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Role of xanthine oxidoreductase in cardiac nitroso-redox imbalance

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Abstract

Emerging evidence supports the importance of nitroso-redox balance in the cardiovascular system. Xanthine oxidoreductase (XOR) is a major oxidative enzyme and increased XOR activity, leading to both increased production of reactive oxygen species and uric acid, is implicated in heart failure. Within the heart, XOR activity stimulates cardiomyocyte hypertrophy, apoptosis, and impairs matrix structure. The underpinnings of these derangements can be linked not solely to oxidative stress, but may also involve the process of nitroso-redox imbalance. In this regard, XOR interacts with nitric oxide signaling at numerous levels, including a direct protein-protein interaction with neuronal nitric oxide synthase (NOS1) in the sarcoplasmic reticulum. Deficiency or translocation of NOS1 away from this microdomain leads to increased activity of XOR, which in turn impairs excitation-contraction coupling and myofilament calcium sensitivity. There is a mounting abundance of preclinical data supporting beneficial effects of inhibiting XOR, but translation to the clinic continues to be incomplete. A growing understanding of XOR and its role in nitroso-redox imbalance has great potential to lead to improved pathophysiologic insights and possibly therapeutic advances.

Keywords

Xanthine Oxidoreductase; Nitric Oxide; Nitroso-Redox Imbalance; Heart Failure; S-nitrosylation; Review

2. INTRODUCTION

Heart failure (HF) is an important cause of morbidity and mortality worldwide (1, 2), and currently represents the leading cause of hospitalization in individuals older than 65 years (2, 3). Despite the significant progress in its management, 5-year mortality rates are still as high as 50% and are even greater in the more advanced stages of the disease (1, 2). The prevalence of HF increases with age and therefore the number of patients with HF is expected to rise with the progressive aging of the population (3). The improved survival of patients with myocardial infarction (MI) is an added driver of the increasing prevalence of HF (1, 4).

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The regulated production of reactive oxygen species (ROS) is crucial for cellular homeostasis (5). In this regard, there is accumulating evidence that ROS play signaling roles and participate in the regulation of many cellular functions (5). However, oxidative stress denotes a state of imbalance between the production of ROS and antioxidant mechanisms in which the former prevail over the latter (6). Oxidative stress is intimately involved in the pathogenesis of HF, regardless of its underlying cause (coronary heart disease or non-ischemic cardiomyopathy) (7). Elevated levels of a variety of markers of oxidative stress have been reported in both plasma and pericardial fluid in patients with HF and positively correlate with the degree of left ventricular dilation and the severity of HF (8–11).

The imbalance between ROS production and antioxidant mechanisms leads to myocyte dysfunction, injury and necrosis resulting in the development and progression of HF (7). ROS reduce Ca^{+2} entry in the cardiomyocytes, attenuate myocardial contractility, and augment cardiomyocyte apoptosis (5, 12). Oxidative stress also contributes to the development of HF by inducing heart remodeling (13). ROS are potent stimulators of matrix metalloproteinases (MMP), which in turn mediate left ventricular (LV) remodeling (13–19).

A variety of sources contribute to the increased ROS production in HF, including xanthine oxidoreductase (XOR) (20, 21), the mitochondria (22, 23), nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (24–26) and uncoupled nitric oxide synthase (NOS, both neuronal (NOS1) and endothelial (NOS3)) (27, 28) (Figure 1). In contrast, reduced levels of antioxidant mechanisms have been reported in these patients and are inversely associated with the stage of HF (11, 20, 29). Among oxidative enzymes, XOR has been the focus of significant research as a principal culprit participating in the propagation of oxidative stress in HF (20). Here we review existing data on the role of XOR in the pathogenesis of HF and cardiac remodeling, and address the potential therapeutic implications of XOR inhibition.

3. XOR – BIOCHEMISTRY AND FUNCTION

Among oxidative enzymes, XOR is a key regulator of oxidative stress (30). XOR belongs to the molybdoenzyme family and exists as a homodimer in 2 potentially interconvertible forms, xanthine dehydrogenase (XDH) and xanthine oxidase (XO) (30, 31). Each monomer consists of a molybdenum center, 2 distinct iron-sulfur centers and a flavin adenine dinucleotide (FAD) center (30, 31) (Figure 2). XOR catalyzes the conversion of hypoxanthine to xanthine and xanthine to uric acid (30). During XOR reoxidation, both hydrogen peroxide (H_2O_2) and superoxide (O_2^-) are produced (30) (Figure 3). XO appears to be more potent ROS generator than XDH since both can react with either NAD^+ or molecular oxygen but the reactivity of XO toward NAD^+ is negligible (32). However, XDH can be converted to XOR either by a reversible process involving thiol oxidation or by proteolytic cleavage (30).

O_2^- was identified as a product of XOR activity in 1968 and is a potent free radical produced by the 1-electron reduction of molecular oxygen (33). Except from its direct effects, O_2^- can also transform to other potent ROS (5, 34). O_2^- can react with H_2O_2 leading to the formation of hydroxyl radicals (OH^-) (Haber-Weiss reaction) (34). Production of OH^-

through this reaction has also been demonstrated in the failing myocardium (35). O_2^- can also generate peroxynitrite by interacting with NO (5). O_2^- is converted by superoxide dismutase (SOD) to H_2O_2, which is less potent but can also exert cardiotoxic effects (5, 36). H_2O_2 can also convert to OH^- through the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow OH^- + OH^- + Fe^{3+}$) and this conversion was shown to occur in myocardial tissue obtained from animal models of heart failure (22, 36).

4. XOR IS UPREGULATED IN HF

An upregulation of cardiac XOR expression and activity has been consistently reported in various animal models of HF (28, 37–40) and in humans with HF (21). This upregulation could be a result of the activation of the renin-angiotensin system – a hallmark of HF (41) – since angiotensin II appears to stimulate XOR (42). Moreover, HF is characterized by increased circulating levels of proinflammatory cytokines (43, 44), which could also augment XOR activity (45, 46). Hypoxia also stimulates XOR activity (47–49) and might therefore play a role in XOR upregulation in HF, particularly in patients with ischemic cardiomyopathy.

5. EFFECTS OF XOR IN MYOCARDIAL CONTRACTILITY

5.1. *Ex vivo* studies

Whether XOR is implicated in the pathogenesis of HF and the specific mechanisms involved have been evaluated in several studies. XOR-produced O_2^- diminishes maximal Ca^{2+}-activated force in isolated cardiac myofilaments (50). Moreover, XOR inhibition with allopurinol augments contractile force (51). A XOR-induced decrease in the Ca^{2+} sensitivity of cardiac myofilaments could help explain this negative inotropic effect of XOR (51–53). Furthermore, XOR reduces myofibrillar Ca^{2+}-ATPase activity (54). An increase in the maximal force-generating capacity of cardiomyocytes may also contribute to the positive inotropic action of XOR inhibition (55). Maximal force-generating capacity is greatly reduced in ventricular muscle isolated from rats with HF (56). It should also be noted that allopurinol had no significant effect in the contractility of ventricular trabeculae isolated from normal hearts (55). This finding supports the importance of XOR upregulation in HF in the pathogenesis of myocardial dysfunction (55). In addition to O_2^-, OH^-, produced by the interaction of O_2^- with H_2O_2, can also reduce myocardial contractility (13, 57).

5.2. *In vivo* studies

The negative inotropic effects of XOR in the *in vitro* studies were also assessed in animal models of HF. In an early experiment from our lab, acute inhibition of XOR with allopurinol (200 mg infused as 3.3. mg/min in the right atrium) improved myocardial contractility in dogs with pacing-induced HF (38). These observations were subsequently reproduced by other investigators (58). In accordance with the results of the *ex vivo* studies, allopurinol did not substantially modulate the contractility of normal hearts (38, 58).

In order to decipher the mechanisms accounting for these beneficial actions of XOR inhibition, we compared the effects of allopurinol (200 mg given as 6.6. mg/min intravenously for 30 min) with intravenously administered vitamin C – an antioxidant agent

– in dogs with pacing-induced HF (28). Allopurinol exerted a positive inotropic effect to a degree equivalent to vitamin C (28). In addition, when allopurinol was infused immediately after vitamin C, it had no additional effect on myocardial contractility (28). These findings supported the idea that XOR-induced oxidative stress is primarily responsible for the detrimental effects of XOR on myocardial systolic function and that the latter effects can be abrogated with allopurinol treatment (28).

In a genetic model of dilated cardiomyopathy, the spontaneously hypertensive/HF (SHHF) rat, oxypurinol – the active metabolite of allopurinol – when administered po for 4 weeks (1 mmol/l in drinking water) improved fractional shortening and increased LV ejection fraction (LVEF) (59). Again, these effects of oxypurinol appeared to result from the amelioration of XOR-induced oxidative stress (59). Indeed, SHHF rats showed an increase in oxidative stress and more specifically, in the production of O_2^- in their cardiomyocytes; this altered redox state was restored with oxypurinol treatment (59). We also observed that oxidative stress was primarily due to increased XOR activity whereas NADPH oxidase activity was similar in HF and control rats (59).

5.3. Mechanisms involved in the negative inotropic action of XOR

Overall, there is now considerable evidence supporting the importance of XOR in the impairment of myocardial contractility in HF. XOR-induced oxidative stress emerges as the principal mechanism of this effect of XOR. We will next discuss in more detail the putative mechanisms that could contribute to this negative inotropic action of XOR-induced oxidative stress. We will schematically divide them in effects on myocardial energetics, Ca^{+2} cycling and structural changes in the myofilaments.

5.3.1. Myocardial energetics—XOR could contribute to the development of HF through its adverse effects on myocardial energetics (60). Phosphocreatine (PCr) is the principal energy reserve molecule in the heart (61). Creatine kinase (CK) uses PCr as a substrate in order to produce ATP, the cellular energy “currency” (61). HF is characterized by a depletion of both ATP and PCr and the reduction in the levels of these energy molecules correlates directly with the severity of HF (62). The PCr/ATP ratio is also reduced in HF, suggesting an imbalance between energy demand and supply (63). In patients with dilated cardiomyopathy, the decreased PCr/ATP ratio represents an independent predictor of mortality (64). Energy depletion in the failing myocardium appears to result principally from a reduction in CK activity (65, 66). In turn, inhibition of CK activity in isolated rat hearts diminishes contractile reserve (67, 68) and it has been proposed that the same may apply in the failing human heart (61). Therefore, a tight link appears to exit between energy starvation in the failing myocardium and the development and progression of heart failure (61).

A number of *ex vivo* studies supports a role for XOR in the impairment of myocardial energetics in HF (36, 57). XOR reduces CK activity in rat hearts *in vitro* through the production of O_2^- (36). H_2O_2 also suppressed CK activity in the same report (36). Moreover, OH^- reduced the ATP content of cardiomyocytes *in vitro* (57).

There are preliminary findings that suggest that XOR inhibition can prevent the deterioration of myocardial energy status and thus prevent the development of HF. Treatment with either allopurinol or oxypurinol (0.5. mmol/l and 1 mmol/l in the drinking water for 4 weeks, respectively) after the induction of MI in mice prevented the decrease in the PCr/ATP ratio (60). This resulted in attenuation of ventricular dilation and of the fall in LVEF (60).

