available at www.sciencedirect.com

SCIENCE DIRECT®

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

Superoxide radical production by allopurinol and xanthine oxidase

Chiara Galbusera [a,*], Peter Orth [a], David Fedida [a,b], Thomas Spector [c]

[a] Cardiome Pharma Corp., 6190 Agronomy Rd., Vancouver, BC, Canada V6T 1Z3
[b] Department of Cellular and Physiological Sciences, University of British Columbia, 2146 Health Sciences Mall, Vancouver, Canada V6T 1Z3
[c] Spector Consulting Services, 5216 Old Chapel Hill Rd., Durham, NC 27707, USA

ARTICLE INFO

Article history:
Received 12 December 2005
Accepted 7 February 2006

Keywords:
Allopurinol
Oxypurinol
Xanthine oxidase
Superoxide radical
Heart failure

Abbreviations:
ROS, reactive oxygen species
SOD, superoxide dismutase
XO, xanthine oxidase
XOR, xanthine oxidoreductase
XDH, xanthine dehydrogenase
FAD, flavine–adenine dinucleotide

ABSTRACT

Oxypurinol, an inhibitor of xanthine oxidase (XO), is being studied to block XO-catalyzed superoxide radical formation and thereby treat and protect failing heart tissue. Allopurinol, a prodrug that is converted to oxypurinol by xanthine oxidase, is also being studied for similar purposes. Because allopurinol, itself, may be generating superoxide radicals, we currently studied the reaction of allopurinol with xanthine oxidase and confirmed that allopurinol does produce superoxide radicals during its conversion to oxypurinol.

At pH 6.8 and 25 °C in the presence of 0.02 U/ml of XO, 10 and 20 μM allopurinol both produced 10 μM oxypurinol and 2.8 μM superoxide radical (determined by cytochrome C reduction). The 10 μM allopurinol was completely converted to oxypurinol, while the 20 μM allopurinol required a second addition of xanthine oxidase to complete the conversion. Fourteen percent of the reducing equivalents donated from allopurinol or xanthine reacted with oxygen to form superoxide radicals. Superoxide dismutase prevented the reduction of cytochrome C by these substrates. At higher xanthine oxidase concentrations, or at lower temperatures, more of the 20 μM allopurinol was converted to oxypurinol during the initial reaction. At lower xanthine oxidase concentrations, or higher temperatures, less conversion occurred. At pH 7.8, the amount of superoxide radicals produced from allopurinol and xanthine was nearly doubled. These results indicate that allopurinol is a conventional substrate that generates superoxide radicals during its oxidation by xanthine oxidase. Oxypurinol did not produce superoxide radicals.

1. Introduction

Xanthine oxidoreductase (XOR) is implicated in generating reactive oxygen species (ROS) that contribute to the biology associated with ischemia and reperfusion injury as well as to the pathology of failing cardiac tissues [1,2], reviewed in [3–5]. XOR exists as either an oxidase (XO) that transfers reducing equivalents to oxygen, or as a dehydrogenase (XDH) that utilizes NAD or oxygen as the final electron acceptor [3,6–9]. Both forms contain an internal electron transport system that is capable of producing ROS [7,10,11]. The physiological substrates, xanthine and hypoxanthine, bind oxidized enzyme and donate two electrons into the molybdenum cofactor reducing it from Mo^{VI} to Mo^{IV}. Substrates are hydroxylated by H_2O at the molybdenum site as the electrons travel via two iron-sulfide residues to flavine–adenine dinu-

* *Corresponding author*. Tel.: +1 604 677 6905; fax: +1 604 677 6915.
E-mail address: cgalbusera@cardiome.com (C. Galbusera).
0006-2952/$ – see front matter © 2006 Published by Elsevier Inc.
doi:10.1016/j.bcp.2006.02.008

cleotide (FAD). Reduced FAD can be divalently reoxidized by oxygen to produce hydrogen peroxide, or univalently reoxidized in two steps to generate two equivalents of superoxide radical (reviewed in [3,12–14]). Superoxide in particular has been identified as the probable reactive oxygen species that contributes to cardiac dysfunction in failing myocardium [1,2], reviewed [3–5].

