO in vascular ageing.

Materials and methods

Reagents

Chemicals were obtained from Boehringer (Mann heim, Germany), Merck (Darmstadt, Germany), Sigma Chemical (St. Louis, USA) and Pharmacia Fine Chemicals (Uppsala, Sweden).

Human studies

This is a prospective blind study. Peripheral blood (2 ml) was obtained from nine healthy subjects (five men and four women, from 38–65 years old) in the Clinical Hospital of Valencia. Exclusion criteria were smoking, antioxidant administration, obesity, strenuous exercise and pregnancy. Subjects gave informed consent and the study was approved by the Ethics Committee of the Clinical Hospital of Valencia. Blood samples were centrifuged at 2500 rpm for 10 min to obtain blood plasma which was stored at -80°C.

Analysis of human plasma. Xanthine oxidase activity was measured in human plasma in duplicate in 50 µl of plasma using the Amplex® Red Xanthine/Xanthine Oxidase Assay Kit (Molecular Probes, Eugene, OR).

Animal studies

Experiments were conducted on adult Wistar rats that had free access to food and water and were kept on a 12:12-h light-dark cycle. Handling of animals was performed in accordance with the 86/609/CEE European Community regulations and the *Guiding Principles for Research Involving Animals and Human Beings* of the American Physiological Society.

Animals were divided in two groups: a group of young adult rats (4–6 months old) and a group of old rats (23–25 months old).

Tissue preparation. The rats were anaesthetized with an intraperitoneal dose of sodium Thiopental. Blood samples were obtained by venous puncture from the cava vein after laparotomy. Then samples from the aorta and the gastrocnemius muscle were obtained. Blood samples were centrifuged as previously mentioned to obtain plasma, which was stored at -80°C.

Tissues were quickly freeze-clamped in liquid nitrogen and stored at -80°C. Tissues were powdered thoroughly in liquid nitrogen using a pestle and a mortar and homogenized with a motor-driven Potter-Elveljem glass homogenizer at 0–4°C at low speed using the corresponding medium.

Analysis of samples. Xanthine oxidase and xanthine dehydrogenase activities were measured in plasma and tissue homogenates as previously described [16]. Briefly, isoxanthopterin formation from pterine was monitored by fluorimetry (excitation at 345 nm and emission at 390 nm).

Western immunoblotting analysis was performed by using an anti-xanthine oxidase antibody from Chemicon International and chemiluminescent detection kits (Cell Signalling Technology, Beverly, MA). Immunoblotting was performed under non-reducing conditions. Immunoreactive bands showing a 150-kDa band corresponding to each of the two sub-units of the dimer is shown. Western blots were quantified by densitometry.

Superoxide-dependent lucigenin chemiluminescence of aortic rings was measured following our protocol [5] which is a modification of that of White et al. [17]. The rat thoracic aorta was excised, cleaned of fat and adhering tissue and divided into two rings of 4–5 mm each. Chemiluminescence was measured in one of these rings in a vial containing 3 ml PBS with 0.25 mmol/l lucigenin and 50 µmol/l xanthine. In the second ring, chemiluminescence was determined under the same conditions plus 100 mmol/l allopurinol. It is well-known that high concentrations of lucigenin may produce redox cycling leading to artificial increases in $O_2^{\bullet -}$. The low concentration of lucigenin used in our assays rules out this possibility.

Statistics

Results are expressed as mean ± SD. Statistical analyses were performed by the least-significant difference test. The null hypothesis was accepted for all numbers in which F was non-significant at the level of $p < 0.05$. The sets of data in which F was significant were examined by the modified t-test, using $p < 0.05$ as the critical limit.

Results

Plasma XO activity in human ageing

We determined XO activity in plasma from healthy volunteers (from 38–65 years old). Figure 1 shows that XO activity correlates positively with age: this correlation is highly significant ($R = 0.82$, $p = 0.007$).