It has been proposed that energy depletion in the failing human heart has also deleterious consequences on mechanical efficiency (61). In isolated rat hearts, inhibition of CK activity induces mechanoenergetic uncoupling (67, 68). Mechanoenergetic uncoupling is a core characteristic of HF and refers to a mismatch between the depressed myocardial contractility and the energy consumption, which is disproportionately high (38, 69). The pivotal role of XOR in the regulation of myocardial energetics is supported by the findings of several studies that evaluated the effects of XOR inhibition on mechanical efficiency. We showed that allopurinol consistently improves mechanical efficiency in animal models of HF (28, 38). In dogs with pacing-induced HF, acute inhibition of XOR with allopurinol increased myocardial contractility; at the same time, a reduction in myocardial oxygen consumption was observed and therefore an improvement in mechanical efficiency was achieved with allopurinol treatment (38). Again, attenuation of XOR-induced oxidative stress appears to be implicated in this improvement in myocardial energetics with allopurinol treatment (28). Indeed, in dogs with pacing-induced HF, allopurinol and vitamin C ameliorated mechanoenergetic uncoupling to a similar extent (28). In addition, when allopurinol was infused immediately after vitamin C, it did not yield any further improvement in cardiac mechanical efficiency (28).

The amelioration of myocardial energetics with allopurinol appears paradoxical in view of its positive inotropic action. Conventional positive inotropic agents disproportionately increase myocardial energy consumption and further aggravate the mechanoenergetic uncoupling in the failing heart (69). In dogs with HF, we reported that dobutamine improves contractility but at the same time induces a significant increase in myocardial oxygen consumption culminating in a decrease in mechanical efficiency (38) (Figure 4). A similar effect in humans was theorized as an explanation for the increased morbidity and mortality of patients with HF treated with positive inotropic agents (70, 71). The Ca^{2+}-sensititizing effect of XOR-inhibition was proposed to account for the improvement in mechanoenergetic uncoupling with allopurinol despite the increase in contractility (39).

5.3.2. Ca^{2+} cycling—Perturbations in physiological cardiomyocyte Ca^{+2} cycling might be involved in the pathogenesis of HF (39, 72). The major players in Ca^{+2} cycling are the cardiac calcium release channel (ryanodine receptor, RyR), the sarcoplasmic reticulum (SR) Ca^{2+}-ATPase 2alpha (SERCA2alpha)/phospholamban (PLB) complex and the Na^{+}-Ca^{+2} transporter (NCX) (72) (Figure 5). RyR is responsible for the release of Ca^{+2} from the SR in response to the influx of Ca^{+2} through the L-type (or voltage dependent) Ca^{+2} channels (LTCC) (72). SERCA2alpha controls Ca^{2+} uptake in to the SR during diastole after its release from the myofilaments (72). SERCA2alpha activity is directly associated with cardiac contractility (72). PLB is another integral protein of the SR and plays an important role in the regulation of cardiac contractility (72). PLB inhibits SERCA in the

unphosphorylated state; in contrast, phosphorylation of PLB is associated with enhanced SERCA activity (72). NCX participates in diastolic Ca^{2+} extrusion in the extracellular space (72).

HF is characterized by abnormalities in the abundance and/or the activity of all major Ca^{+2} cycling proteins. More specifically, impairment in the RyR function has been reported in human HF resulting in uncoupling with the LTCC (73). SERCA2alpha levels and activity are reduced in human HF and result in both systolic and diastolic dysfunction (74, 75). In addition, PLB phosphorylation is impaired in HF and this leads to a further suppression of SERCA2alpha function (75). Regarding NCX, an upregulation in its expression has been reported in the failing human myocardium (74). It has been hypothesized that the subsequent increased Ca^{2+} extrusion in the extracellular space combined with a reduced SR Ca^{2+} uptake contributes to the pathogenesis of HF (76).

XOR appears to affect Ca^{2+} cycling. Early studies showed that XOR inhibition blunts Ca^{2+} amplitude (51). It was suggested that this effect might stem from increased binding of Ca^{2+} to the myofilaments and that this decrease in intracellular Ca^{2+} concentration may be beneficial (51). More recent studies offer new insights in the interaction of XOR with Ca^{2+} cycling proteins. We found that SERCA levels decrease whereas NCX levels increase in SHHF rats during the development of HF; more importantly, oxypurinol normalized NCX levels and blunted the reduction in SERCA levels (59). In order to identify the level where XOR controls the synthesis of Ca^{2+}-cycling proteins, we studied the effects of XOR inhibition in dogs with pacing-induced HF (77). Allopurinol treatment (100 mg po daily) during the HF-induction period attenuated the decrease in myocardial contractility (77). In accordance with our earlier findings in mice, allopurinol attenuated the increase in NCX mRNA and protein levels in dogs as well (77). Furthermore, allopurinol prevented the upregulation of PLB mRNA and protein levels and the fall in phosphorylated PLB levels (77). In rats with established diastolic HF, XOR inhibition with oxypurinol also increased phosphorylated PLB levels (40). Therefore, it is intriguing to speculate that XOR-derived ROS could influence transcription of genes encoding the synthesis of Ca^{2+}-cycling proteins. In addition, it controls Ca^{2+}-cycling by post-translational mechanisms as well, such as the regulation of PLB activity by affecting its phosphorylation.

5.3.3. Structural changes in the myofilaments—XOR upregulation in HF also has detrimental effects on myofilament structure that apparently contribute to its negative inotropic action. In mice with ischemic cardiomyopathy, allopurinol reduced the levels of 4-hydroxy-2-nonenal (HNE)-modified myocardial proteins (39). HNE is a lipid peroxidation product that can disrupt the structure of myocardial proteins (78, 79). In another study, transgenic mice with truncated troponin I, a model of dilated cardiomyopathy, exhibited a 3-fold increase in XOR activity compared with wild type littermates (80). These transgenic mice showed excessive oxidative damage in myofibrillar proteins, particularly actin, that was attenuated with allopurinol treatment (26 mg/dl in the drinking water for 2 months) (80). In the same report, XOR inhibition with allopurinol restored cardiac muscle force in isolated cardiac muscle and prevented LV dilation (80). These findings support the notion that the myofibrillar damage induced by XOR is implicated in the pathogenesis of contractile dysfunction in HF.

6. CARDIAC REMODELING – THE ROLE OF XOR

Cardiac remodeling is the intermediate step in the development of overt HF regardless of its underlying cause (81). The principal mechanisms leading to remodeling are cardiomyocyte hypertrophy and apoptosis as well as alterations in matrix structure (81). Oxidative stress is intimately implicated in the development of cardiac remodeling by stimulating all 3 pathways (13). Among the various sources of ROS, XOR is a key factor in the development of remodeling. Several studies showed that allopurinol prevents LV remodeling both after experimental MI in mice (39, 60, 82) as well as in established HF (59, 83).

We will next briefly discuss the pathophysiologic mechanisms culminating in cardiac remodeling, with a specific focus on the existing data implicating XOR in these pathways.

6.1. Hypertrophy

Cardiac hypertrophy represents, to some extent, a physiological adaptive response to hemodynamic stress (84). However, it might also progress to overt HF and is associated with increased cardiovascular morbidity and mortality in the general population (84, 85). Significant progress has been made during the last decade in elucidating the signaling pathways involved in the pathogenesis of myocardial hypertrophy (86). In this labyrinth of interacting control mechanisms, the mitogen-activated protein kinase (MAPK) pathway appears to have a prominent role (86). The final effectors in this system are p38, c-Jun N-terminal kinases (JNK) and extracellular signal-regulated kinases (ERK) (86). Among these, ERK was proposed to be particularly significant in terms of regulating cardiac hypertrophy (86, 87).

Recent studies propose a role for XOR in the stimulation of ERK-induced myocardial hypertrophy (Figure 6). In isolated rat cardiomyocytes exposed to hypoxia-reoxygenation, there was an upregulation of XOR activity and the resulting oxidative stress induced ERK phosphorylation (88). In addition, allopurinol dose-dependently reduced this ERK activation (88). OH^- also induce myocardial hypertrophy after experimental MI in mice (13). In Dahl salt-sensitive hypertensive rats with established diastolic HF, an increase in ERK phosphorylation was noticed that was attenuated with oxypurinol treatment (40). In SHHF rats, we also reported an increased phosphorylated/unphosphorylated ERK ratio and a restoring of this imbalance with oxypurinol (59). More importantly, in the same study, XOR inhibition induced a regression in cardiomyocyte hypertrophy and reduced LV mass (59) (Figure 7). These preliminary observations implicate XOR in the pathophysiology of cardiac hypertrophy through the activation of ERK.

Cardiac hypertrophy is also characterized by an upregulation of a series of genes that is collectively characterized as the fetal gene program and includes atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), beta-isoform of myosin heavy chain (beta-MHC) and the alpha skeletal muscle isoform of actin (alphaSA) (89). This overexpression might represent a causative agent for myocardial hypertrophy (89). We found that mRNA levels of all these genes are increased in SHHF rats with established HF; we also showed that oxypurinol blunted the upregulation of ANP and alphaSA and completely normalized BNP and beta-MHC expression (59).

6.2. Apoptosis

In animal models of heart failure, apoptotic cardiomyocyte death is a precursor of overt heart failure (90). Increased apoptosis of cardiomyocytes is also frequently observed in patients with HF and may play a role in its progression (91, 92). Our knowledge of the signaling routes that regulate cardiomyocyte apoptosis has increased exponentially during the last decade (84). These routes are often identical or tightly linked to the ones controlling myocardial hypertrophy (84, 86). Thus, apoptosis signal-regulating kinase 1 (ASK1), which stimulates cardiomyocyte apoptosis and contributes to the pathogenesis of LV remodeling (93, 94), belongs to the above-mentioned MAPK family and is a selective upstream activator of p38 and JNK (86, 93, 95). Both p38 and JNK have also been implicated as apoptosis-promoting kinases, although the existing data are conflicting (86, 96). The final effectors of apoptotic cell death are caspases, a group of proteolytic enzymes, which are under the control of the Bcl-2 family of proteins (97, 98). The Bcl-2 family includes members with both pro- and anti-apoptotic actions (97, 98). Among the former, the Bax subfamily was recently shown to interact with p38 resulting in the induction of cardiomyocyte apoptosis (99).

Oxidative stress is intimately involved in the stimulation of apoptotic death of cardiomyocytes (12, 90). XOR was also shown to stimulate apoptosis of rat cardiomyocytes *in vitro* by increasing Bax mRNA levels (100). Oxidative stress was also shown to activate ASK (95, 101) and ASK appears to be essential for the oxidative stress-induced apoptotic cell death (94, 95). In Dahl salt-sensitive hypertensive rats with established diastolic HF, an increase in ASK1 phosphorylation has been reported and was reversed with oxypurinol treatment (40). Moreover, XOR inhibition prevented LV dilation and improved the survival rate in the same study (40). It is apparent that more studies are necessary in order to clarify the role of ASK in XOR-mediated prevention of cardiac remodeling.

6.3. Alterations in matrix structure

In the heart, extracellular matrix is not a static skeleton but a dynamic system (102–104). In contrast, it has become clear that altered matrix turnover is an active component of the process of cardiac remodeling (102–104). MMP is a family of enzymes that includes more than 20 members (collagenases, gelatinases and stromelysins) and is the major regulator of extracellular tissue breakdown (102–104). In this context, it is of interest that XOR activates MMP-2, MMP-9 and MMP-13 in cardiac fibroblasts and vascular smooth muscle cells *in vitro* (16, 17). OH^- is also a major player in the pathogenesis of post-MI remodeling in mice, possibly by activating MMP-2 (13). Interestingly, myocardial MMP-2, MMP-9 and MMP-13 protein levels and activity are elevated in patients with HF compared with controls (19, 105, 106). In addition, in animal models, non-selective inhibition of MMP activity prevented the development of cardiac remodeling (15, 18). MMP-9 knockout mice are protected from post-MI remodeling (107) whereas transgenic mice overexpressing MMP-2 develop advanced remodeling (108). Preliminary studies in humans also suggest that suppression of MMP-2 and MMP-9 expression in patients with acute MI prevents remodeling (109). Therefore, the *in vitro* stimulatory effects of XOR on MMP-2, MMP-9 and MMP-13 could be implicated in the beneficial effects of XOR-inhibitions on cardiac remodeling.