Several studies in tissue culture, isolated hearts, laboratory animals and in cardiac patients have indicated that the XOR inhibitors, allopurinol and oxypurinol, are useful in preventing the formation of superoxide and improving cardiac function [1,2], reviewed in [3–5]. However, allopurinol by itself cannot prevent the generation of superoxide by XOR [15]. It is an efficient alternative substrate of XOR that must first be converted to oxypurinol, the actual inhibitor [16–22]. In contrast to allopurinol, which binds to the oxidized Mo^{VI}, oxypurinol binds to reduced Mo^{IV}. The reduced XOR–oxypurinol complex then slowly rearranges into a tightly bound inhibitory complex. Consequently, during the course of the reaction (with and without the physiological substrates) the rate of product formation prematurely decelerates as the enzyme becomes strongly inhibited by oxypurinol.

Although the inhibition appears to be irreversible, oxypurinol can slowly dissociate from XOR. Oxypurinol is referred to as a pseudo irreversible inhibitor that ''inactivates'' the enzyme.

In 1970 [18], allopurinol was shown to reduce cytochrome *C* while being oxidized by XO. A contemporary study demonstrated that the reduction of cytochrome *C* by xanthine and XO was mediated by superoxide [11]. Because allopurinol may be generating superoxide, we have studied the reaction of allopurinol with XO and confirmed that allopurinol does produce superoxide during its conversion to oxypurinol. We also examined the variables that determine the quantity of superoxide formed during the reaction.

2. Materials and methods

2.1. Materials

Xanthine, allopurinol, uric acid, 8-methyl xanthine, EDTA disodium salt and horse heart Cytochrome *C* type III were purchased from Sigma (St. Louis, MO). Oxypurinol was obtained from Sigma (St. Louis, MO) and from DSM Pharmaceuticals (Greenville, NC). Analytical grade phosphate buffer, acetonitrile, tetrabutylammonium hydrogen sulfate and trichloroacetic acid were purchased from Fisher. Amicon ultrafree centrifugal filter devices 5000 NMWL were from Millipore Corporation (Bedford, MA). Catalase and superoxide dismutase were purchased from Calbiochem (a brand of EMD Biosciences Inc., La Jolla, CA). XO was purified from unpasteurized cow's milk (obtained from the experimental dairy herd at the Ohio State University) using the modified [23] method of Massey et al. [24] and was kindly provided by Dr. Russ Hille of The Ohio State University, Department of Molecular and Cellular Biology, Columbus OH. A Cary 100 spectrophotometer equipped with a Peltier temperature control system and CaryWin software, was used for the spectrophotometric measurements (Varian, Quebec Canada). HPLC analyses used a Gilson system coupled to an Agilent PDA detector.

2.2. Methods

The XO-catalyzed conversion of xanthine to uric acid was monitored spectrophotometrically at 295 nm, $\Delta\varepsilon_{295} = 9.7\ mM^{-1}\ cm^{-1}$ [25]. Reactions were carried out in Buffer A (50 mM potassium phosphate buffer, pH 6.8, containing 0.1 mM EDTA and 200 U/ml catalase) unless otherwise indicated.

The production of superoxide was monitored by following the superoxide-dependent reduction of cytochrome *C* spectrophotometrically at 550 nm, $\Delta\varepsilon_{550} = 21\ mM^{-1}\ cm^{-1}$ [26]. The reactions were initiated with the addition of xanthine oxidase after the desired temperature was reached.

The conversion of allopurinol to oxypurinol was quantified by HPLC analysis. The concentrations of XO were varied and the initial allopurinol concentration was 10 or 20 μM. The reactions were quenched after product formation had reached a plateau using a 10% TCA solution that contained 12.5 μM 8-methyl xanthine as an internal standard. Protein was removed by centrifugation through the Amicon filters with a 5000 NMWL molecular weight cut-off. Samples were injected (25 μl) onto a Zorbax C8 column (150 × 4.5 mm) with a C8 guard and isocratically eluted with 2% acetonitrile in 50 mM potassium phosphate buffer (pH 5.0) with 0.5 mM tetrabutylammonium-hydrogensulfate, at a flow rate of 1 ml/min at 23 °C. Analytes were quantified by their UV absorbance at 260 nm. Standards and quality control samples contained 2.5–20 μM allopurinol and oxypurinol in Buffer A (without XO). The calibration curves for both analytes were linear (correlation coefficients = $R^2 > 0.99$). The precision of the method was within 10% CV and the accuracy ranged from −8 to +11% of nominal for oxypurinol and from −6 to +4% of nominal for allopurinol.

