J Hepatobiliary Pancreat Sci (2011) 18:137–146
DOI 10.1007/s00534-010-0328-7

REVIEW ARTICLE

Allopurinol and xanthine oxidase inhibition in liver ischemia reperfusion

Stephanie Peglow · Alexander H. Toledo · Roberto Anaya-Prado · Fernando Lopez-Neblina · Luis H. Toledo-Pereyra

Published online: 29 September 2010

Abstract
Introduction Allopurinol was first introduced, in 1963, as a xanthine oxidase inhibitor when it was investigated for concomitant use with cancer chemotherapy drugs. Today it is used in gout and hyperuricemia. Due to its additive benefit in preventing oxidative damage, attention has shifted towards the use of allopurinol in organ ischemia and reperfusion.
Current status Currently, the mechanism by which allopurinol exerts a protective benefit in ischemia reperfusion related events is not fully understood. There are various theories: it may act by inhibiting the irreversible breakdown of purine substrates, and/or by inhibiting the formation of reactive oxygen species, and/or by protecting against damage to the mitochondrial membrane.
Aim This work focuses on liver ischemia and reperfusion injury in an effort to better understand the mechanisms associated with allopurinol and with this pathological entity.
Review of literature The current research, mainly in animal models, points to allopurinol having a protective benefit, particularly if used pre-ischemically in liver ischemia reperfusion injury. Furthermore, after reviewing allopurinol dosing and administration, it was found that 50 mg/kg is statistically the most effective dose in attenuating liver ischemia reperfusion injury. Owing to the limited number of samples, the time of administration did not show statistical difference, but allopurinol was often beneficial when given around 1 h before ischemia.
Conclusion In conclusion, allopurinol, through its known xanthine oxidase inhibitory effect, as only one of the potential mechanisms, has demonstrated its potential application in protecting the liver during ischemia and reperfusion.

Keywords Allopurinol · Xanthine oxidase inhibition · Liver ischemia · Ischemia reperfusion injury · Reactive oxygen species

S. Peglow · L. H. Toledo-Pereyra (✉)
Department of Research, Kalamazoo Center for Medical Studies, Michigan State University, 1000 Oakland Drive, Kalamazoo, MI 49008, USA
e-mail: toledo@kcms.msu.edu

A. H. Toledo
Division of Abdominal Transplantation, University of North Carolina, Chapel Hill, NC, USA

R. Anaya-Prado
Department of Research, IMSS, Guadalajara, Jalisco, Mexico

F. Lopez-Neblina
Department of Biological Sciences, University of Baja California, Mexicali, Mexico

Introduction

Allopurinol, a structural analogue of hypoxanthine and a xanthine oxidase inhibitor, has been utilized experimentally in the attenuation of warm and cold ischemia and reperfusion injury of various organs since 1971 [1]. Initial studies on hemorrhagic shock in 1969 analyzed the effect that this drug had on the potential loss of functional purine bases during hypoxia-related events [2].

Studies in rats have shown a protective benefit when animals that underwent liver ischemia reperfusion injury were pretreated with allopurinol. These studies have

demonstrated that allopurinol's protective mechanism [3–14] may be wholly or partly due to inhibition of the irreversible breakdown of purine metabolites, thereby facilitating intracellular ATP production, inhibition of the formation of reactive oxygen species (ROS), and protection against mitochondrial membrane damage [3–14].

This paper reviews allopurinol's role in warm liver ischemia and reperfusion. Additionally, it identifies the ideal dose of allopurinol in liver ischemia and reperfusion and emphasizes the mechanisms of injury and drug protection.

Brief history on the use of allopurinol in hepatic ischemia reperfusion injury

Allopurinol (Fig. 1) is best known for its use in the treatment of gout with hyperuricemia, even though it was discovered in an effort to develop anticancer drugs. In the 1950s, Hitchings noticed that purine antimetabolites had antitumor activity in cultures of transplanted tumors, but their effect was limited because the tumors were refractory to treatment [15]. Developed in 1963 [16], allopurinol was used in conjunction with the antitumor drug 6-mercaptopurine (6-MP) because it was both a substrate and an inhibitor of the enzyme that metabolized 6-MP [15]. Allopurinol was successful in slowing the degradation of 6-MP in human trials and is used today in secondary gout induced by tumors, radiation, or chemotherapy [17–19]. It was incidentally noted that there was a decrease in the uric acid in serum and the urate in urine accompanied by a concomitant increase in xanthine and hypoxanthine in urine of these same original patients [15].