Plasma XO activity increases with age in rats

Xanthine oxidase activity in plasma from old rats increased with age significantly (see Figure 2). A 25% increase in XO activity was found in plasma from old

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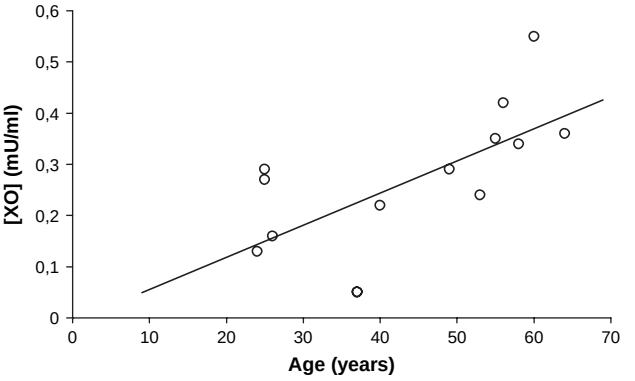

Figure 1. XO activity in human plasma from healthy volunteers. Points represent individual samples.

animals when compared with their young counterparts.

Xanthine oxidase protein level in aged rat aorta

Western blot analysis showed that XO expression in aortas from aged rats is higher ($p < 0.01$) than in samples from young animals (Figure 3A) as demonstrated by densitometric analysis (Figure 3C). Thus, the change in XO expression with age correlates with the reported changes in XO activity.

To assess if there is a relation between aorta and other rat tissues we determined the expression of XO in skeletal muscle from old rats. When we performed a Western blot analysis we observed an increase in XO expression in gastrocnemius muscle from old rats ($n = 4$) when compared with that of young ones ($n = 4$, see Figure 3B and C).

Aorta and skeletal muscle XOR activity in ageing

Xanthine oxidase activity in aorta from old rats was significantly higher ($p < 0.01$) than in their young counterparts (Table I). There were also significant differences in XDH activity (Table I). The percentage of XO activity (i.e. the superoxide generating isoform) with respect to total XOR activity was higher ($p < 0.05$) in aortas from old animals than in those from young ones.

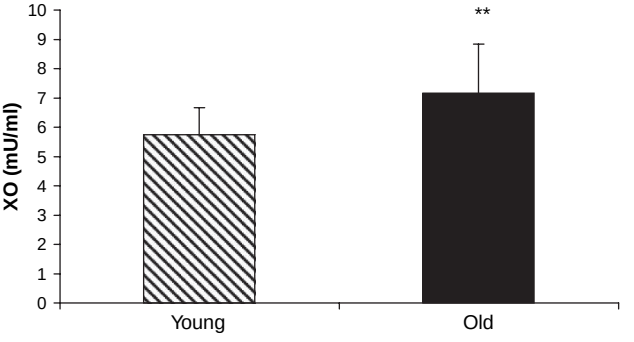

Figure 2. XO activity (mU/ml) in rat plasma from young ($n = 21$) and old ($n = 27$) animals. Results are mean ± SD. ** $p < 0.01$ from young.

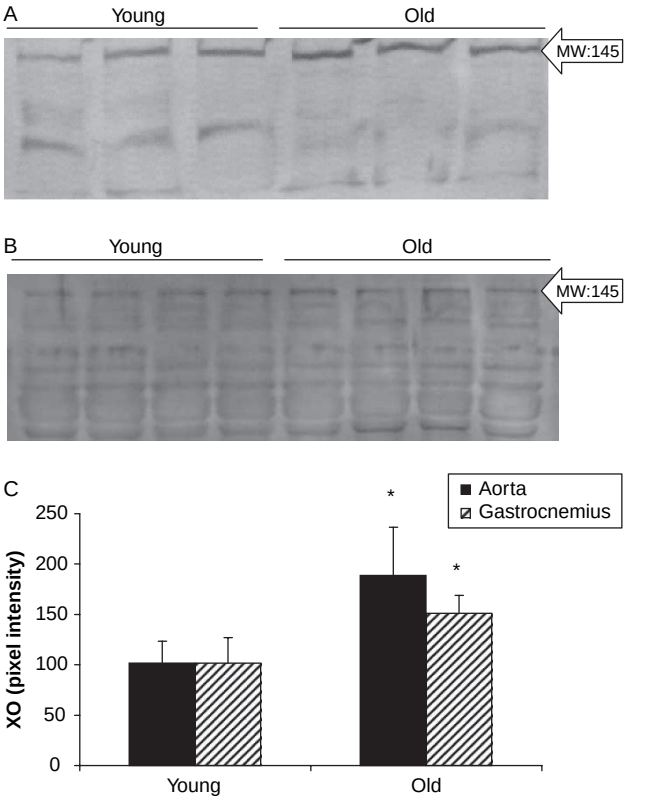

Figure 3. Representative blot of expression of XO in aorta (A) and gastrocnemius muscle (B) homogenates from young and old rats. (C) Grouped densitometric data. * $p < 0.05$ from young.