3. Results

3.1. Superoxide production by xanthine and allopurinol

The production of superoxide from XO was monitored spectrophotometrically by following the reduction of cytochrome *C* in reactions at pH 6.8 and 37 °C. The data in Fig. 1 shows that during the complete conversion of 20 μM xanthine to uric acid (confirmed by the direct spectral assay), approximately 5 μM superoxide was formed. This represents approximately 13% of the total electron flux.[1] At the end of the reaction with xanthine, the addition of 20 μM allopurinol resulted in a similar production of superoxide. However, as previously seen for the oxidation of allopurinol [16,19], the rate

[1] Each mole of substrate donates 2 mol of reducing equivalents. Thus, 20 μM xanthine represents 40 μM reducing equivalents, and 5 μM superoxide (univalent reduction of oxygen) is 13% of this total. Presumably, the remaining 35 μM reducing equivalents (87%) produced 17.5 μM H_2O_2 (divalent reduction of oxygen) superoxide.

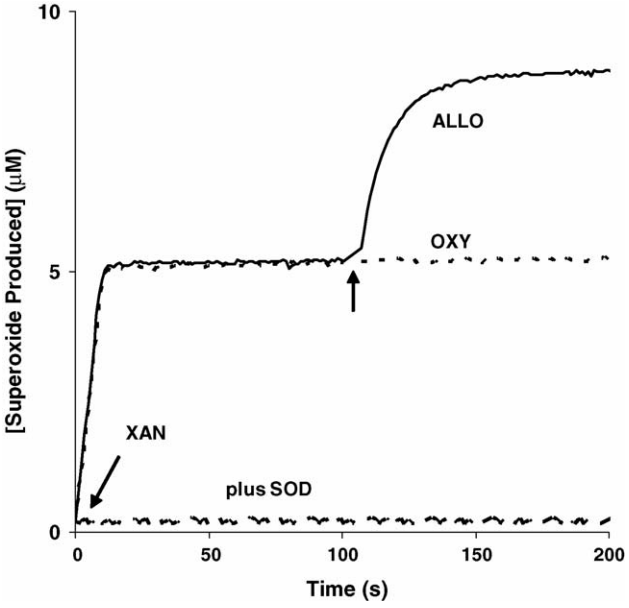

Fig. 1 – Production of superoxide radical during the XO-catalyzed oxidation of xanthine and allopurinol. Cuvettes contained Buffer A with 0.04 U/ml XO, 0.1 mM cytochrome C, and 20 μM xanthine (XAN). Reaction mixture also containing 300 U/ml SOD is labeled accordingly. Reference cuvettes contained the same components less XO. Reactions were initiated by adding XO (first arrow). The addition of 20 μM allopurinol (ALLO) or 20 μM oxypurinol (OXY) to the sample and reference cuvettes at 100 s is indicated by the second arrow. The final volume was 2.4 ml and the temperature was 37 °C.

of product formation prematurely slowed down due to the slow rearrangement of the complex of the reaction product (oxypurinol) with reduced XO into the tightly bound (''inactivated'') form. Under these conditions, approximately all of the allopurinol was converted to oxypurinol within the time course shown in Fig. 1. However, in some of the reactions described below, the conversion was incomplete. Note that in this experiment and in all others performed, oxypurinol never produced any detectable superoxide when incubated with XO. Furthermore, superoxide dismutase (SOD) always completely prevented the reduction of cytochrome C.