With the knowledge that by inhibiting xanthine oxidase, allopurinol inhibited the formation of uric acid from xanthine and xanthine from hypoxanthine, the focus of its use was consequently shifted to the treatment of hyperuricemia in gout.

In 1969, Crowell and associates tested allopurinol pretreatment in hemorrhagic shock. It was their hypothesis that xanthine oxidase prevented the irreversible breakdown of purine substrates that could be used for ATP re-synthesis after the ischemic insult [2]. As early as 1971, the effect of allopurinol in ischemic tissues was tested, starting with the ischemic myocardium by DeWall and group [1]. In 1972, Vasko et al. [20] demonstrated the protective effect of allopurinol in renal ischemia. In 1973, Toledo-Pereyra et al. [21] studied the effect of allopurinol in ischemically preserved kidneys for transplant. Others, like Lazarus et al. [22] used allopurinol in hemorrhagic shock to test hepatic function. The first experimental testing of allopurinol pretreatment in liver donors was performed by Toledo-Pereya et al. [23] in 1975. This experiment found increased survival in canine donor liver recipients if pretreated with isoproterenol, allopurinol, and heparin [23].

In 1968, McCord and Fridovich [24] found that reduction of cytochrome c by xanthine oxidase produced ROS. It was not until the 1980s that it was considered that ischemia reperfusion injury (IR) was associated with ROS as a mechanism of injury [25]. Recalling that reactive oxidative species were formed in both reactions catalyzed by xanthine oxidase, theories arose as to allopurinol's mechanism of protection in IR. Since that time, theories of its usefulness and its mechanism of protection in ischemic and reperfused tissues have continued.

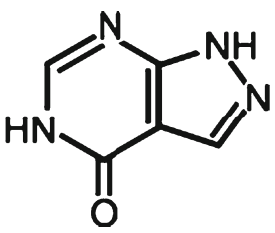

Fig. 1 The chemical structure of allopurinol, [1,4]dihydropyrazolo[4,3-d]pyrimidin-7-one (chemical formula $C_5H_4N_4O$) (mol. wt. 136 g/mol)

Mechanism of allopurinol in ischemia reperfusion injury

Although a beneficial effect of allopurinol in IR injury has been frequently documented, there has not been a general consensus as to the mechanism by which it exerts its beneficial effect. As a way of explanation, we can begin by endeavoring to understand the pathway and metabolites associated with allopurinol and IR.

Xanthine dehydrogenase (XDH) is an oxidizing agent of hypoxanthine to xanthine and xanthine to uric acid. In the creation of these reduced molecules under normal conditions, nicotinamide adenine dinucleotide (NAD+) is used as an electron acceptor. In ischemia, intracellular calcium homeostasis is disturbed and influx of calcium into the cell triggers activation of many enzymes including a protease that changes XDH such that it can no longer reduce NAD+ and is called xanthine oxidase (XO). XO instead transfers the electrons to molecular oxygen creating ROS [26]. ROS are generally accepted as harmful to a cell's homeostasis, and their insult can eventually lead to cell death. This is especially relevant in relation to IR injury, since the liver has the highest concentration of XDH in the body [27] (Fig. 2).

As we analyze the mechanistic approach of allopurinol in liver IR, several theories are readily evident. The first