Both XO and XDH activities were higher in gastrocnemius muscles from old rats than in young ones (79% and 141%, respectively, see Table I).

XO contributes to higher superoxide production in aortic rings from aged rats

We measured superoxide production by chemiluminescence in aortic rings to asses if XO contributes to an enhanced $O_2^{\bullet -}$ production (Figure 4A). As we expected, higher $O_2^{\bullet -}$ production was found in aortic rings from old rats than in those from young ones ($p < 0.05$). In addition, the percentage of inhibition of superoxide production by allopurinol was higher in samples from old rats ($p < 0.05$), see Figure 4B. Thus, XO activity contributes significantly to superoxide production in ageing aortic rings.

Discussion

We found that XO, a source of reactive oxygen species, increases in plasma from aged rats and humans and in skeletal muscle and aorta from old rats and could act as a systemic factor contributing to ageing.

Data from the CDC of the US has recently shown that, in the year 2000, only 3–4% of deaths, of subjects aged between 20–24 years, were due to cardiovascular diseases. This rose to 39% at the age of 85 or over. This kind of disease becomes more frequent with age, even

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Table I. XO, XDH and percentage of XO with respect to total XOR activity in aorta and gastrocnemius muscle homogenates from young and old rats.

		Young	Old
Aorta	*n*	4	4
	XO (mU/mg)	0.065±0.013	0.135±0.035**
	XDH (mU/mg)	0.174±0.037	0.243±0.111
	XO (% of total XOR)	27.16±0.08	38.04±12.40*
Gastrocnemius muscle	*n*	8	6
	XO (mU/mg)	0.042±0.017	0.075±0.027*
	XDH (mU/mg)	0.027±0.011	0.065±0.018*
	XO (% of total XOR)	59.44±12.06	52.76±10.74

Results are mean±SD. *$p<0.05$ from young; **$p<0.01$ from young.

in the absence of established risk factors [18]. It has been suggested that ageing in itself alters vascular function and it is known that endothelium-dependent relaxation declines with age [19]. It seems that ageing affects vascular tissue and this ageing effect is related to oxidative stress, more specifically to $O_2^{\bullet -}$. Nitric oxide (NO) levels are lower in aged rat aortas, in spite of a 7-fold expression and activity of endothelial NO synthase [20]. This is because of an age-associated enhanced ($O_2^{\bullet -}$) production (3-fold) with concomitant quenching of NO and formation of peroxynitrite. Recent studies also point to peroxynitrite as the intracellular mediator of SERCA (sarco/endoplasmic reticulum calcium ATPase) glutathiolation, which leads to arterial relaxation [21].

Our results show that aortas from aged rats have higher XO (the isoform that produces $O_2^{\bullet -}$) than those from young ones. Numerous studies have demonstrated that the local $O_2^{\bullet -}$ concentration is the main limiting factor for the availability of bioactive NO in healthy and diseased vessels [22].

Xanthine oxidase has a complex regulation and its activity is altered by many factors, including hypoxia, which leads to increased activity [23–26], mRNA expression [23,26] and protein level *in vivo* [27]. Our results show that old rats have higher XO protein levels and enzyme activity than young animals.

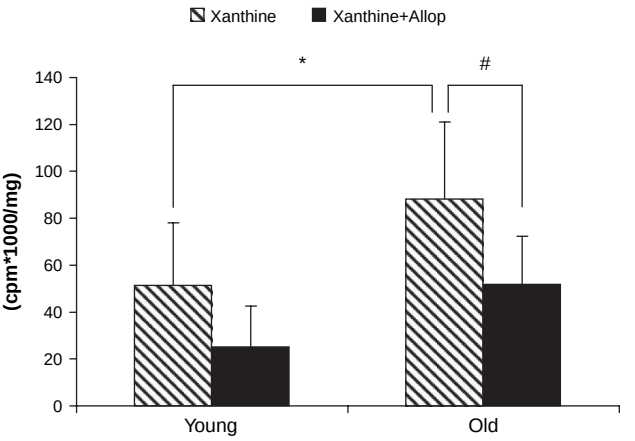

Figure 4. Total maximum lucigenin chemiluminescence in aortic rings from young and old rats with and without allopurinol incubation ($n=3$). Results are mean±SD. *$p<0.05$ from xanthine young group; #$p<0.05$ from xanthine old group.