7. ISCHEMIC CARDIOMYOPATHY – THE ROLE OF XOR

The impact of XOR inhibition in ischemic cardiomyopathy deserves special mention, since this is the commonest cause of HF (4, 110). There is considerable evidence suggesting that allopurinol preserves myocardial contractility and improves survival after experimental MI in mice (39, 60, 82) (Figure 8). Interestingly, an increased XOR activity was also demonstrated in the remote LV myocardium in these reports (39, 82). Even more importantly, in rats with established ischemic HF, chronic administration of allopurinol (50 mg/kg/day po for 10 weeks) induced reverse remodeling and decreased LV weight and fibrosis (83). Therefore, XOR inhibition seems not only to prevent the development of HF after MI but is also effective in stimulating reverse remodeling in established ischemic HF.

8. DIASTOLIC DYSFUNCTION AND XOR

It has already been mentioned that XOR inhibition exerts a Ca^{2+} sensitizing action in cardiac myofilaments (39, 51–53). One could anticipate impairment in diastolic relaxation as a result of this effect; indeed, other Ca^{2+} sensitizing agents induce diastolic dysfunction by lowering the threshold of $(Ca^{2+})_I$ where myocardial contraction is activated (111). In contrast, XOR inhibition does not shift the range of Ca^{2+} activation and can actually improve diastolic dysfunction (39, 40). In an early study in mice with post-MI HF, allopurinol improved diastolic relaxation (39). In a more recent report, Dahl salt-sensitive hypertensive rats with established diastolic HF were treated with oxypurinol (40 mg/kg per day for 4 weeks) (40). XOR inhibition reduced interstitial fibrosis, prevented LV dilation and the survival rate of rats treated with this agent was significantly ($p < 0.01$) greater than rats given vehicle (0.5% carboxymethyl cellulose) (40) (Figure 9). As already mentioned, a reduction in ERK and ASK1 phosphorylation, an augmentation in PLB phosphorylation and an attenuation in cardiomyocyte apoptosis were observed with oxypurinol treatment and could explain its beneficial effects (40). The role of renin-angiotensin system inhibition in patients with systolic HF is well established and they might also be useful in diastolic HF (4). It is thus of interest that the beneficial effects of candesartan in this study were mediated through its effects on cardiac XOR (40). Therefore, XOR inhibition might partly contribute to the salutary effects of angiotensin receptor blockers in patients with HF.

9. INTERACTION OF XOR WITH OTHER SOURCES OF ROS IN HF

NADPH oxidases are family of 5 enzymes that transfer electrons from NADPH to molecular oxygen leading to the formation of O_2^-. Similar to XOR, NADPH oxidases are also stimulated – among other agonists – by cytokines and angiotensin II (112). We recently showed that NADPH oxidase activity is similar in HF and control rats (59). In the same study we observed an upregulation of the NADPH oxidase subunits $Gp91^{phox}$ and $p67^{phox}$ in HF rats whereas $p22^{phox}$ and $p47^{phox}$ did not change significantly (59). However, NADPH oxidase activity is increased in the failing human myocardium (26, 113, 114) and may promote the development of cardiac hypertrophy, remodeling and ultimately HF (112, 115). It should be noted that there appears to be a cross-talk between NADPH and XOR in that the former increases the production of O_2^- from the latter by inducing the conversion of XDH to XO (116).

The mitochondria might also generate ROS in the failing heart (22). This was attributed to a decreased activity of complex I leading to an impairment in electron transport (22). However, the mitochondria also represent the main energy source through oxidative phosphorylation and this essential function is disrupted by XOR-produced O_2^- (117).

10. INTERACTION OF XOR WITH ANTIOXIDANT SYSTEMS IN HF

SOD converts O_2^- to H_2O_2 and exists in 3 isoforms, cytosolic or copper-zing SOD (SOD1), manganese SOD (SOD2, localized in mitochondria) and extracellular SOD (SOD3) (118). A relative deficit of SOD appears to be implicated in the development and progression of HF (23, 100, 119). Heart/muscle-specific SOD2-deficient mutant mice demonstrate increased production of O_2^- leading to HF (23). In humans, a polymorphism in the SOD2 gene that reduces the antioxidant activity of SOD also increases the risk of developing HF (119). Regarding the implicated mechanisms, inhibition of SOD1 and SOD3 induced apoptosis of rat cardiomyocytes *in vitro* and increased Bax mRNA levels (100). SOD2-deficient mutant mice showed suppressed oxidative phosphorylation and decreased levels of ATP in the heart (23). These detrimental consequences of the deficiency of SOD, the principal superoxide-inactivating antioxidant enzyme, further support the pivotal role of O_2^- in the pathogenesis of HF.

11. CROSS-TALK OF OXIDATIVE AND NITROSATIVE PATHWAYS IN HF – THE ROLE OF XOR

Since its original description as endothelium-derived relaxing factor in the pivotal experiments of Furchgott and Zawadski in 1980 (120), intense interest has been focused on the nitric oxide (NO) signaling pathway (5). Prototypically, NO exerts signaling by activation of guanylyl cyclase, which in turn converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), an important second messenger (121). However, another mechanism of action of NO has been the topic of intensive research during the last decade, namely S-nitrosylation, which is the covalent attachment of NO to cysteine thiol (5). This ubiquitous signaling mode represents a post-translational modification system akin to phosphorylation, and is regulated and capable of tight molecular regulation by virtue of its reversibility. An important corollary to the molecular regulation exerted by SNO, is that NOSs are frequently found in signaling modules participating in protein-protein interactions with effector proteins. This is particularly true in the cardiomyocyte in which NOSs are compartmentalized in various key organelles (122). NOS1 is situated in the SR and mitochondria and NOS3 in specialized sarcolemmal signal-transducing domains termed caveolae (123–125). In this context, it is also important to stress that, in HF, NOS1 redistributes from the SR to the cell membrane (126) and this could have important implications in NO signaling in the failing myocardium (122).

NO is an important modulator of excitation/contraction coupling and myocardial energetics (127, 128) (Figure 5). NO regulates all major Ca^{+2} cycling proteins. NOS1 progressively activates RyR, possibly by S-nitrosylation (129, 130). NOS1 inhibits SERCA (123). NOS3 inhibits the LTCC in a cGMP-dependent way; however, it can also regulate it through S-

nitrosylation and whether it exerts stimulatory or inhibitory actions depends on the redox milieu (131, 132).

Overall, NO appears to protect against the development of myocardial hypertrophy (133, 134). However, it has been reported that NO activates the hypertrophy-inducing MAPK, ERK1/2, in animal and human cell lines (135, 136). More specifically, NO activates Ras through S-nitrosylation and this leads to ERK1/2 activation (137). It was also suggested that NO mediates the stimulating effect of vascular endothelial growth factor (VEGF) on ERK1/2 in human endothelial cells; this effect was cGMP-dependent (138, 139). This is controversial as others did not observe effects of NO on ERK1/2 in murine macrophages (140). More recent studies reported that NOS1 inhibits Ras activity by S-nitrosylation and impairs downstream activation of RAF and ERK1/2 (141).

Besides these effects of NO on hypertrophy-regulating kinases, NO modulates the activity of specific phosphatases that are implicated in myocardial hypertrophy. Calcineurin is a Ca^{+2}-activated phosphatase that dephosphorylates nuclear factor of activated T-cells (NFAT), which then translocates to the nucleus and stimulates hypertrophic gene expression (142). In cardiomyocytes and in vascular smooth muscle cells, NO prevents the activation of calcineurin and exerts anti-hypertrophic actions by regulating Ca^{+2} concentration (143, 144). This was mediated through a cGMP-dependent inhibition of Ca^{+2} entry via the LTCC (144).

NO can both induce and inhibit apoptosis depending on its concentration, cell type and redox status (145). It has already been mentioned that XOR can activate ASK-1, which then stimulates p38 and JNK resulting in increased apoptosis. In contrast, NO inhibits ASK-1 through S-nitrosylation (146). It was also recently reported that NOS inhibition induces cardiomyocyte apoptosis in wild type mice but not in ASK-1 deficient mice (147). These findings highlight the significance of ASK-1 inhibition in the anti-apoptotic actions of NO. Despite the inhibitory action of NO on ASK-1, the effects of NO on the downstream effectors of ASK-1 in the MAPK pathway, p38 and JNK, might promote apoptosis. Thus, NO activates p38 in various cell lines resulting in pro-apoptotic effects (135, 136, 140, 148). NO-induced p38 activation is both cGMP-dependent and independent (149, 150). Regarding the effect of NO on JNK, both inhibitory and stimulating actions have been reported, depending on the cell line assessed (136, 140, 151–153). Again, these actions of NO were either mediated through the guanylyl cyclase pathway or through S-nitrosylation (151–153). Overall, the effects of NO on both the apoptosis-stimulating (p38/JNK) and the hypertrophy-inducing branches (ERK1/2) of the MAPK pathway are complex and require further study.

ROS and NO interact in multiple levels (Figure 1). On one hand, XOR has multiple effects on NO-signaling pathways. As previously mentioned, O_2^- interacts with NO to form peroxynitrite; thus, elevated O_2^- levels decrease NO bioavailability (5). XOR also appears to regulate the S-nitrosylation pathway. In recent years it has become clear that nitrosative and oxidative pathways share similar targets and therefore compete for the same binding sites (5). Moreover, the modification of a protein by one signaling route could alter this protein's susceptibility to the effects of the other route (5). It was also shown that XOR reduces S-nitrosothiols and particularly S-nitrosoglutathione (GSNO), leading to the regeneration of glutathione, the enzyme that reduces SNO moieties in proteins and preserves

the S-nitrosylation equilibrium (154). Interestingly, the reduction of GSNO by XOR depends on the production of O_2^- (154). However, XOR can also augment NO production by catalyzing nitrite reduction, particularly in ischemic conditions (155).

On the other hand, NO regulates XOR activity. Earlier biochemical studies showed that NO can inhibit both XO and XDH (156, 157). More specifically, NO reacts with an essential sulphur in the molybdenum center of XOR and removes it (156). Furthermore, NOS1 deficiency augments the production of O_2^- by XOR in the cardiomyocytes; in contrast, NOS3 deficiency has no impact on XOR expression (52, 158, 159). This effect of NOS1 deficiency is due to an increase in XOR activity whereas XOR mRNA and protein levels are not affected; this suggests that NOS1 via a post-translational effect (very possibly S-nitrosylation) constrains XOR activity (52, 159). NOS1 deficiency also results in increased activation of p38 kinase, and it has been proposed that the latter could activate XOR by phosphorylation (158). It is also of interest that XOR and NOS1 coimmunoprecipitate in the SR of cardiomyocytes, suggesting a direct protein-protein interaction (52). The co-localization of XOR and NOS1 could also stem from an attachment to a common adapter protein (52). Therefore, inside the cardiomyocytes, this NO-XOR interaction appears to be NOS1-specific and spatially confined to the SR (52). It is also of interest that NOS3 suppresses NADPH activity conferring cardioprotection (160) whereas NADPH-generated O_2^- can induce NOS3 uncoupling thereby augmenting O_2^- production and reducing NO bioavailability (161). It is apparent that the cross-talk between oxidative and nitrosative pathways is not limited to XOR and NOS1 but extends to other major players of these systems.