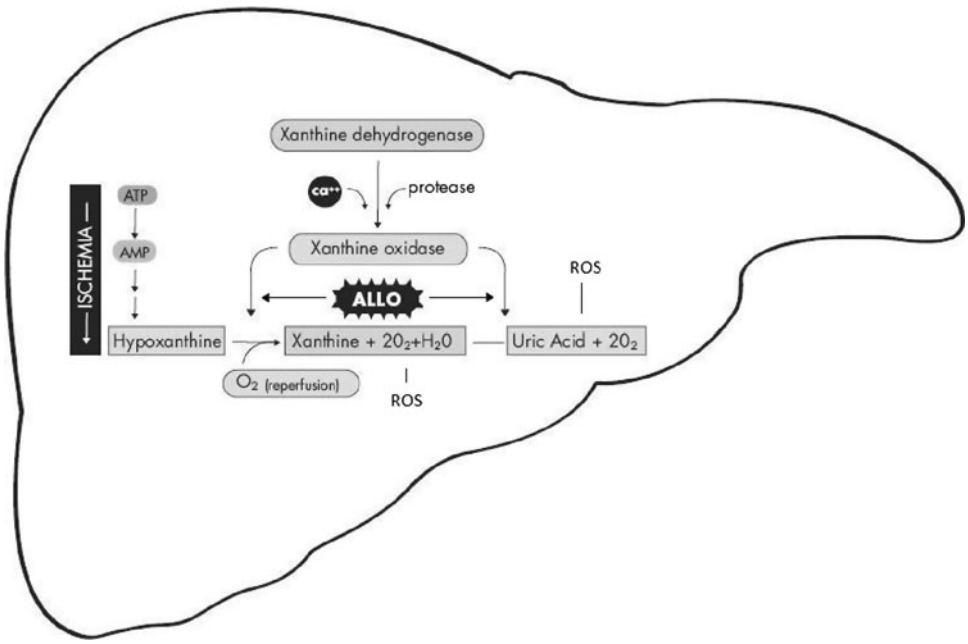

Fig. 2 Xanthine oxidase inhibition, free radicals (ROS), and liver ischemia

theory can be represented by the initial work of Crowell and associates in 1969 [2]. They hypothesized that allopurinol inhibited the irreversible breakdown of hypoxanthine during ischemia to be later used for ATP re-synthesis. The evidence that dogs with induced hemorrhage had increased survival when pretreated with allopurinol indirectly supported this theory [2]. Since then, additional formal experiments testing this theory have been performed. Several researchers have found that allopurinol pretreatment before hepatic IR insult resulted in increased hypoxanthine accumulation [14, 28], decreased xanthine [10, 29], increased adenosine/xanthine ratio [7], increased total adenine nucleotide concentration during ischemia [10], decreased total purine catabolites [10], and a resultant increase in energy phosphates after reperfusion [7, 14, 29]. In some cases, increases in hypoxanthine did not translate into increased ATP synthesis after reperfusion [4, 28]. Additionally, when hypoxanthine was added to allopurinol pretreatment, no additive benefit was found, suggesting that purine catabolite conservation may not be allopurinol's only mechanism in IR injury [6].

Using the knowledge that XO produces ROS, the second theory suggests that allopurinol was protective in liver IR by inhibiting the formation of ROS [6]. This was demonstrated by the addition of XO pre-ischemically. The additional increased damage suggests it as a primary mechanism in IR injury [6]. In a study that tested preconditioning with allopurinol compared to free radical scavengers, the protective benefit was greatest with allopurinol, indicating that XO and XDH are a primary source of ROS during IR injury [13]. Interestingly, the addition of free radical scavengers pre-ischemically is protective to IR damage, implicating ROS production as the mechanism of IR injury [30, 31].

Other findings describes a different protective effect. Conversion from XDH to XO occurs in ischemia, but the exact timing has been shown to range from 10 min [32] to 2 h [33, 34]. Since it may take 2 h for the enzyme to convert from XDH to XO, and ROS appear long before 2 h, it is difficult to support the role of XO as the primary source of ROS. However, if the conversion occurred in the first 10 min as suggested by Della Corte's studies, this could account for ROS formation by XO as a means of IR injury [32].

Another argument that might not be in accordance with the inhibition of XO as the reason for the protective effect is that allopurinol in very high concentrations is needed to prevent IR injury, concentrations much higher than those needed to completely inhibit the enzyme in vivo [35]. Explanations for this finding are two-fold. First, rats have higher concentrations of XO and XDH in blood and liver than humans [36]. Second, as suggested by Godin et al. [36], allopurinol's mechanism of protection may not be singularly related to the ability to inhibit the formation of ROS by XO. Godin et al. [36] found that in rabbit ischemic myocardium, which has practically undetectable XO activity, allopurinol has a protective effect in IR injury. This research considers that the protective mechanism of allopurinol may not be totally related to the inhibition of ROS generation by XO.