3.2. *Effect of allopurinol concentration*

The amount of superoxide produced by allopurinol and XO was dependent on several factors, including the concentration of allopurinol. Fig. 2 shows the amount of superoxide produced from 10 and 20 μM allopurinol. These concentrations were chosen for display because they clearly demonstrate the basic effects and are the maximum plasma concentrations that result from oral allopurinol therapy. Note that the concentration of XO was one-half that used in Fig. 1 and the temperature was lowered to 25 °C (see below for the effects of these variables). Under the conditions in Fig. 2, 10 and 20 μM allopurinol both produced approximately 2.8 μM superoxide and 10 μM oxypurinol (confirmed by HPLC analyses). After the formation of superoxide from 10 μM allopurinol reached a plateau (left panel), a second addition of XO did not increase the amount of superoxide formed, while the second addition of 10 μM allopurinol resulted in another burst of superoxide production. Therefore, all of the 10 μM allopurinol was converted to oxypurinol and XO was reoxidized before oxypurinol could form the tight inhibitory complex with all the reduced XO.

In contrast, in the reaction with 20 μM allopurinol, the second addition of allopurinol did not create another burst of superoxide production, while a second addition of XO enabled the reaction to go to completion. Clearly, the oxypurinol generated from 20 μM allopurinol greatly inhibited the reaction by the time only half of the allopurinol was converted to oxypurinol.

The data in Figs. 1 and 2 show that the complete conversion of 10 or 20 μM allopurinol to oxypurinol occurred with about 14% of the reducing equivalents being transferred to oxygen in univalent steps to generate superoxide. Moreover, 10 μM (data

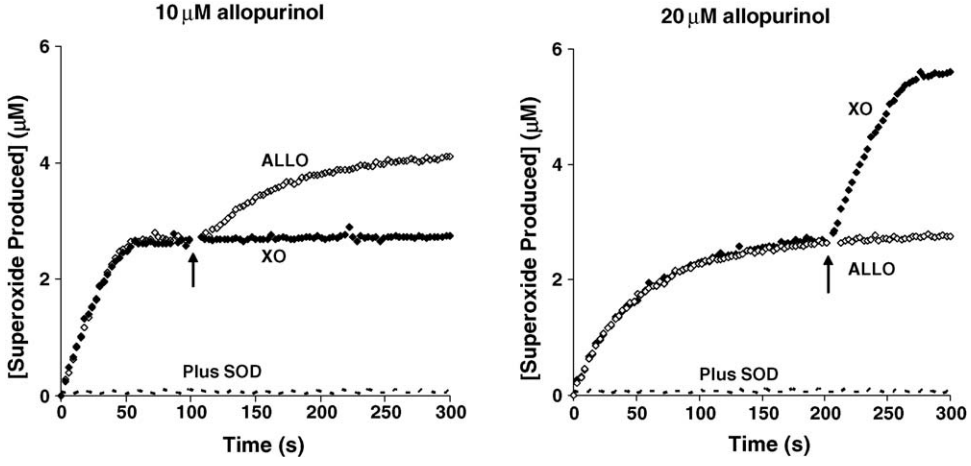

Fig. 2 – Effect of allopurinol concentration on superoxide radical production. Cuvettes contained Buffer A with 0.02 U/ml XO, 0.1 mM cytochrome C, and 10 μM allopurinol (left panel) or 20 μM allopurinol (right panel). The timing of addition of allopurinol (ALLO) or XO is indicated by the arrow. Reaction mixtures also containing 300 U/ml SOD are labeled accordingly. The final volume was 2.4 ml and the temperature was 25 °C. Other conditions are described in Fig. 1.