The NO-XOR interplay is an important regulator of myocardial function (5, 162). It has been already mentioned that S-nitrosylation progressively activates RyR; however, oxidation hampers this regulatory action by inducing irreversible activation of RyR (129, 130). In addition, the effect of NOS3 on LTCC depends on the redox status (131, 132). Peroxynitrite, the product on NO and O_2^- reaction, suppresses myocardial contractility by impairing Ca^{+2} responsiveness of myofilaments (57). This was attributed to a reduction in the activity of NCX resulting in an increase in intracellular Ca^{+2} concentration (57). Peroxynitrite also inhibits mitochondrial function and this could result in a reduction in ATP levels that could also be implicated in its negative inotropic actions (163). Besides systolic function, peroxynitrite increases resting tension in isolated heart muscle and this could potentially result in impairment in diastolic function (164). The suppressing effects of peroxynitrite on myocardial contractility could also be related to its proapoptotic actions on cardiomyocytes (165). In addition, peroxynitrite also triggers the cell death of endothelial cells (165) and this might adversely affect coronary vasculature, myocardial perfusion, and inotropy.

Overall, these findings suggest that XOR has an important role in the interplay between oxidative and nitrosative mechanisms and the preservation of nitroso-redox balance (6). *In vivo* studies lend further support to this hypothesis. In dogs with HF, we showed that pretreatment with the non-specific NOS-inhibitor N^G-monomethyl-$_L$-arginine (L-NMMA) prevents the increase in myocardial contractility and the improvement in mechanoenergeting uncoupling induced by allopurinol (28). This finding implies that suppression of O_2^-

production is not sufficient to restore myocardial function but additionally requires an intact NO-signaling pathway (28). We extended these findings by studying NOS1 knockout mice; these mice exhibited impaired contractility that was reversed by allopurinol (52). This suggests that part of the positive inotropic effects of allopurinol stem from a reduction in O_2^- levels–induced rise in the bioavailability of NO (52). In addition, in dogs with normal heart function, L-NMMA increased myocardial oxygen consumption and depressed myocardial efficiency and this effect was alleviated by either vitamin C or allopurinol coinfusion (28). These findings suggest that a physiological NO signaling is essential for the control of XOR-induced oxidative stress in normal hearts and prevents XOR from depressing cardiac efficiency (28).

The pivotal role of nitroso-redox balance in cardiovascular homeostasis and more specifically in HF is also supported by clinical studies showing that combined administration of nitrates and hydralazine reduced mortality in HF regardless of its severity (166, 167). Since nitrates act as NO donors (168) and hydralazine scavenges ROS and is implicated as possibly inhibiting NADPH oxidases (169, 170), it is attractive to contemplate that their beneficial effects in HF are mediated through the restoration of nitroso-redox balance (162).

12. PERIPHERAL EFFECTS OF XOR INHIBITION IN HF

Even though XOR is located in cardiomyocytes and exerts a multitude of autocrine effects as delineated above, the liver and small intestine show the most abundant expression of this enzyme in humans (30). In addition, XOR is expressed in endothelial cells and can also be released in the circulation (30). Therefore, apart from the direct effects of XOR on myocardial structure and function, peripheral mechanisms may also be implicated in the XOR-induced impairment of myocardial function. In dogs with pacing-induced HF, allopurinol treatment (100 mg po daily during the pacing period) attenuates the increase in arterial elastance, an index of afterload, as well as the decrease in ventricular elastance, a marker of contractility (37). Together these effects lead to the preservation of ventricular-vascular coupling ratio (ventricular/arterial elastance) and subsequently to an improved systolic function compared with the placebo group (37). Similar results were observed with both acute (5-day) and chronic (10-week) administration of allopurinol in rats with left coronary artery ligation-induced HF (83).

Studies in both animals and humans have shown that oxidative stress plays a role in the development of endothelial dysfunction (ED) in HF (171). ED is frequently present in HF and correlates with its severity ((172–177). Endothelium-bound XOR has a critical role in the pathogenesis of ED in these patients since it is more than 2-fold more active in HF and its activity strongly correlates with the degree of ED (20). In addition, several studies showed an improvement in ED in patients with HF with XOR inhibition (178–180). It must be emphasized that systemic ED is associated with arterial stiffening and increased vascular tone which in turn increase afterload and lead to HF progression (181–183). In addition, ED in the coronary circulation might compromise myocardial oxygenation and could also contribute to the worsening of myocardial function (184, 185). More importantly, prospective studies showed that ED in HF represents an independent risk factor for

readmission with worsening HF, heart transplantation and mortality (186–188). Therefore, it is reasonable to assume that the salutary effects of XOR inhibition on ED could translate into long-term clinical benefits in patients with HF; however, this remains to be established in prospective studies.

Another important aspect of XOR inhibition is the reduction in serum uric acid (SUA) levels. Uric acid *per se* is a powerful ROS-scavenger (189) and also activates other endogenous antioxidant systems, particularly SOD (190). Interestingly, uric acid is metabolized by uricase in most mammals whereas in humans the uricase gene is nonfunctional (191). It has thus been proposed that this might confer a survival advantage to humans due to increased antioxidant defense (192). However, uric acid can also promote the development of cardiovascular disease since it has proinflammatory actions (193–195), activates platelets (196), stimulates the growth of vascular smooth muscle cells (197, 198) and induces ED (199). Prospective studies suggested that elevated SUA levels represent an independent risk factor both in the general population (200–202) and in patients with established cardiovascular disease, including coronary heart disease (203, 204) and acute stroke (205). Elevated SUA levels are frequently observed in patients with HF and directly correlate with its severity (206–208). The upregulation of XOR in HF might contribute to this phenomenon; decreased renal excretion and increased XOR substrate resulting from enhanced ATP breakdown are other putative explanations (209). Elevated SUA levels in HF reflect – to a certain degree – the activation of the XOR oxidative pathway; however, uric acid *per se* may also contribute to HF pathophysiology (209). In patients with HF, elevated SUA levels are associated with systemic inflammation (207), increased peripheral vascular resistance (210, 211), decreased functional capacity (206) and with the presence of diastolic dysfunction (212). More importantly, prospective studies showed that elevated SUA levels in HF independently predict mortality and the need for transplantation (213–215). Interestingly, elevated SUA levels also predict allograft vasculopathy in cardiac transplant recipients (216). In addition, in the Losartan Intervention For Endpoint reduction in hypertension (LIFE) study, a losartan-induced reduction in SUA levels in patients with left ventricular hypertrophy reduced cardiovascular morbidity and mortality (217). Therefore, by reducing SUA levels, XOR inhibition might offset an important risk factor in patients with HF.

13. XOR AND ADIPOGENESIS

Our knowledge of the mechanisms through which XOR contributes to the pathogenesis of cardiovascular disease continues to expand. A recent study showed that XOR can stimulate adipogenesis and that this effect is mediated through a XOR-induced increase in the activity of peroxisome proliferator-activated receptor gamma (PPARgamma) (218). PPARgamma is an important regulator of adipose differentiation and when activated, enhances adipogenesis through the induction of transcription of specific adipogenic genes (219). In the same study, a reduction in adipose mass was observed in XOR null mice, further supporting the importance of XOR in adipogenesis (218). Interestingly, XOR null mice do not live more than 40 days (220) and this is additional evidence that XOR serves important physiological roles.

If the association of XOR with adipogenesis is confirmed in humans, XOR inhibition could emerge as a promising strategy in the management of obesity and obesity-related disorders, including type 2 diabetes mellitus and the metabolic syndrome. Paradoxically, obesity is associated with improved survival in patients with HF, a phenomenon termed reverse epidemiology (221). However, it appears that obesity is not truly protective but merely reflects a lower inflammatory response in obese patients with HF (221). HF is increasingly being recognized as an inflammatory state and increased inflammation contributes to the development and progression of HF (222). Clearly, more studies are required to unravel the complex links between obesity and HF and the potential role of XOR in this interplay.

14. CLINICAL STUDIES OF XOR INHIBITION IN HF

The beneficial effects of XOR inhibition in the above-mentioned studies in a variety of animal models of HF support the pivotal role of XOR in the pathogenesis of HF. They also suggest that XOR inhibition might represent a valuable adjunct in the treatment of HF in humans as well. Based on these promising results, a number of studies have evaluated the effect of this treatment strategy in the clinical setting. In a preliminary study from our group in patients with dilated cardiomyopathy, intracoronary infusion of allopurinol (0.5., 1.0. and 1.5. mg/min, each administered for 15 min) decreased myocardial oxygen consumption without affecting myocardial contractility (21). Therefore, XOR is involved in the pathogenesis of mechanoenergetic uncoupling in HF i.e. the discordance between impaired LV work and myocardial energy consumption, which impairs the mechanical efficiency of contraction (21).

In another study assessing acute XOR inhibition, a single intravenous dose of oxypurinol (400 mg) significantly reduced LV end-systolic volume and increased LVEF ($p = 0.03$ and $p = 0.003$, respectively) in patients with ischemic cardiomyopathy (223).

Short-term XOR inhibition has also been evaluated in HF. In a randomized placebo-controlled trial ($n = 50$), allopurinol (300 mg/day po for 3 months) significantly ($p = 0.035$) reduced plasma BNP concentrations (224). It is well established that elevated BNP concentrations represent a strong and independent risk factor in HF (225, 226). In another randomized placebo-controlled trial ($n = 60$), oxypurinol (600 mg/day po for 1 month) induced a non-significant increase in LVEF ($p = 0.08$) (227). However, when patients with baseline LVEF ≤ 40% were analyzed separately, there was a significant ($p < 0.02$) improvement in LVEF with oxypurinol treatment (227) (Figure 10).

The largest study of the effects of an XO inhibitor in HF patients is the Oxypurinol Therapy for Chronic HF study (OPT-CHF) (228, 229). In this double-blind study, 405 patients with NYHA class III-IV heart failure were randomized to receive either oxypurinol 600 mg/day or placebo for 24 weeks (229). Overall, there was no difference beween groups in the composite endpoint comprising CHF morbidity, mortality, and quality of life (229). However, in a post-hoc exploratory analysis oxypurinol improved the outcome of patients with elevated SUA levels at baseline (> 9.5. mg/dl) (229). This cutoff value was selected on the basis of a previous study in patients with CHF which showed that these SUA levels are the best for predicting mortality (213). In addition, within the entire oxypurinol group, a

larger decrease in SUA levels was associated with a better outcome (229). These data strongly support the concept that patients with CHF and elevated SUA levels, potentially a surrogate for increased XOR activity, might benefit the most from XOR inhibition. Finally, in the placebo group of the OPT-CHF study, baseline SUA levels were associated with adverse outcome, further supporting earlier reports on the predictive role of SUA levels in CHF and other cardiovascular disorders (213–216).

It should be noticed that the effects of XOR inhibition in the aforementioned studies were observed in the context of standard pharmacological treatment for HF, including beta-blockers and rennin-angiotensin system inhibitors (223, 227, 229). Therefore, it appears that XOR inhibition can provide additive benefits to those of current drug therapy for HF.

An important limitation of all studies employing allopurinol treatment is that this agent has other effects besides XOR inhibition, including antioxidant action, copper chelation, inhibition of lipid peroxidation and down-regulation of heat shock protein expression (230). Allopurinol can also directly scavenge OH^- (231). Therefore, not all effects of allopurinol can be attributed to XOR inhibition. On the other hand, it has been suggested that cell-associated XOR may be less susceptible to allopurinol inhibition (232). This finding could imply that the effects of allopurinol administration cannot totally capture the significance of XOR.