The third theory indicates allopurinol's beneficial effect as the preservation of mitochondrial function by protecting

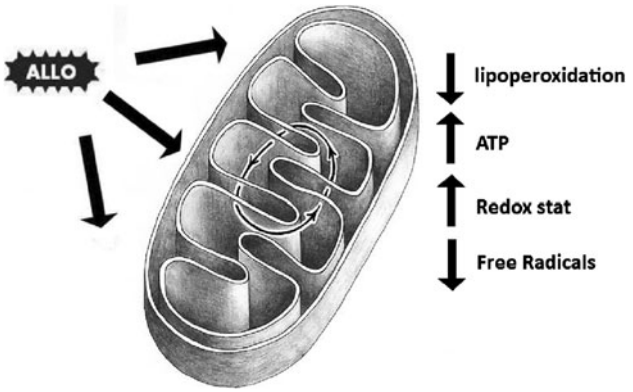

Fig. 3 Xanthine oxidase inhibition and preservation of mitochondrial function

mitochondrial membrane integrity [7, 37]. Support for this theory has been demonstrated by the decreased mitochondrial swelling and cell death [7] and decreased lipid peroxidation [10] with allopurinol pretreatment. It is also suggested that allopurinol has scavenging properties of its own, either by hydroxyl radical [38] or as an electron transfer agent [39] (Fig. 3).

Even though none of these arguments can confirm or refute any one mechanism for allopurinol's role in IR injury, the evidence remains that allopurinol appears to be protective for IR injury.

Allopurinol in liver ischemia-reperfusion injury

Allopurinol appears to be hepato-protective for IR injury as measured by markers of liver injury and function, histopathology, and markers of oxidative stress as reviewed below (Table 1). This work will review studies from all animal models, but primarily the rat as it is the predominant model in this field of research. This presents a challenge in generalizing these findings and predicting results in humans as rodents have higher levels of XO activity [27]. However, using rat models has some advantages, such as the low expense involved, extensive past research in this area for comparison, and avoiding the many ethical hurdles of other models. Since many studies do not utilize the same tests to measure liver injury, this work will systematically review those results in groups by the mechanism of injury measured.

Allopurinol protection in liver IR as measured by markers of liver injury

Liver injury can be measured with traditional markers of tissue injury including alanine aminotransferase (ALT), aspartate aminotransferase (AST), which are both specific to the liver, and lactate dehydrogenase (LDH), which is not. Measured after reperfusion, along different time intervals, allopurinol has been shown to attenuate the IR induced rise in both transaminases [7–10, 13, 37, 40–43] and LDH [9, 40]. There are a few studies that have shown allopurinol has no effect on transaminases [5, 44] and that LDH [42, 45] increases. In most cases, if the attenuation in transaminase increases was not seen immediately, it occurred at least 5 h after reperfusion had elapsed [10, 37, 41, 42]. However when the period was extended to 24 h, no effect was observed by Metzger et al. [5]. The majority of these studies have shown that allopurinol has a protective effect on liver tissue injury based on transaminases and LDH.

Allopurinol protection in liver IR injury as measured by markers of liver metabolic response

Liver injury can also be measured indirectly by detecting the liver's ability to perform other metabolic responses, for example, the ability to conjugate bilirubin [13]. Allopurinol's effect on this is divided into no effect or a significant increase in conjugated bilirubin [29]. Bile flow has been shown in experiments to cease after 10 min of ischemia [11]. However, measuring the return of bile flow in reperfusion can determine the liver's ability to secrete bile into ducts. This was measured by cannulating the efferent bile duct [11, 37] and by measuring alkaline phosphatase [13]. In these experiments, it was shown that allopurinol pretreatment significantly restored bile flow in reperfusion of IR injury [11, 13, 37].

It is worth mentioning that early studies on the effect of allopurinol on hepatic injury after reperfusion were measured in terms of RNA synthesis in dogs with induced hemorrhage [4]. It was found that pretreated animals had improved RNA synthesis after hemorrhage compared to controls [4].