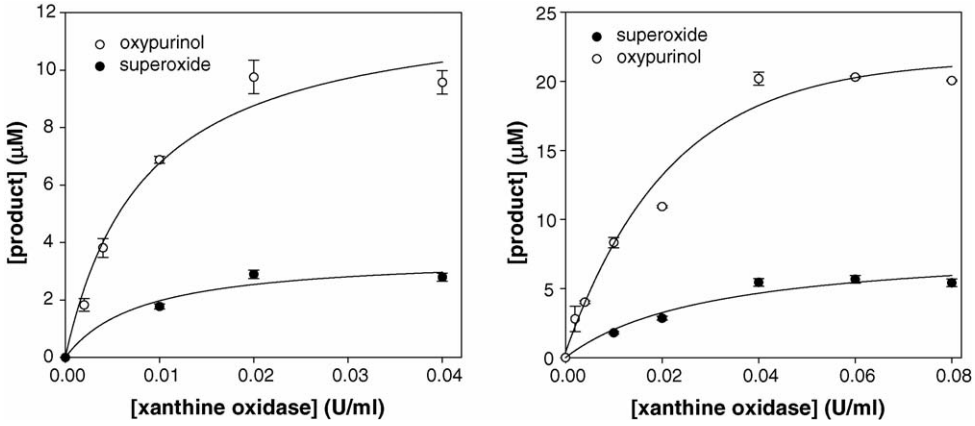

Fig. 3 – Effect of XO concentration on superoxide radical and oxypurinol production by allopurinol. Cuvettes contained Buffer A with 0.1 mM cytochrome *C*, 10 or 20 μM allopurinol and the indicated concentrations of XO. The temperature was 25 °C. Reactions were terminated with TCA (see Section 2.2) once the production of superoxide had reached a plateau. Superoxide was determined spectrophotometrically for reaction mixtures with ≥XO 0.01 U/ml as in Fig. 2. Oxypurinol production was determined by HPLC analysis. The values are the average of triplicate determinations. Other conditions are described in Fig. 1.

not shown) and 20 μM xanthine (Fig. 1) generated the same proportional amount (13–14% of the reducing equivalents) of superoxide.

3.3. *Effect of XO concentration*

The production of superoxide and oxypurinol from 10 and 20 μM allopurinol were studied at different XO concentrations at 25 °C and pH 6.8. Reactions were terminated and analyzed after the production of superoxide had reached a plateau. Fig. 3 shows that oxypurinol and superoxide are produced in proportional amounts with approximately 0.28 mol (14% of the total reducing equilavents) of superoxide generated for every mole of oxypurinol formed. Moreover, the amount of superoxide and oxypurinol produced from the reaction with 10 μM allopurinol was less than complete at 0.01 U/ml XO, but was complete at 0.02 and 0.04 U/ml. Superoxide and oxypurinol production from 20 μM allopurinol greatly increased with the XO concentration and reached complete formation at 0.04 U/ml XO.

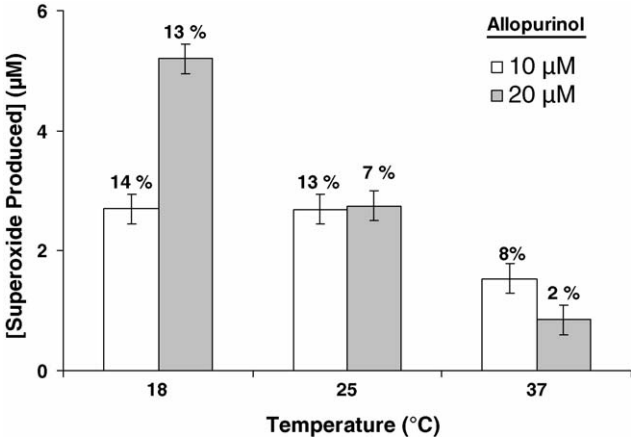

Fig. 4 – Effect of temperature on superoxide radical production by allopurinol. Cuvettes contained Buffer A with 0.1 mM cytochrome *C*, 10 or 20 μM allopurinol and 0.02 U/ml XO. Reaction temperatures are indicated. The final volume was 2.4 ml. The values are the average of triplicate determinations. The percent of the total reducing equivalents that reacted with oxygen to produce superoxide is shown above the error bars. Other conditions are described in Fig. 1.

3.4. *Effect of temperature*

Under the conditions described in Fig. 2, 10 and 20 μM allopurinol produced less superoxide at 37 °C than at 25 °C (Fig. 4). Ten micromolar allopurinol generated 1.7 μM superoxide before the product formation reached a plateau, indicating that allopurinol was nearly completely converted to oxypurinol and XO was nearly completely inhibited during the reaction. Twenty micromolar allopurinol produced only 0.8 μM superoxide before product formation reached a plateau. At 18 °C, the conversion of 20 μM allopurinol to oxypurinol was nearly complete. Thus, the earlier observation that the rate-constant for the rearrangement of the reduced XO–oxypurinol complex into the "inactivated" species is highly temperature dependent [18,21] is also confirmed with respect to superoxide formation.