It is worth noting that the presence of XO in vascular endothelium [28–30] has been involved in many pathophysiological conditions, especially vascular disorders, including ischaemia-reperfusion [31,32], congestive heart failure [33–36], atherosclerosis [37], diabetes [6], hypercholesterolemia [8,38], smoking [39,40] and hypertension [41,42]. Consequently, XO may be associated with the four major risk factors for coronary heart disease. Moreover, the presence of XO has been demonstrated in atherosclerotic plaque [43,44]. A number of interesting data correlate ageing with the end product (uric acid) of the two reactions catalysed by XO. These are: (i) uricaemia increases with age and (ii) hyperuricaemia is associated with hypertension, vascular disease, renal disease and cardiovascular events [45]. Uric acid has been proposed as a risk factor for coronary heart disease [46] and an independent marker of worse prognosis in patients with moderate-to-severe chronic heart failure [47].

Very recently a number of reports have emphasized the role of vascular sources of ROS other than from XO. Eskurza et al. [15] reported that XO expression in the endothelial wall of the vein is similar in young and old volunteers. According to these authors a single dose of allupurinol could not decrease age-associated vascular oxidative stress. Cardillo et al. [48] reported that inhibition of XO did not improve endothelium-dependent relaxation in patients with essential hypertension. This is in constrast to recent findings that XO inhibition with acute or chronic oral allopurinol administration or intravenous treatment improves endothelial function [7,12,40,49]. Our results give a molecular insight and provide additional information supporting the importance of XO activity in age-associated vascular impairment, showing that XO, an important source of free radicals, increases with ageing and that it could be correlated with vascular biology disorders.

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References

[1] Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol 1956;11:298–300.

[2] Barja G. Rate of generation of oxidative stress-related damage and animal longevity. Free Radic Biol Med 2002;33: 1167–1172.

[3] Newaz MA, Yousefipour Z, Oyekan A. Oxidative stress-associated vascular aging is xanthine oxidase-dependent but not NAD(P)H oxidase-dependent. J Cardiovasc Pharmacol 2006;48:88–94.

[4] Landmesser U, Spiekermann S, Dikalov S, Tatge H, Wilke R, Kohler C, Harrison DG, Hornig B, Drexler H. Vascular oxidative stress and endothelial dysfunction in patients with chronic heart failure: role of xanthine-oxidase and extracellular superoxide dismutase. Circulation 2002;106:3073–3078.

[5] Viña J, Gimeno A, Sastre J, Desco C, Asensi M, Pallardó FV, Cuesta A, Ferrero JA, Terada LS, Repine JE. Mechanism of free radical production in exhaustive exercise in humans and rats; role of xanthine oxidase and protection by allopurinol IUBMB. Life 2000;49:539–544.

[6] Desco MC, Asensi M, Marquez R, Martinez-Valls J, Vento M, Pallardo FV, Sastre J, Vina J. Xanthine oxidase is involved in free radical production in type 1 diabetes: protection by allopurinol. Diabetes 2002;51:1118–1124.

[7] Butler R, Morris AD, Belch JJ, Hill A, Struthers AD. Allopurinol normalizes endothelial dysfunction in type 2 diabetics with mild hypertension. Hypertension 2000;35: 746–751.

[8] White CR, Darley-Usmar V, Berrington WR, McAdams M, Gore JZ, Thompson JA, Parks DA, Tarpey MM, Freeman BA. Circulating plasma xanthine oxidase contributes to vascular dysfunction in hypercholesterolemic rabbits. Proc Natl Acad Sci USA 1996;93:8745–8749.

[9] Netea MG, Kullberg BJ, Blok WL, Netea RT, van der Meer JW. The role of hyperuricemia in the increased cytokine production after lipopolysaccharide challenge in neutropenic mice. Blood 1997;89:577–582.

[10] Zou Y, Jung KJ, Kim JW, Yu BP, Chung HY. Alteration of soluble adhesion molecules during aging and their modulation by calorie restriction. FASEB J 2004;18:320–322. Epub 19 December 2003.

[11] Csiszar A, Ungvari Z, Edwards JG, Kaminski P, Wolin MS, Koller A, Kaley G. Aging-induced phenotypic changes and oxidative stress impair coronary arteriolar function. Circ Res 2002;90:1159–1166.

[12] Farquharson CA, Butler R, Hill A, Belch JJ, Struthers AD. Allopurinol improves endothelial dysfunction in chronic heart failure. Circulation 2002;106:221–226.