Allopurinol protection in liver IR injury as measured by histopathology

Measuring cellular damages histologically can be undertaken in many different ways. Allopurinol has been shown to have a protective effect on hepatic congestion [40, 41], fatty degeneration [40], necrosis [8, 13, 40], apoptosis [8, 9], lesions in central lobule [9], mitochondrial swelling by inhibiting mitochondrial pore opening [7, 37], and intracellular [4] and extracellular water accumulation [40] as a measurement of membrane damage. However, one experiment showed no protective benefit of allopurinol pretreatment on lipid droplets in cytoplasm, glycogen granule

Table 1 Allopurinol (ALO) in liver ischemia reperfusion injury experiments

Year	Model	MOA[a]	Conclusions	References
1975	Dog	Liver transplant	Pretreatment of donor liver with a combination of isoproterenol, allopurinol, and heparin increased survival of recipient dogs.	Toledo-Pereyra et al. [23]
1979	Rat	IR total	Allopurinol enhanced significantly the ischemia tolerance of the liver in vitro.	Kupcsulik and Kokas [55]
1979	Rat	Liver IR	Allopurinol, dibenxyline, ATP + $MgCl_2$, glucagon, and aspartic acid reduce ischemic lesions of liver in vivo and in vitro.	Kupcsulik and Kokas [56]
1982	Rat	Liver IR	Pretreatment with allopurinol resulted in hypoxanthine accumulation in ischemic liver but did not have an effect on ATP recovery during reperfusion.	Kamiike et al. [28]
1983	Rat	Liver extirpated, perfused, and stored	Nucleotide degradation, especially steps catalyzed by XO and uricase, is main pathway for ROS in ischemia.	Siems et al. [3]
1985	Rat	Liver IR	Study suggests ALLO produced a beneficial effect by inhibition of production of ROS, not by improved protein synthesis and reduced tissue water.	Nordstom et al. [4]
1986	Rat	Perfusion with donor blood	Implicates oxygen-derived free radicals in hepatocellular injury by IR.	Adkinson et al. [43]
1988	Rat	Liver IR	Study suggests XO may not play a critical role in ROS production during IR injury.	Metzger and Lauterburg [5]
1988	Rat	Liver IR	Oxidant stress may result from activation of leukocytes and macrophages rather than by XO.	Metzger et al. [44]
1989	Rat	Liver IR total	Study suggests ROS as mechanism of IR injury.	Toledo-Pereyra et al. [6]
1989	Rat	Liver IR total	ALLO showed same protective benefit with pretreatment, but no additional benefits conferred when adding a pre-reperfusion dose of ALLO.	Castillo et al. [40]
1990	Rat	Liver IR	Study suggests XDH is a primary source of ROS.	Nauta et al. [13]
1990	Rat	Liver IR	Data suggest ALLO pretreatment preserved hypoxanthine, which was used for ATP resynthesis during reperfusion.	Karwinski et al. [14]
1991	Mouse	Liver IR	Prime mechanism of ALLO may be preservation of hypoxanthine. Protective effect of ALLO can be potentiated by pretreatment before ischemia and reperfusion.	Karwinski et al. [29]
1993	Rat	Liver IR	Study suggests XO may not be the primary source of ROS production.	Karwinski et al. [37]
1994	Guinea pig	Liver IR	Study suggests ALLO has a protective effect on cell membrane during IR.	Durmus et al. [12]
1997	Rat	Liver IR	Study suggests oxidant stress is a mechanism in attenuating liver function after IR.	Karwinski and Søreide [11]
2000	Rat	Liver IR	ALLO protective on hepatic IR in rats.	Rhoden et al. [41]
2001	Rat	Liver IR	ALLO pretreatment protects against mitochondrial injury, which prevents mitochondrial oxidant stress and lipid peroxidation and preservation of hepatic energy metabolism.	Jeon et al. [10]
2002	Human	Liver IR during partial liver resection	Because IR injury was modest, ALLO pretreatment showed no benefit.	Virens et al. [51]
2002	Rat	Liver IR total	Pretreatment with ALLO or pentoxifilline results in lower hepatic enzyme elevation in IR, but together they do not confer any added benefit.	Yildirim et al. [42]
2005	Rat	Liver IR total	Indirectly demonstrated that NO_2^- derived NO by XOR in hepatic IR protects the liver against IR injury in vivo.	Lu et al. [45]
2006	Rat	Liver IR and IPC	ALLO and IPC may benefit during IR by increasing adenosine, lowering xanthine, or facilitating intracellular production of ATP.	Lee and Lee [7]
2008	Mouse	Liver IR total	Both ALLO and apocynin pretreatment were protective, by means of blocking generation of ROS during IR.	Liu et al. [8]
2009	Rabbit	Liver IR total	Protective effect of ALLO pretreatment was probably associated with blocking generation of ROS during IR by inhibiting XO activity.	Taha et al. [9]