3.5. *Effect of pH*

The production of superoxide by allopurinol was also studied at pH 7.2, 7.4, and 7.8 at 25 °C, using 0.04 U/ml XO to ensure complete conversion of allopurinol to oxypurinol (Fig. 5). Under these conditions, after the production of superoxide reached a plateau, adding more allopurinol produced another burst of superoxide formation, while adding XO had no effect, thus confirming the complete conversion of allopurinol to oxypurinol. The data in Fig. 5 show that allopurinol generated considerably more superoxide at higher pH. The amount of

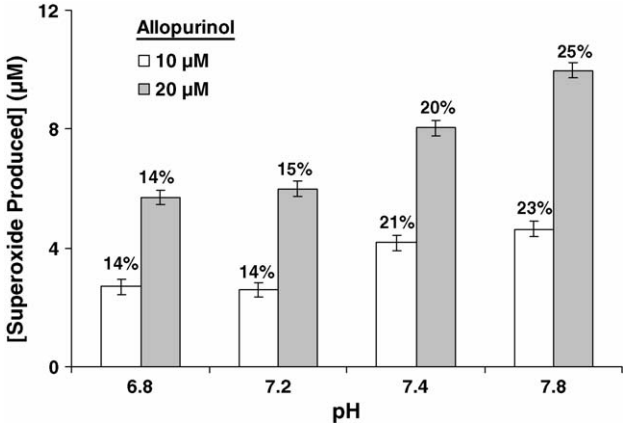

Fig. 5 – Effect of pH on superoxide radical production by allopurinol. Cuvettes contained Buffer A at the indicated pH with 0.04 U/ml XO, 0.1 mM cytochrome *C*, and 10 or 20 μM allopurinol. The final volume was 2.4 ml and the temperature was 25 °C. The values are the average of triplicate determinations. The percent of the total reducing equivalents that reacted with oxygen to produce superoxide is shown above the error bars. Other conditions are described in Fig. 1.

superoxide generated for 10 and 20 μM allopurinol at pH 7.8 was nearly double the amount formed at pH 6.8. Moreover, at pH 6.8, 7.2, 7.4, and 7.8, allopurinol and xanthine produced similar amounts of superoxide.

4. Discussion

The XO-catalyzed conversion of allopurinol to oxypurinol clearly generates superoxide during the reaction. Furthermore, the complete conversion of allopurinol to oxypurinol or xanthine to uric acid produced similar amounts of superoxide. Ironically, allopurinol is one of the inhibitors of XO commonly studied to prevent its production of superoxide, which is deleterious to inflamed tissue [1,2] (reviewed in [3–5]). We found that the amount of superoxide produced depended on the concentrations of allopurinol and XO as well as the temperature and pH of the reaction.

At 25 °C, pH 6.8, in reactions with 0.02 U/ml XO, 10 and 20 μM allopurinol produced the same amount of superoxide before the rate of product formation reached a plateau. In both cases, 10 μM oxypurinol was concomitantly formed. Interestingly, the enzyme exposed to 10 μM allopurinol retained sufficient activity to catalyze a second burst of superoxide production following a second addition of allopurinol. However, the enzyme from the reaction with 20 μM allopurinol was ''inactivated'' by the time the 10 μM oxypurinol was generated. A second addition of XO was required to complete the conversion. Apparently, 20 μM allopurinol is more capable than 10 μM allopurinol of sustaining XO in the reduced state and thereby allowing oxypurinol to form the tightly inhibited enzyme complex. The formation of this ''inactivated'' enzyme species requires that the Mo of the transport chain remain in the electronically reduced state (Mo^{IV}) while the XO complex with oxypurinol is transformed into the more tightly bound complex [16–22]. The amount of ''inactivated'' enzyme is determined by the rate of reoxidation of the enzyme-bound Mo versus the rate of rearrangement of the reduced XO–oxypurinol complex into the ''inactivated'' species. Moreover, the rate-constant of the formation of the tight complex is highly temperature dependent [16,18,21]. Therefore, raising the temperature to 37 °C tilted the advantage to ''inactivation'' over superoxide formation, resulting in the production of less oxypurinol and less superoxide. Lowering the temperature to 18 °C gave the advantage to superoxide formation and allowed for the nearly complete conversion of even 20 μM allopurinol to oxypurinol and the maximum formation of superoxide prior to ''inactivation''.