15. PERSPECTIVE

XOR is intimately involved in the pathophysiology of HF and exerts regulatory actions in multiple levels of both ROS and NO signaling pathways. Preclinical studies and preliminary clinical studies assessing XOR inhibition showed promising findings. Given the rising burden of HF and its high morbidity and mortality rates, there is a clear rationale for pursuing further prospective, large-scale clinical studies designed to evaluate the potential therapeutic role of XOR inhibition in these patients. Finally, in this emerging era of individualized medicine, identifying and targeting specific subgroups of patients – such as patients with elevated SUA levels as the OPT-CHF study suggests – might also maximize the benefits of XOR inhibition in the management of HF.

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Abbreviations

ANP	atrial natriuretic peptide
aSA	a skeletal muscle isoform of actin
ASK1	apoptosis signal-regulating kinase 1
β-MHC	β-isoform of myosin heavy chain
BNP	brain natriuretic peptide

cGMP	cyclic guanosine monophosphate
CK	creatine kinase
ED	endothelial dysfunction
ERK	extracellular signal-regulated kinases
GTP	guanosine triphosphate
HF	heart failure
HNE	4-hydroxy-2-nonenal
JNK	c-Jun N-terminal kinases
L-NMMA	NG-monomethyl-L-arginine
LTCC	L-type Ca+2 channels
LV	left ventricular
LVEF	left ventricular ejection fraction
MAPK	mitogen-activated protein kinase
MI	myocardial infarction
MMP	matrix metalloproteinases
NADPH	nicotinamide adenine dinucleotide phosphate
NCX	Na+-Ca+2 transporter
NFAT	nuclear factor of activated T-cells
NO	nitric oxide
NOS	nitric oxide synthase
NOS1	neuronal nitric oxide synthase
NOS3	endothelial nitric oxide synthase
PCr	Phosphocreatine
PLB	phospholamban
PPARgamma	peroxisome proliferator-activated receptor gamma
ROS	reactive oxygen species
RYR	ryanodine receptor
SERCA	sarcoplasmic reticulum Ca2+-ATPase
SOD	superoxide dismutase
SR	sarcoplasmic reticulum
SUA	serum uric acid
VEGF	vascular endothelial growth factor

XDH	xanthine dehydrogenase
XO	xanthine oxidase
XOR	xanthine oxidoreductase

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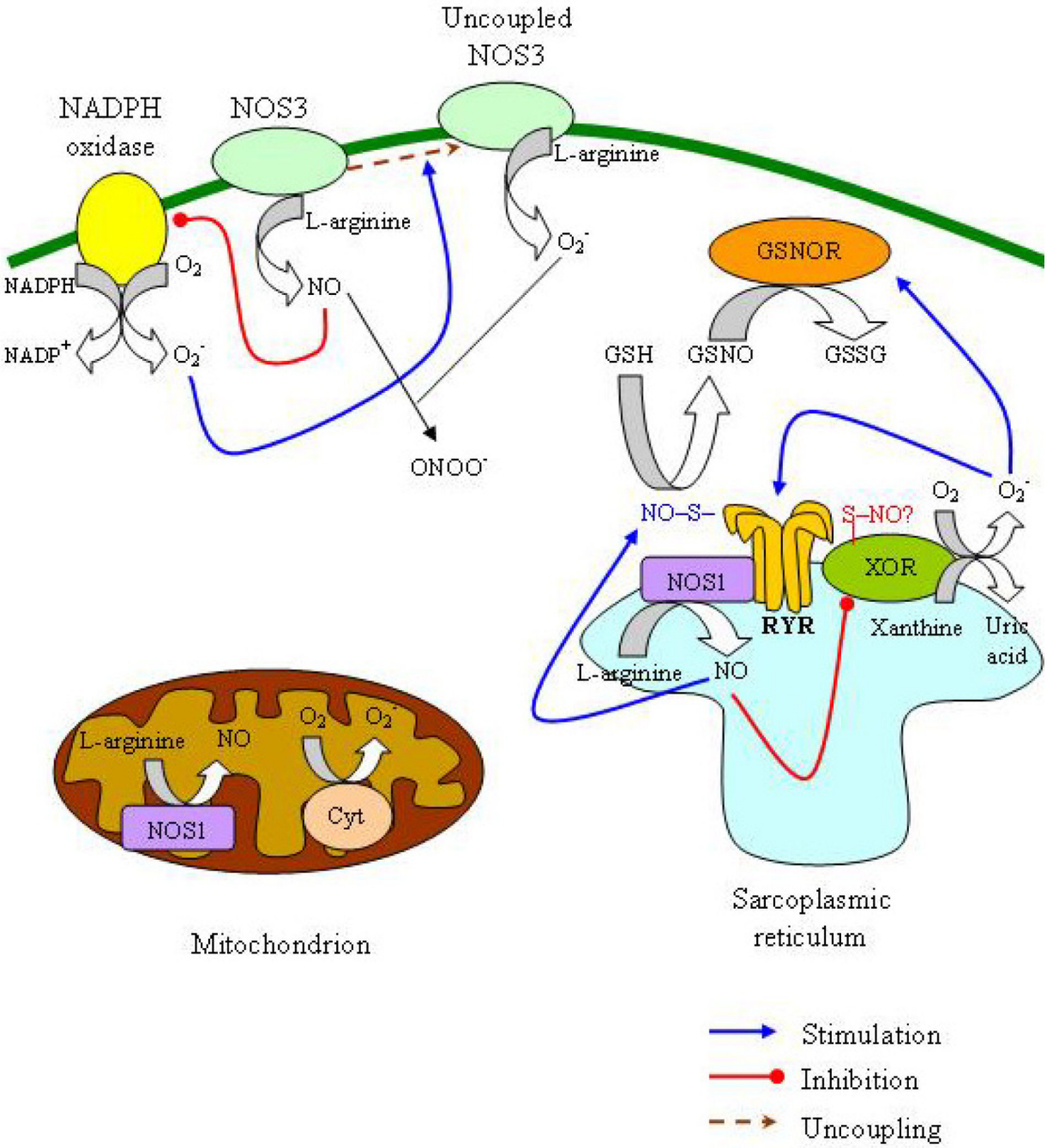

Figure 1.

Interaction of oxidative and nitrosative pathways in heart failure. The principal sources of reactive oxygen species in the cardiomyocyte are xanthine oxidoreductase (XOR), nicotinamide adenine dinucleotide phosphate oxidase (NADPH) and the mitochondria. Nitric oxide (NO) is produced by neuronal nitric oxide synthase (NOS1, situated in the sarcoplasmic reticulum (SR) and in the mitochondria) and endothelial nitric oxide synthase (eNOS, situated in the caveolae in the cell membrane). XOR and NOS1 colocalize in the SR and this allows the inhibition of XOR by NOS1, possibly through S-nitrosylation In turn,

XOR reduces S-nitrosoglutathione (GSNO), leading to the regeneration of glutathione (GSH), the enzyme that reduces SNO moieties in proteins and preserves the S-nitrosylation equilibrium. In SR, NOS1 regulates the activity of the ryanodine receptor (RyR) through S-nitrosylation; in contrast, XOR-generated superoxide (O_2^-) irreversibly activates RyR, precluding this regulatory action of NO. In the cell membrane, NOS3 suppresses NADPH activity whereas NADPH-produced O_2^- can induce NOS3 uncoupling resulting in O_2^- production and reduced NO synthesis. O_2^- interacts with NO and generates peroxynitrite ($ONOO^-$). (GSSG, oxidized GSH; GSNOR, GSNO reductase; Cyt, cytochrome).

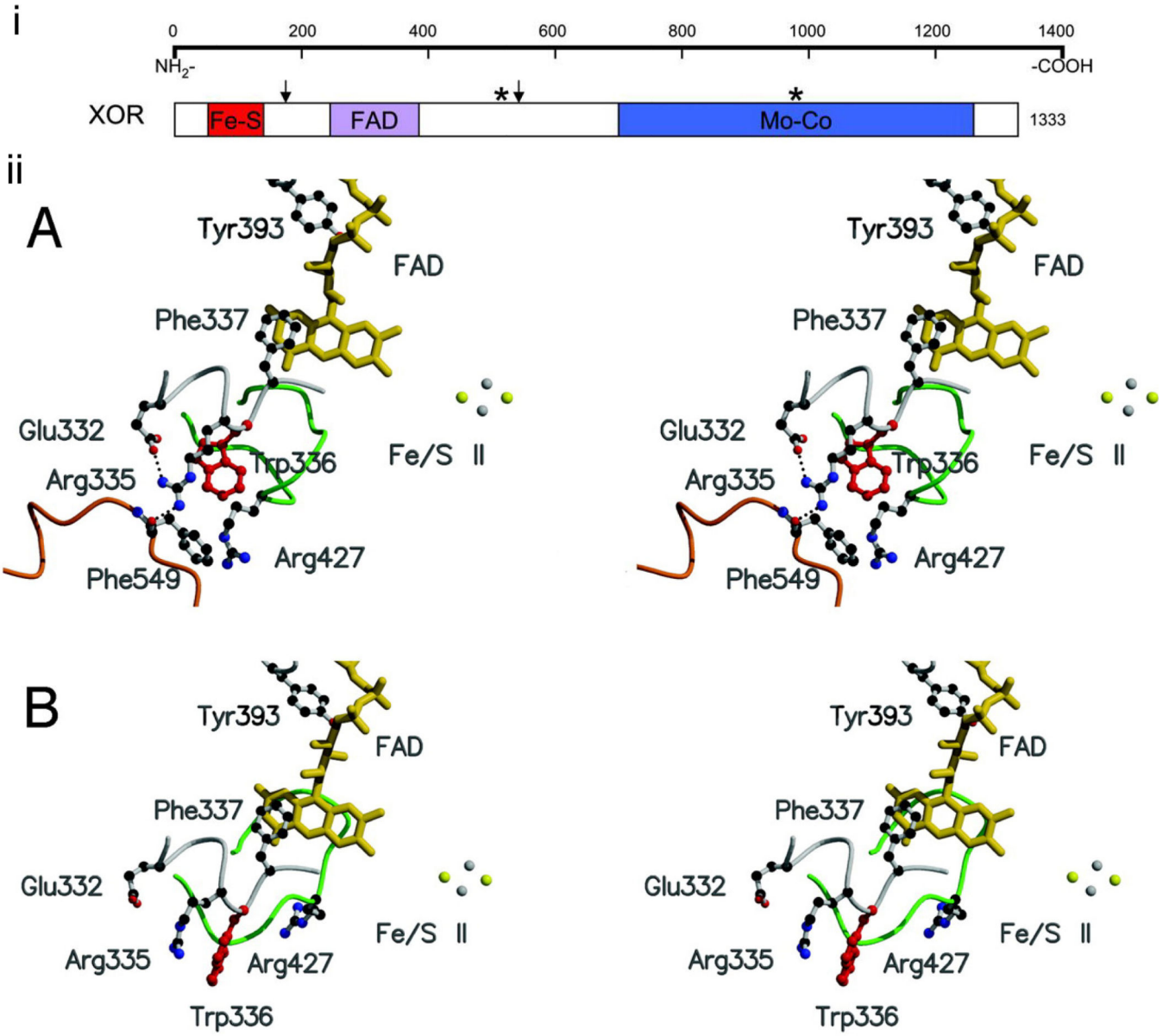

Figure 2.

i) Secondary structure of xanthine oxidoreductase (XOR). Arrows designate trypsin sites; stars designate cysteine residues modified in reversible XOR conversion. Reproduced with permission from (30). Copyright 2004, The Physiological Society. ii) Structure of the active-site loop (Gln-423–Lys-433, shown in green) of the xanthine dehydrogenase (A) and xanthine oxidase (B) forms of bovine xanthine oxidoreductase. Reproduced with permission from (31). Copyright 2003, National Academy of Sciences, U.S.A.