ALLO Allopurinol, *IPC* ischemic preconditioning, *IR* ischemia reperfusion injury, *ROS* reactive oxygen species, *XDH* xanthine dehydrogenase, *XO* xanthine oxidase

[a] Unless otherwise noted, ischemia was produced by clamping hepatic veins and arteries to medial and left lobes of the liver only

disappearance, loss of normal endoplasmic reticulum architecture, and disappearance of outer nuclear membrane [12]. The tissue samples in this study were taken after 45 min of ischemia but before reperfusion. In all of the other histological studies where significant findings were produced with allopurinol pretreatment, samples were taken after reperfusion began. These findings are good evidence that damage from oxygen species occurs after reperfusion begins. Most studies show allopurinol improves liver response to IR injury histopathologically (Fig. 2).

Allopurinol protection in liver IR injury as measured by markers of oxidative stress

Oxidative stress is a direct measure of tissue injury as it is known that it will greatly injure or ultimately destroy cells. Intracellular ROS can be measured directly by liver peroxide levels, or indirectly by malondialdehyde (MDA), a byproduct of lipid peroxidation. Several studies show that allopurinol pretreatment significantly decreased liver peroxidation [7, 8, 10, 12, 37, 41]. However, other studies dispute these findings, indicating other extracellular sources, such as Kupffer cells and polymorphonuclear cells, as the primary means of ROS production [46–48].

Reducing hydrogen peroxide by glutathione peroxidases is a mechanism of protecting cells from oxidative stress. In this process, glutathione (GSH) is oxidized to glutathione disulfide (GSSG). Measuring GSSG concentration in bile has been shown in animal models to be a sensitive index of oxidant stress in vivo [49, 50], but the GSSG/GSH ratio is a more sensitive index of intrahepatic oxidative stress [50]. GSH can be used as a marker of membrane damage based on leakage into the extracellular space [50]. Results of experimental models after hepatic IR injury disagreed, suggesting allopurinol pretreatment decreased oxidant stress [7, 8, 11, 12, 37] or did not have an effect on oxidant stress [5, 44, 51] as measured by intracellular GSH stores, biliary GSH, and GSSG efflux, and decreases in mitochondrial glutamate dehydrogenase.

Oxidative stress causes a halt in cellular aerobic respiration and hence depletion of energy stores. Therefore, oxidative stress can be measured indirectly by changes in cellular ATP during hepatic IR injury, or by measuring byproducts of anaerobic respiration including lactic acid. Allopurinol pretreatment attenuated the rise in lactic acid formation [40], a sign that hepatic energy stores were more plentiful and anaerobic respiration was less necessary. Allopurinol also showed a beneficial effect in hepatic IR injury by increasing ATP stores after reperfusion, increasing total adenine nucleotide concentration, and decreasing purine catabolites compared to controls. Accordingly, these studies show that allopurinol improves resynthesis of ATP during reperfusion [7, 10, 14, 29, 52]. One study showed a detrimental effect; allopurinol pretreated animals had decreased ATP compared to controls [45]. In this study the dose of allopurinol was minimal at 1.5 mg/kg in a rat model. It has been shown by Jeon et al. [10] that the hepatoprotective dose in rats is 50 mg/kg. These data suggest that part of allopurinol's beneficial mechanism is increased aerobic respiration and a reciprocal decrease in anaerobic response.