Complete conversion of 20 μM allopurinol to oxypurinol and maximum superoxide production could also be achieved in reactions at 37 °C by increasing the concentration of XO to 0.04 U/ml. Therefore, the higher XO concentration overrode the capacity of 20 μM allopurinol to retain enzyme in the electronically reduced state during the reaction. Conversely, in the presence of 0.01 U/ml XO, less than complete conversion of allopurinol to oxypurinol and less superoxide was generated even in reactions with 10 μM allopurinol at 25 °C. Thus, at lower concentrations of XO, the lower concentration of allopurinol was capable of sustaining the enzyme in the reduced state.

The pH of the reaction was found to be another important factor. At pH 6.8, 14% of the reducing equivalents from either allopurinol or xanthine reacted with oxygen univalently to produce superoxide. This value nearly doubled at pH 7.8. Fridovich has previously shown that the amount of superoxide generated from the oxidation of xanthine by XO increases with pH [27]. Thus, these data provide more evidence that allopurinol is a conventional substrate of XO.

It would be difficult to speculate how much superoxide would be produced by allopurinol in vivo or whether the superoxide generated from allopurinol contributes to tissue pathology. However, it is known that XOR is significantly elevated in failing cardiac tissue [1,28–31]. We also know that patients treated with allopurinol excrete about 50–70% of the dose as oxypurinol and less than 10% as allopurinol [32,33]. Thus, the vast majority of allopurinol is converted to oxypurinol, which could result in significant superoxide production. Because oxypurinol does not generate superoxide, it may be the drug of choice to prevent superoxide production from XOR.

Oxypurinol may have another advantage over allopurinol. Because the physiological substrates (xanthine and hypoxanthine) and allopurinol compete for binding to oxidized XO, high concentrations of these substrates can impede the binding of allopurinol and prevent the generation of oxypurinol [15]. In contrast, the physiological substrates aid the binding of oxypurinol by generating the reduced enzyme species to which it binds. Consequently, in the presence of high concentrations of xanthine and hypoxanthine, oxypurinol is considerably more effective than allopurinol as an inhibitor to superoxide production [15].

In conclusion, allopurinol can generate superoxide during its conversion to oxypurinol as catalyzed by XO. Moreover, although both allopurinol and oxypurinol are inhibitors of

XOR that can block the formation of uric acid (reviewed in [20]), only oxypurinol can inhibit the formation of superoxide.

Acknowledgements

We gratefully acknowledge Dr. Russ Hille for providing the xanthine oxidase used in these studies and for helpful discussion and Dr. Alan Ezrin for contributions and guidance.