[13] Doehner W, Schoene N, Rauchhaus M, Leyva-Leon F, Pavitt DV, Reaveley DA, Schuler G, Coats AJ, Anker SD, Hambrecht R. Effects of xanthine oxidase inhibition with allopurinol on endothelial function and peripheral blood flow in hyperuricemic patients with chronic heart failure: results from 2 placebo-controlled studies. Circulation 2002;105:2619–2624.

[14] O'Driscoll JG, Green DJ, Rankin JM, Taylor RR. Nitric oxide-dependent endothelial function is unaffected by allopurinol in hypercholesterolaemic subjects. Clin Exp Pharmacol Physiol 1999;26:779–783.

[15] Eskurza I, Kahn ZD, Seals DR. Xanthine oxidase does not contribute to impaired peripheral conduit artery endothelium-dependent dilatation with ageing. J Physiol 2006;571: 661–668.

[16] Beckman JS, Parks DA, Pearson JD, Marshall PA, Freeman BA. A sensitive fluorimetry assay for measuring xanthine dehydrogenase and oxidase in tissues. Free Radical Biol Med 1989;6:607–615.

[17] White CR, Darley-Usmar V, Berrington WR, McAdams M, Gore JZ, Thompson JA, Parks DA, Tarpey MM, Freeman BA. Circulating plasma xanthine oxidase contributes to vascular dysfunction in hypercholesterolemic rabbits. Proc Natl Acad Sci USA 1996;93:8745–8749.

[18] Lüscher T, Noll G. The endothelium in coronary vascular control. Heart Dis 1995;3:1–10.

[19] Tschudi M, Barton M, Bersinger N, Moreau P, Cosentino F, Noll G, Malinski T, Lüscher T. Effect of age on kinetics of nitric oxide release in rat aorta and pulmonary artery. J Clin Inves 1996;98:899–905.

[20] van der Loo B, Labugger R, Skepper JN, Bachschmid M, Kilo J, Powell JM, Palacios-Callender M, Erusalimsky JD, Quaschning T, Malinski T, Gygi D, Ullrich V, Lüscher TF. Enhanced peroxynitrite formation is associated with vascular aging. J Exp Med 2000;192:1731–1744.

[21] Adachi T, Weisbrod RM, Pimentel DR, Ying J, Sharov VS, Schoneich C, Cohen RA. S-Glutathiolation by peroxynitrite activates SERCA during arterial relaxation by nitric oxide. Nat Med 2004;10:1200–1207.

[22] Cai H, Harrison D. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 2000;87: 840–844.

[23] Hassoun PM, Yu FS, Shedd AL, Zulueta JJ, Thannickal VJ, Lanzillo JJ, Fanburg BL. Regulation of endothelial cell xanthine dehydrogenase/xanthine oxidase gene expression by oxygen tension. Am J Physiol 1994;266:L163–L171.

[24] Kayyali US, Donaldson C, Huang H, Abdelnour R, Hassoun PM. Phosphorylation of xanthine dehydrogenase/oxidase in hypoxia. J Biol Chem 2001;276:14359–14365.

[25] Poss WB, Hueckstaeadt TP, Panus PC, Freeman BA, Hoidal JR. Regulation of xanthine dehydrogenase and xanthine oxidase activity by hypoxia. Am J Physiol 1996;270:L941–L946.

[26] Terada LS, Piermattei D, Shibao GN, McManaman JL, Wright RM. Hypoxia regulates xanthine dehydrogenase activity at pre and posttranslational levels. Arch Biochem Biophys 1997;348:163–168.

[27] Hassoun PM, Yu FS, Cote CG, Zulueta JJ, Sawhney R, Skinner KA, Skinner HB, Parks DA, Lanzillo JJ. Upregulation of xanthine oxidase by lipopolysaccharide, interleukin-1, and hypoxia. Role in acute lung injury. Am J Respir Crit Care Med 1998;158:299–305.

[28] Jarasch ED, Grund C, Bruder G, Heid HW, Keenan TW, Franke WW. Localization of xanthine oxidase in mammary-gland epithelium and capillary endothelium. Cell 1981;25: 67–82.

[29] Kooij A, Schijns M, Frederiks WM, Van NCJ, James J. Distribution of xanthine oxidoreductase activity in human tissues: a histochemical and biochemical study. Virchows Arch B Cell Pathol 1992;63:17–23.