A newer field of study is exploring allopurinol's effect on molecular signaling pathways within the liver. Since it is known that ROS can activate inflammation, some have investigated if allopurinol would have an inhibitory effect on the inflammatory pathways by decreasing ROS production. Liver production of TNF-α, an inflammatory cytokine, as measured by ELISA, was shown to be decreased with allopurinol pretreatment [8]. Allopurinol pretreatment in lobar liver ischemia reduced NF-kappa B activation [53]. NF-kappa B is a quick responder to inflammatory stimulus, activating the transcription of genes that are pro-inflammatory, cell proliferative, and anti-apoptotic [53]. Both of these studies suggest that ROS produced by XO may have an impact on NF-kappa activation. High mobility group box 1 (HMGB1) is a nuclear factor released extracellularly as an early mediator of IR injury. In liver IR, it is released as early as 1 h after reperfusion, and inhibition of HMGB1 significantly decreased liver damage after IR [54]. Science is sure to benefit as molecular biology explores allopurinol's effect in cell signaling during IR injury.

Human study of allopurinol pretreatment in ischemia reperfusion injury

In the period of 1992–1994, Vriens and group [51] prospectively followed a group of 16 human patients with IR injury to the liver induced by liver resection of five or fewer colorectal metastases confined to the liver. Half of the patients were pretreated with 10 mg/kg of allopurinol given p.o. the day before the operation with two additional doses of 5 mg/kg just before the operation and just before reperfusion. Ischemia to the liver was induced for a minimum of 30 min. Measurements of liver injury were determined in terms of MDA, GSH, GSSG, vitamin C, coagulation studies, ALT/AST, LDH, and albumin.

Within 24 h after reperfusion, MDA, GSH/GSSG, and vitamin C were not significantly different between control and allopurinol groups. PT, PTT, fibrinogen, and factor V were not significantly different between groups and in most cases were not elevated. Albumin, AST, ALT, and LDH were measured up to 10 days postoperatively, and no

significant differences were seen between the two groups, although trends were identified in each. An almost double increase of AST in the control group occurred from post-operative day 1 through 4 compared to the allopurinol pretreated group. The ALT levels of the treated group decreased drastically on post-operative day 2 compared to a decrease on day 3 for the control group. LDH levels were twice as high in the control group compared to the allopurinol treated group [51].

The authors concluded that liver tissue must be more resistant to IR injury since no significant elevation in markers that measure oxidative damage was found in the control group. This model therefore was not an appropriate one to test the effect of allopurinol in liver IR.

There are several issues that need to be critically considered prior to defining the role of allopurinol in human liver ischemic injury. One, it is related to the model utilized; in the case of Vriens et al. [51], the time of liver ischemia was variable and at times suboptimal at 30 min. Ideal times for assessing ischemia should be 60 min or more to allow for enough oxidative damage for the allopurinol to exert its antioxidant effect. Two, the amount of allopurinol used was extremely low at 10 mg/kg orally and 5 mg/kg twice intravenously. The recommended dose is 50 mg/kg for the treatment of experimental livers subjected to warm ischemic injury. However, caution should be exercised when allopurinol is given to humans in spite of the fact that no single side effect has been observed in animals treated at this dose. Third, the time of administration, although more variable, should remain at around 1 h before ischemia.

For a successful clinical trial, liver resection with the characteristics mentioned above would be the best suitable model. In human liver transplantation, the best model would be on livers obtained from cardiac death donors with approximately 20 min of warm time or those recovered from living liver donors with ischemic injury present. It is unknown in this last circumstance what the time of ischemia should be. At any event, in short, the dose and time of administration are essential when considering the preferred model for the use of allopurinol in human ischemic injured livers.

Ideal dose and timing of allopurinol pretreatment for liver IR injury

Tables 2, 3, and 4 address the effect of the dose of allopurinol given to rats undergoing liver IR. In order to compare the various doses utilized in the literature, common means of measuring liver injury were chosen. Transaminase studies were selected due to their frequent use by experienced researchers and their acceptance as reliable indicators. Other means of studying liver IR injury, such as GSH, have not yielded significant results due to lack of data.