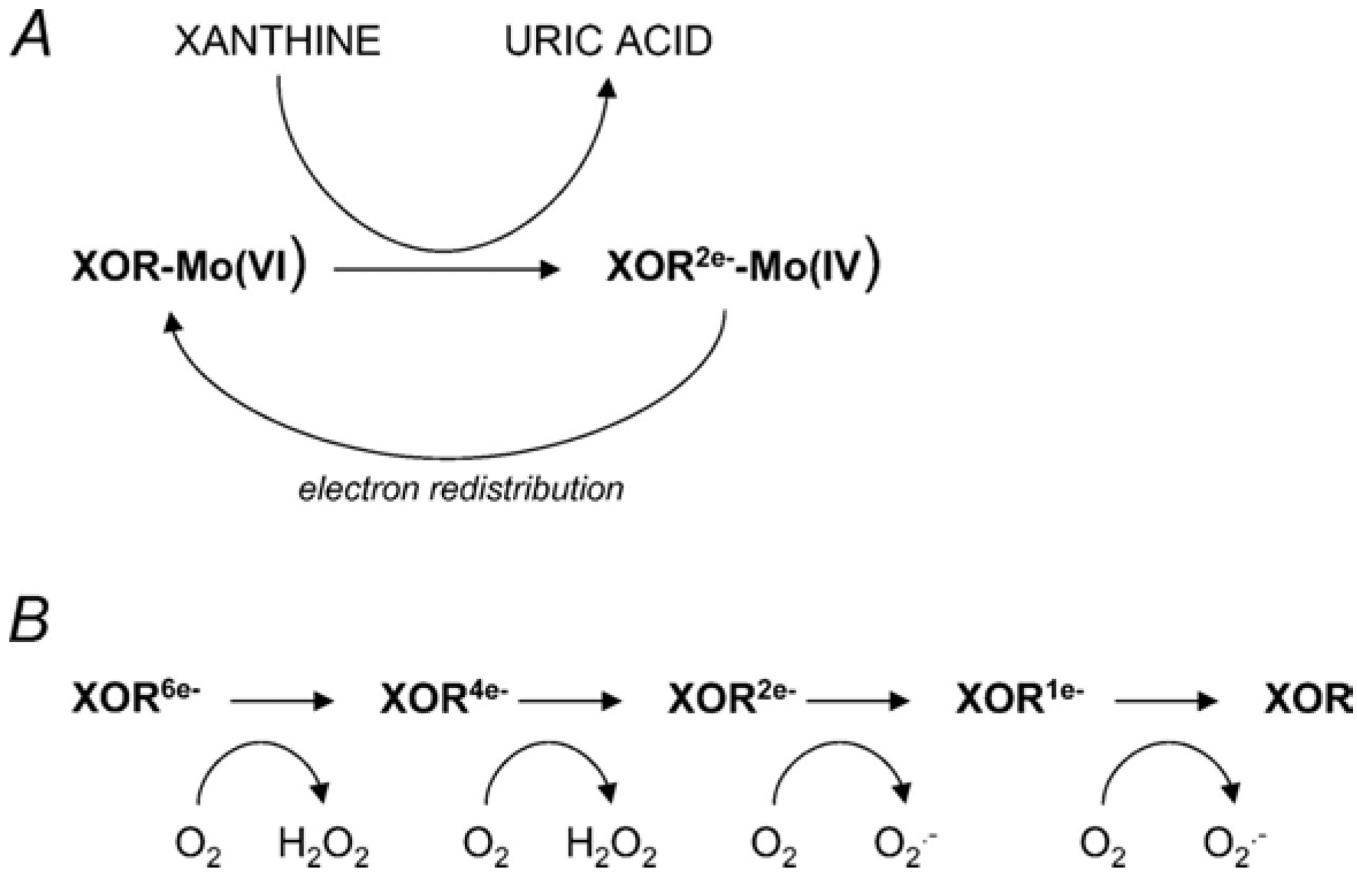

Figure 3.
Mechanism of superoxide production by xanthine oxidoreductase (XOR). Reproduced with permission from (30). Copyright 2004, The Physiological Society.

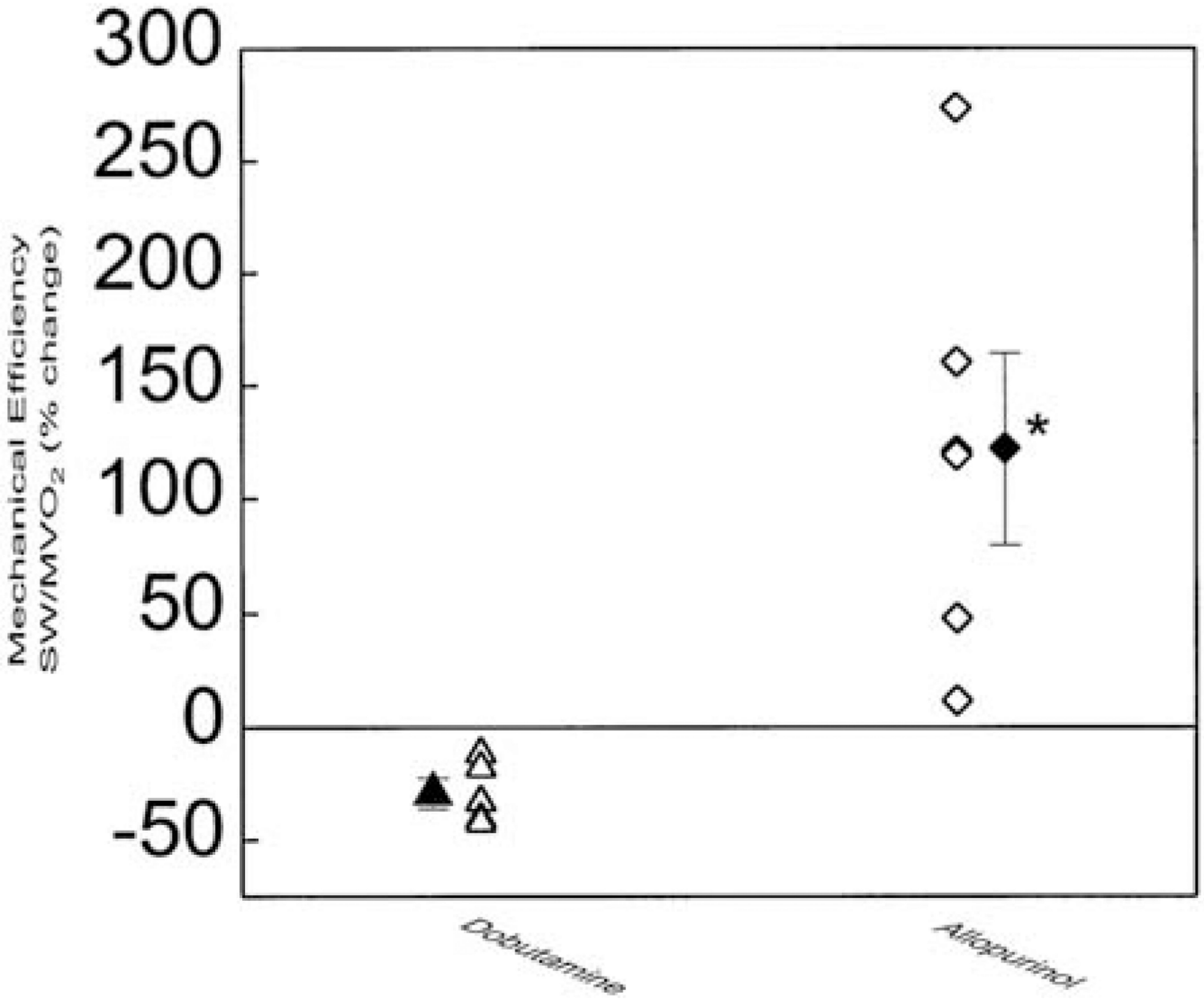

Figure 4.

Comparison of the effects of allopurinol and dobutamine on myocardial efficiency in dogs with pacing-induced heart failure. Compared with baseline, allopurinol significantly improves efficiency whereas dobutamines significantly decreases it. Solid symbols represent mean±SEM. *$p < 0.05$ vs dobutamine. (SW, stroke work; MVO_2, myocardial oxygen consumption per unit time). Reproduced with permission from (38). Copyright 1999, American Heart Association.

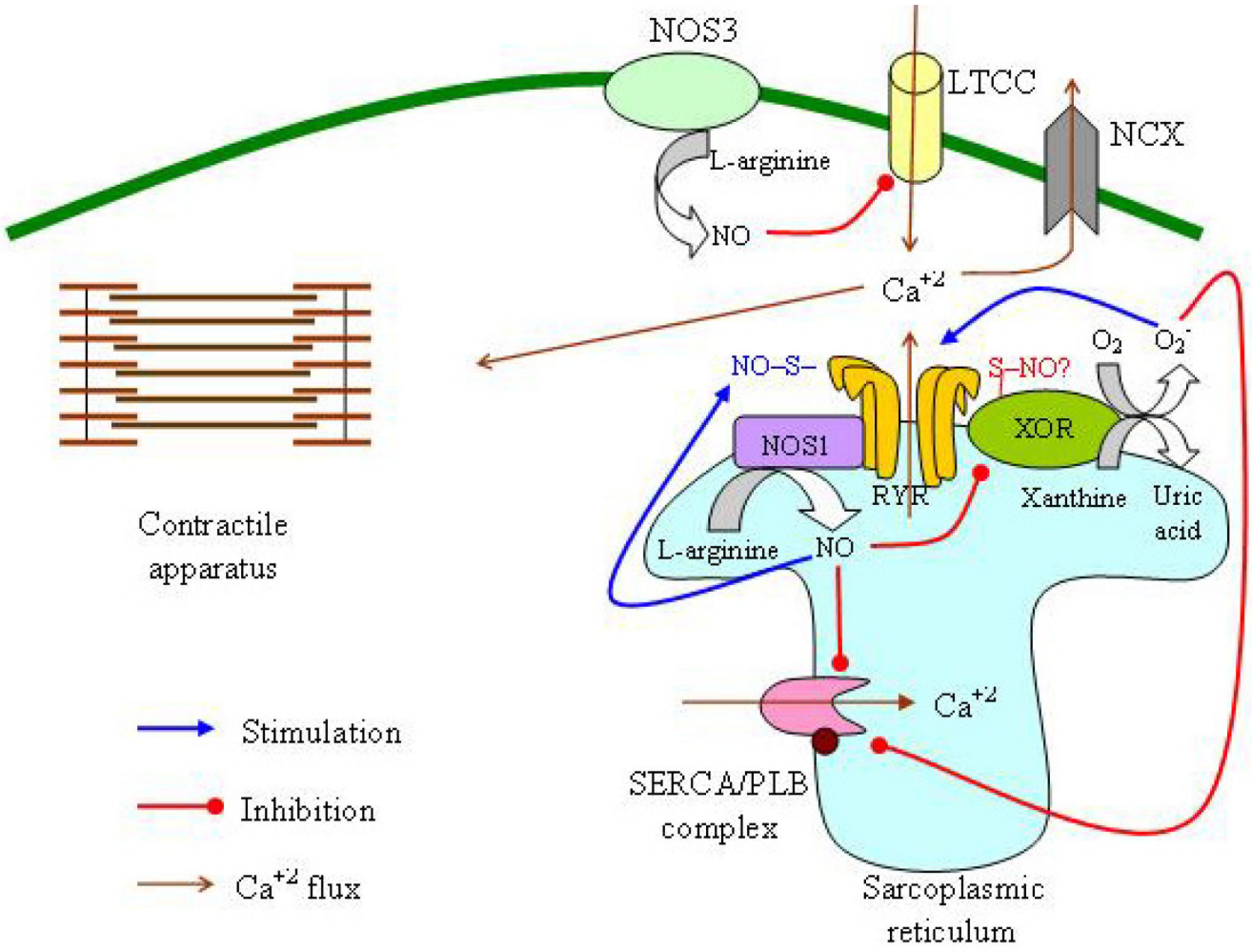

Figure 5.