REFERENCES

[1] 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 (epub ahead of print).
[2] 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;95:1005–11.
[3] Berry CE, Hare JM. Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. J Physiol 2004;555:589–606.
[4] Doehner W, Anker SD. Xanthine oxidase inhibition for chronic heart failure: is allopurinol the next therapeutic advance in heart failure? Heart 2005;91:707–9.
[5] Kittleson MM, Hare JM. Xanthine oxidase inhibitors: an emerging class of drugs for heart failure. Eur Heart J 2005;26:1458–60.
[6] Krenitsky TA, Tuttle JV. Xanthine oxidase activities: evidence for two catalytically different types. Arch Biochem Biophys 1978;185:370–5.
[7] Sanders SA, Eisenthal R, Harrison R. NADH oxidase activity of human xanthine oxidoreductase — generation of superoxide anion. Eur J Biochem 1997;245:541–8.
[8] Stirpe F, Della CE. The regulation of rat liver xanthine oxidase. Conversion in vitro of the enzyme activity from dehydrogenase (type D) to oxidase (type O). J Biol Chem 1969;244:3855–63.
[9] Waud WR, Rajagopalan KV. Purification and properties of the NAD^+-dependent (type D) and O_2-dependent (type O) forms of rat liver xanthine dehydrogenase. Arch Biochem Biophys 1976;172:354–64.
[10] Harris CM, Massey V. The reaction of reduced xanthine dehydrogenase with molecular oxygen. Reaction kinetics and measurement of superoxide radical. J Biol Chem 1997;272:8370–9.
[11] McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969;244:6049–55.
[12] Hille R. Molybdenum-containing hydroxylases. Arch Biochem Biophys 2005;433:107–16.
[13] Massey V, Harris CM. Milk xanthine oxidoreductase: the first one hundred years. Biochem Soc Trans 1997;25:750–5.
[14] Bray RC. The Enzymes. Part XII New York: Academic Press; 1975. p. 303–387.
[15] Spector T. Oxypurinol as an inhibitor of xanthine oxidase-catalyzed production of superoxide radical. Biochem Pharmacol 1988;37:349–52.
[16] Elion GB. Enzymatic and metabolic studies with allopurinol. Ann Rheum Dis 1966;25:608–14.
[17] Massey V, Komai H, Palmer G, Elion GB. On the mechanism of inactivation of xanthine oxidase by allopurinol and other pyrazolo[3,4-d]pyrimidines. J Biol Chem 1970;245: 2837–44.
[18] Spector T, Johns DG. Stoichiometric inhibition of reduced xanthine oxidase by hydroxypyrazolo [3,4-d]pyrimidines. J Biol Chem 1970;245:5079–85.
[19] Spector T, Johns DG. 4-Hydroxypyrazolo (3,4-d)pyrimidine as a substrate for xanthine oxidase: loss of conventional substrate activity with catalytic cycling of the enzyme. Biochem Biophys Res Commun 1970;38:583–9.
[20] Spector T. Inhibition of urate production by allopurinol. Biochem Pharmacol 1977;26:355–8.
[21] Spector T, Hall WW, Krenitsky TA. Human and bovine xanthine oxidases. Inhibition studies with oxipurinol. Biochem Pharmacol 1986;35:3109–14.
[22] Williams JW, Bray RC. Kinetic and e.p.r. studies on the inhibition of xanthine oxidase by alloxanthine (1H-pyrazolo [3,4-d]pyrimidine-4,6-diol). Biochem J 1981;195:753–60.
[23] Kim JH, Ryan MG, Knaut H, Hille R. The reductive half-reaction of xanthine oxidase. The involvement of prototropic equilibria in the course of the catalytic sequence. J Biol Chem 1996;271:6771–80.
[24] Massey V, Brumby PE, Komai H. Studies on milk xanthine oxidase. Some spectral and kinetic properties. J Biol Chem 1969;244:1682–91.
[25] Avis PG, Bergel F, Bray RC. Cellular constituents. The chemistry of xanthine oxidase. Part III. Estimation of the cofactors and the catalytic activities of enzyme fractions from cow's milk. J Chem Soc 1956;1219–26.
[26] Massey V. The microestimation of succinate and the extinction coefficient of cytochrome C. Biochim Biophys Acta 1959;34:255–6.
[27] Fridovich I. Quantitative aspects of the production of superoxide anion radical by milk xanthine oxidase. J Biol Chem 1970;245:4053–7.
[28] Cappola TP, Kass DA, Nelson GS, Berger RD, Rosas GO, Kobeissi ZA, et al. Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. Circulation 2001;104:2407–11.
[29] de Jong JW, Schoemaker RG, de Jonge R, Bernocchi P, Keijzer E, Harrison R, et al. Enhanced expression and activity of xanthine oxidoreductase in the failing heart. J Mol Cell Cardiol 2000;32:2083–9.
[30] Ekelund UE, Harrison RW, Shokek O, Thakkar RN, Tunin RS, Senzaki H, et al. Intravenous allopurinol decreases myocardial oxygen consumption and increases mechanical efficiency in dogs with pacing-induced heart failure. Circ Res 1999;85:437–45.
[31] 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;59:582–92.
[32] Elion GB, Kovensky A, Hitchings GH. Metabolic studies of allopurinol, an inhibitor of xanthine oxidase. Biochem Pharmacol 1966;15:863–80.
[33] Reiter S, Simmonds HA, Zollner N, Braun SL, Knedel M. Demonstration of a combined deficiency of xanthine oxidase and aldehyde oxidase in xanthinuric patients not forming oxipurinol. Clin Chim Acta 1990;187: 221–34.