[30] Rouquette M, Page S, Bryant R, Benboubetra M, Stevens CR, Blake DR, Whish WD, Harrison R, Tosh D. Xanthine oxidoreductase is asymmetrically localised on the outer surface of human endothelial and epithelial cells in culture. FEBS Lett 1998;426:397–401.

[31] Granger DN. Role of xanthine oxidase and granulocytes in ischemia-reperfusion injury. Am J Physiol 1994;266:H1269–H1275.

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[32] Grace PA. Ischaemia-reperfusion injury. Br J Surg 1994;81: 637–647.
[33] Ukai T, Cheng C, Tachibana H, Igawa A, Zhang Z, Cheng H, Little W. Allopurinol enhances the contractile response to dobutamine and exercise in dogs with pacing-induced heart failure. Circulation 2001;103:750–755.
[34] Kogler H, Fraser H, McCune S, Altschuld R, Marban E. Disproportionate enhancement of myocardial contractility by the xanthine oxidase inhibitor oxypurinol in failing rat myocardium. Cardiovasc Res 2003;59:582–592.
[35] Ekelund UE, Harrison RW, Shokek O, Thakkar RN, Tunin RS, Senzaki H, Kass DA, Marban E, Hare JM. Intravenous allopurinol decreases myocardial oxygen consumption and increases mechanical efficiency in dogs with pacing-induced heart failure. Circ Res 1999;85:437–445.
[36] Cappola T, Kass D, Nelson G, Berger R, Rosas G, Kobeissi Z, Marban E, Hare J. Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. Circulation 2001;104:2407–2411.
[37] Schwartz CJ, Valente AJ, Sprague EA. A modern view of atherosclerosis. Am J Cardio 1993;71:9B–14B.
[38] O'Hara Y, Peterson T, Harrison D. Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest 1993;91:2546–2551.
[39] Kayyali U, Budhiraja R, Pennella C, Cooray S, Lanzillo J, Chalkley R, Hassoun P. Upregulation of xanthine oxidase by tobacco smoke condensate in pulmonary endothelial cells. Toxicol Appl Pharmacol 2003;188:59–68.
[40] Guthikonda S, Sinkey C, Barenz T, Haynes W. Xanthine oxidase inhibition reverses endothelial dysfunction in heavy smokers. Circulation 2003;107:416–421.
[41] McNally J, Davis M, Giddens D, Saha A, Hwang J, Dikalov S, Jo H, Harrison D. Role of xanthine oxidoreductase and the NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress. Am J Physiol 2003;285: H2290–H2297.
[42] Taddei S, Virdis A, Mattei P, Ghiadoni L, Fasolo C, Sudano I, Salvetti A. Hypertension causes premature aging of endothelial function in humans. Hypertension 1997;29: 736–743.
[43] Patetsios P, Song M, Shutze W, Papas C, Rodino W, Ramírez J, Panetta T. Identification or uric acid and xanthine oxidase in atherosclerotic plaque. Am J Cardiol 2001;88:188–191.
[44] Swain J, Gutteridge JMC. Prooxidant iron and copper, with ferroxidase and xanthine oxidase activities in human atherosclerotic material. FEBS Lett 1995;368:513–515.
[45] Johnson R, Kang D, Feig D, Kivlighn S, Kanellis J, Watanabe S, Tuttle K, Rodríguez-Iturbe B, Herrera-Acosta J, Mazzali M. Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease? Hypertension 2003;41: 1183–1190.
[46] Fang J, Alderman M. Serum uric acid and cardiovascular mortality. The NHANES Iepidemiologic follow-up study. JAMA 2000;283:2404–2410.
[47] Anker S, Doehner W, Rauchhaus M, Sharma R, Francis D, Knosalla C, Davos C, Cicoira M, Shamim W, Kemp M, Segal R, Osterziel K, Leyva F, Hetzer R, Ponikowski P, Coats A. Uric acid and survival in chronic heart failure. Circulation 2003;107:1991–1997.
[48] Cardillo C, Kilcoyne CM, Cannon RO 3rd, Quyyumi AA, Panza JA. Xanthine oxidase inhibition with oxypurinol improves endothelial vasodilator function in hypercholesterolemic but not in hypertensive patients. Hypertension 1997; 30:57–63.
[49] Guthikonda S, Woods K, Sinkey CA, Haynes WG. Role of xanthine oxidase in conduit artery endothelial dysfunction in cigarette smokers. Am J Cardiol 2004;93:664–668.

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