Regulation of excitation-contraction coupling. The ryanodine receptor (RyR) is responsible for the release of Ca^{+2} from the SR in response to the influx of Ca^{+2} through the L-type Ca^{+2} channels (LTCC). Sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (SERCA) controls Ca^{2+} uptake in to the SR during diastole after its release from the myofilaments. Phospholamban (PLB) is another integral protein of the SR and inhibits SERCA in the unphosphorylated state. Na^{+}-Ca^{+2} transporter (NCX) participates in diastolic Ca^{2+} extrusion in the extracellular space. In HF, there is a reduction in SERCA levels, an increase in NCX levels and an increase in PLB levels and activity. There is also impairment in the RyR function resulting in its uncoupling with the LTCC. Xanthine oxidoreductase (XOR) upregulation appears to contribute to these changes in Ca^{+2} cycling. On the other hand, nitric oxide (NO) synthase 1 (NOS1) inhibits SERCA and activates RyR whereas NOS3 inhibits LTCC. The actions of NOS1 and NOS3 on Ca^{+2} flux are based on their close proximity with Ca^{+2} cycling proteins and are dependent on the redox milieu.

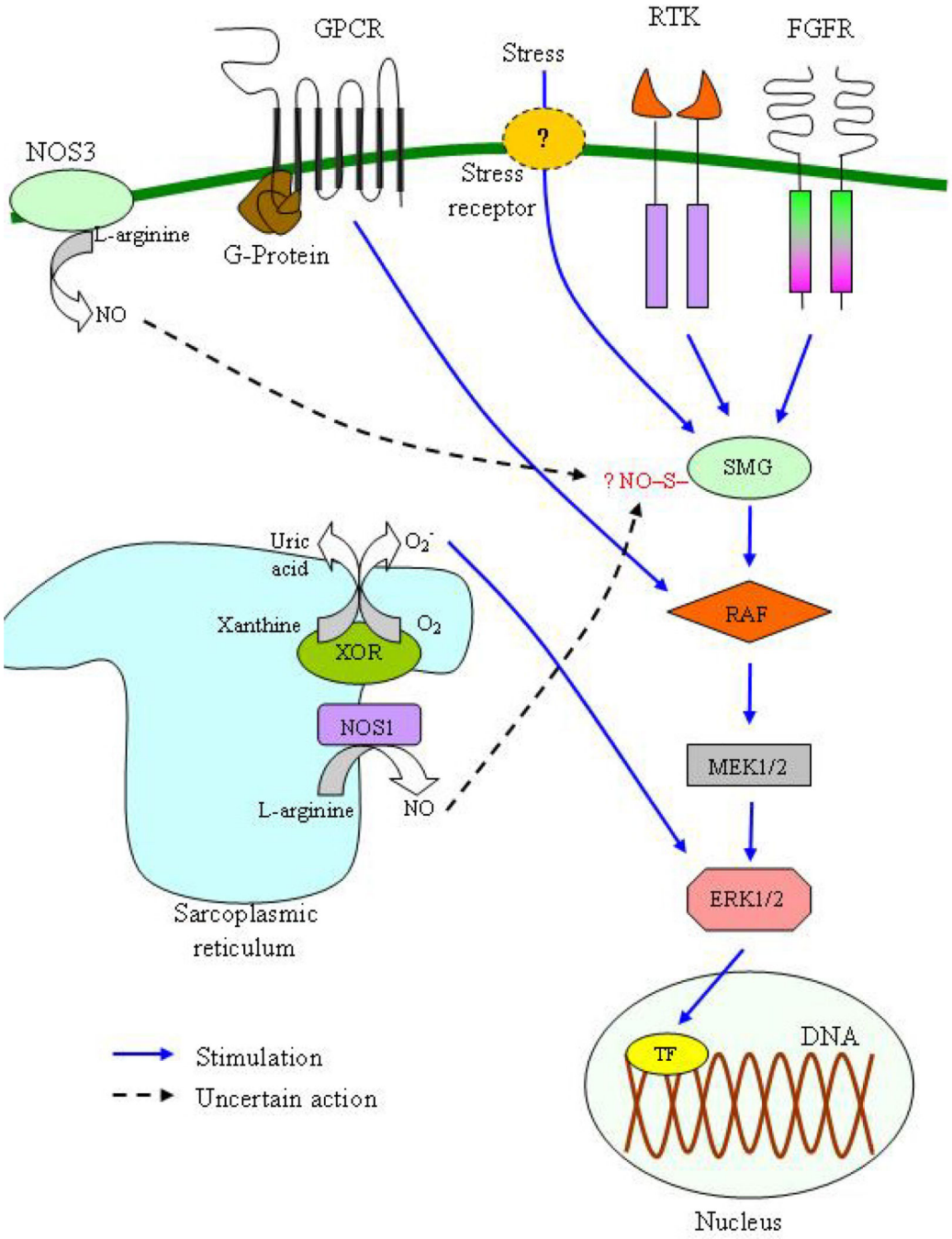

Figure 6.

Simplified representation of the extracellular signal-regulated kinases (ERK)1/2 branch of the mitogen-activated protein kinase (MAPK) signaling pathway and its interaction with xanthine oxidoreductase (XOR) and nitric oxide (NO). The activation of cell membrane receptors (including G-protein coupled receptors (GPCR), receptors tyrosine kinase (RTK) and fibroblast growth factor receptors (FGFR)) by receptor-specific external stimuli (e.g. endothelin-1 for GPCR, epidermal growth factor for RTK) leads to the sequential activation of a series of intracellular messengers. Stress acts either directly or through a putative

receptor. The first intracellular messengers that become activated are the small GTPases (SMG), including members of the Ras (mainly) and Rho/Rac/cdc42 subfamilies, which then activate the RAF family (A-RAF, B-RAF and C-RAF), which in turn activate MEK1/2. The final effectors are ERK1/2, which in turn stimulate hypertrophy by either phosphorylating transcription factors (TF) or by regulating ribosomal RNA transcription and protein translation. ERK1/2 possibly translocates to the nucleus in order to activate TF. XOR activates ERK1/2 and this could partly mediate its hypertrophic actions in the myocardium. The actions of NO on ERK1/2 pathway are a matter of debate. NO appears to act on Ras and both inhibitory and stimulating effects have been reported. These actions are possibly mediated through S-nitrosylation. The relative contribution of nitric oxide synthase 1 (NOS1) and NOS3 on these NO-effects on ERK1/2 pathway is also uncertain.

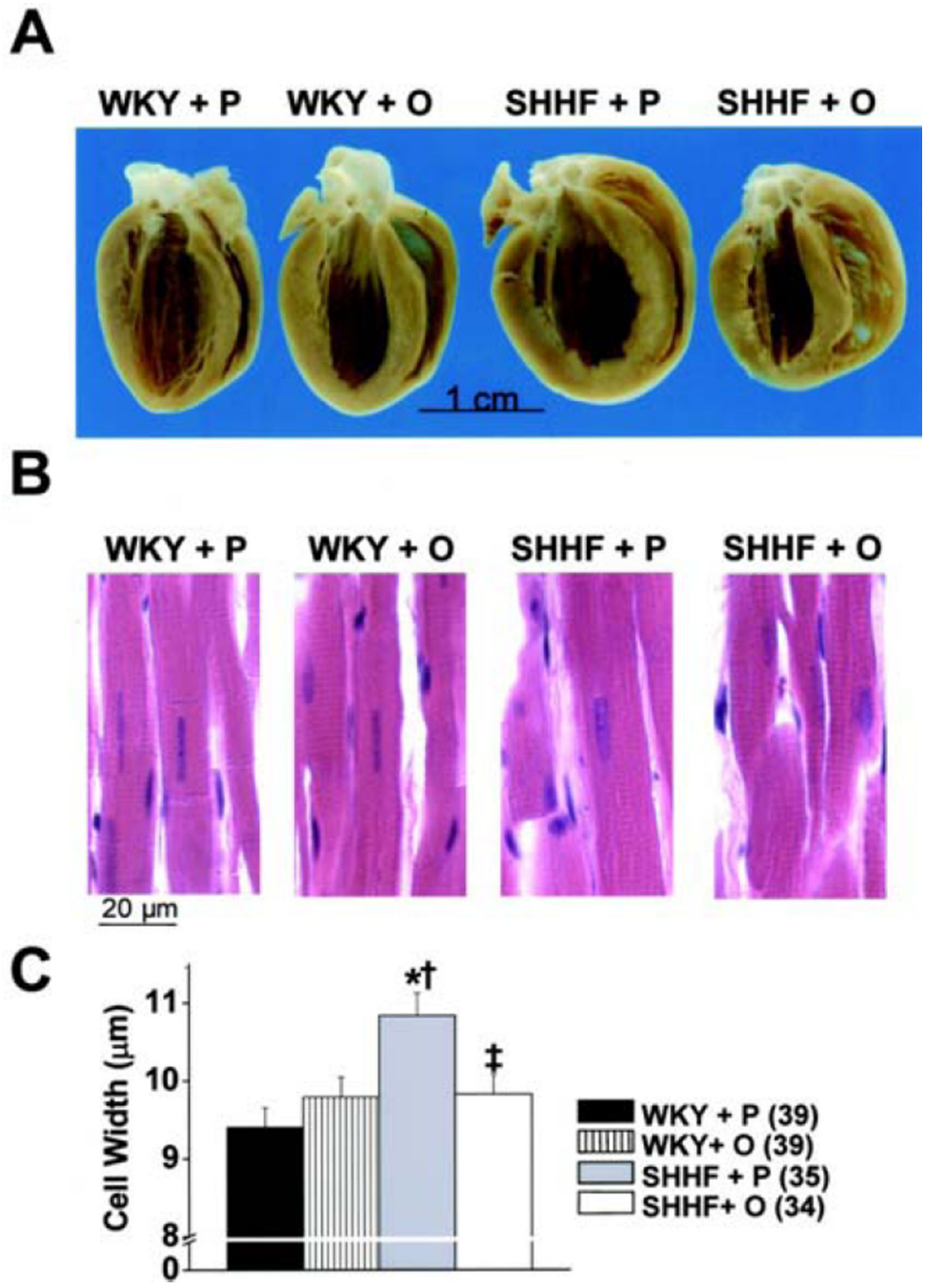

Figure 7.

Oxypurinol induces reverse remodeling in rats with dilated cardiomyopathy. A. Gross pathology showing increased left ventricular (LV) diameter in spontaneously hypertensive/HF (SHHF) rats treated with placebo (SHHF+P); after treatment with oxypurinol (SHHF+O), LV volume is similar to control rats (Wistar Kyoto rats, WKY). B. Light microscopy with hematoxylin/eosin staining shows increased LV myocyte width in SHHF+P rats, which is decreased with oxypurinol treatment (SHHF+O rats) to levels comparable to control rats (WKY). C. Graphic representation of LV myocyte width; there is

a significant increase in SHHF+P rats witch is normalized with oxypurinol treatment. *p < 0.001 compared with WKY+P, †p < 0.05 compared with WKY+O, ‡p < 0.01 compared with SHHF+P. Reproduced with permission from (59). Copyright 2006, American Heart Association.

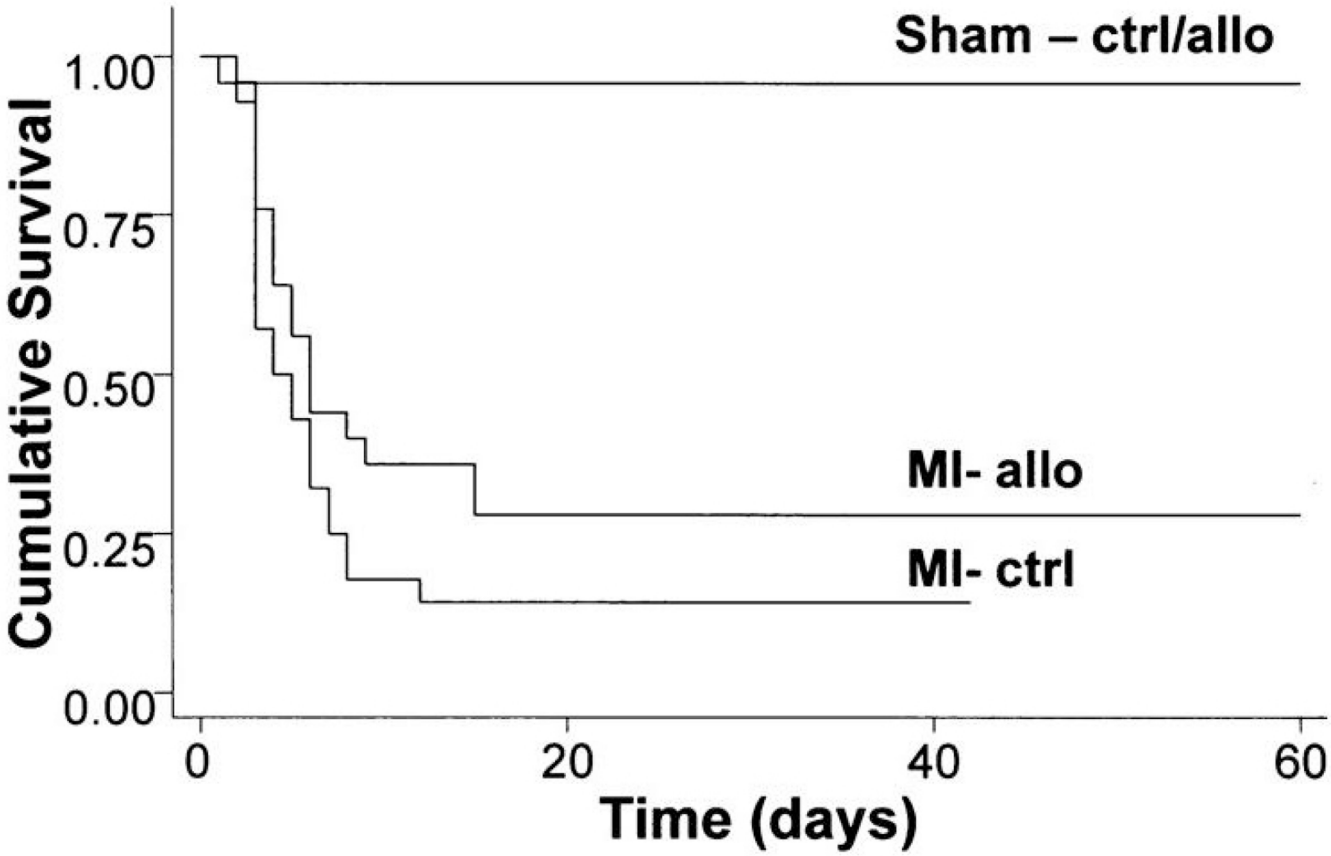

Figure 8.

Allopurinol improves survival after experimental myocardial infarction in mice. Mice subjected to myocardial infarction and treated with allopurinol after recovery (1 mmol/l in drinking water) were twice as likely to survive for 60 days as compared with mice treated with placebo (39% versus 19%). (ctrl, placebo-treated mice; allo; allopurinol-treated mice; MI, myocardial infarction; Sham-ctrl/allo, pooled survival in sham-operated mice receiving either allopurinol or placebo). Reproduced with permission from (39). Copyright 2004, American Heart Association.

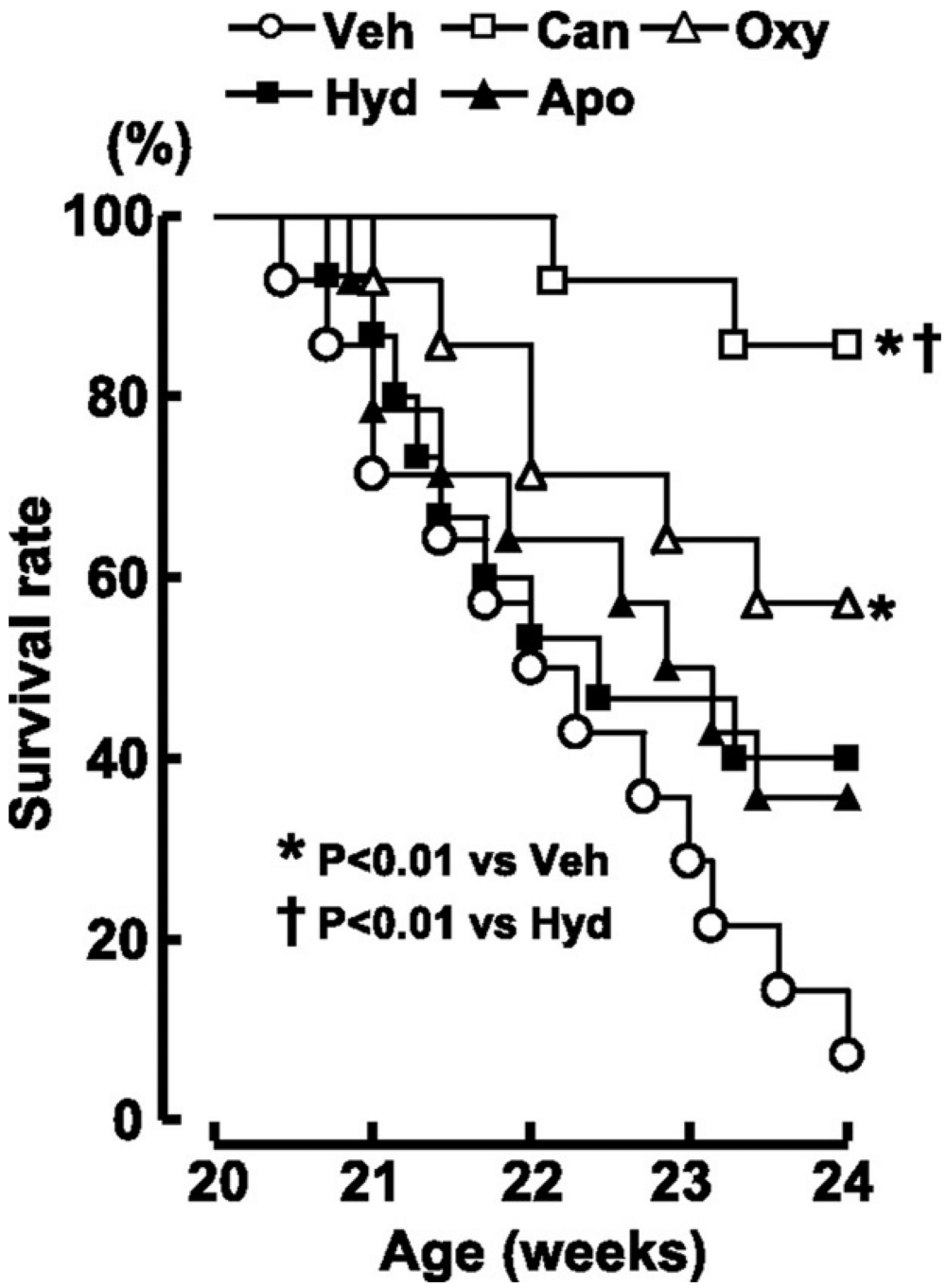

Figure 9.

Treatment with oxypurinol (40 mg/kg per day from 20 to 24 weeks of age) improves survival rate in Dahl salt-sensitive hypertensive rats with established diastolic HF. (veh, vehicle (0.5% carboxymethyl cellulose); can, candesartan; oxy, oxypurinol; hyd, hydralazine; apo, apocynin). Reproduced with permission from (40). Copyright 2007, American Heart Association.

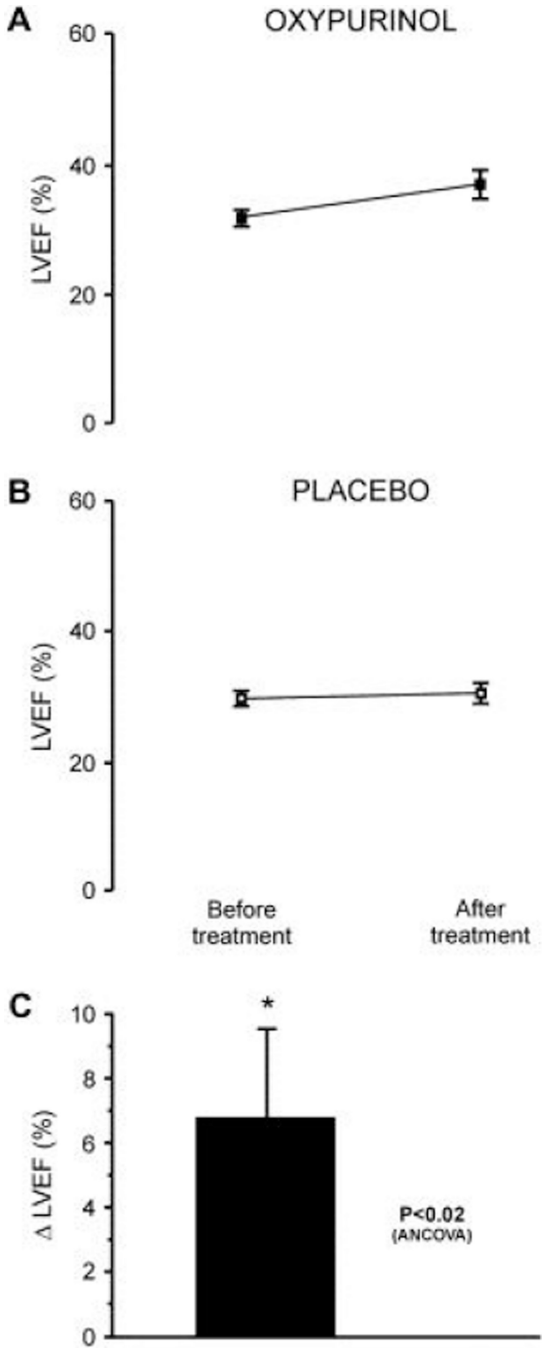

Figure 10.

Treatment with oxypurinol (600 mg/day po for 1 month) significantly ($p < 0.02$) increases left ventricular ejection fraction (LVEF) in patients with heart failure and baseline LVEF ≤ 40% compared with placebo. (A, B) Averaged LVEF data for oxypurinol and placebo, respectively, before and after treatment. (C) Adjusted change in LVEF in the oxypurinol group relative to placebo. * $p < 0.02$ vs. pretreatment value. Reproduced with permission from (227). Copyright 2006, Elsevier Inc.