Table 3 shows the effect of allopurinol dose on liver function tests in liver IR. When the chi-squared test was

Table 2 Effect of dose of allopurinol in liver IR on liver function tests (ALT and AST)

Effect	Dose of allopurinol (mg/kg)	References
Positive effect[a]	10	Taha et al. [9]
	50	Castillo et al. [40]
		Yildirim et al. [42][b]
		Liu et al. [8]
		Rhoden et al. [41][c]
		Jeon et al. [10][d]
		Lee and Lee [7]
		Nauta et al. [13]
Neutral effect[e]	10	Jeon et al. [10]
	25	Jeon et al. [10]
	50	Metzger and Lauterburg [5]
Detrimental effect[f]	1.5	Lu et al. [45]

[a] Positive effect was determined as a significant decrease in transaminases of animals pretreated with allopurinol compared to control animals

[b] Positive effect at 15 and 45 min after reperfusion and neutral effect at 45 min of ischemia

[c] Positive effect at 48 h and neutral effect at 0, 24, or 72 h after reperfusion

[d] Positive effect at 5 h and neutral effect at 0 or 1 h after reperfusion

[e] Neutral effect was determined as no significant difference in transaminases between animals pretreated with allopurinol and controls

[f] Detrimental effect determined as a significant increase in transaminases of animals pretreated with allopurinol compared to control animals, measured at 3 h after reperfusion

Table 3 Chi-squared analysis of the allopurinol dose versus effect on liver IR in liver function tests

	Less than 50 mg/kg	50 mg/kg
Dose versus effect on liver IR in liver function tests		
Positive effect	1	7
Neutral effect	2	1
Negative effect	1	0
Pearson chi-squared		
Value	5.063	
Degrees of freedom	2	
Asymptotic significance (2-sided)	0.080	

When the chi-squared goodness-of-fit test is used for the 50 mg/kg group to test for equal proportions of effects, a p value of 0.004631 was produced. This indicated that, within the 50 mg/kg group, the proportions of samples with each effect were not all the same

Table 4 Effect of timing of administration of allopurinol on liver IR in liver function tests (ALT and AST)

Effect	Timing of administration	References
Positive effect[a]	48 h and 24 h before reperfusion	Nauta et al. [13]
	24 h and 1 h before operation	Liu et al. [8]
	18 h and 1 h before ischemia	Lee and Lee [7]
	18 h and 1 h before ischemia	Jeon et al. [10][b]
	5 h and 1 h before reperfusion	Rhoden et al. [41][c]
	4 h and 10 min before ischemia	Castillo et al. [40]
	4 h before ischemia	Castillo et al. [40]
	15 min before ischemia	Yildirim et al. [42][d]
	10 min before ischemia	Taha et al. [9]
Neutral effect[e]	10 min before ischemia and at reperfusion	Castillo et al. [40]
	At reperfusion	Castillo et al. [40]
Detrimental effect at 3 h after reperfusion[f]	4 h before laparotomy	Lu et al. [45]

[a] Positive effect was determined as a significant decrease in transaminases of animals pretreated with allopurinol compared to control animals

[b] Positive effect at 5 h and neutral effect at 0 or 1 h after reperfusion

[c] Positive effect at 48 h and neutral effect at 0, 24, or 72 h after reperfusion

[d] Positive effect at 15 and 45 min after reperfusion and neutral effect at 45 min of ischemia

[e] Neutral effect was determined as no significant difference in transaminases between animals pretreated with allopurinol and controls

[f] Detrimental effect was determined as a significant increase in transaminases of animals pretreated with allopurinol compared to control animals, measured at 3 h after reperfusion

analyzed with only the 50 mg/kg group, the p values yielded a significant difference at <0.05. From these studies, allopurinol at 50 mg/kg was significantly more likely to result in a high probability of success in liver IR as judged by a significant decrease in liver transaminases.

Table 4 indicates the role of timing of allopurinol administration in rats with liver IR injury as measured by transaminases. Even though there was a clear demonstration of a relationship between timing of administration and the beneficial effect, these studies showed the extensive window of administration in which allopurinol might still be beneficial. There is not a clear explanation for the physiological response as not enough studies included a specific time period of administration (Table 4).

Conclusion

The mechanism by which allopurinol exerts a protective effect on liver IR injury is still under debate, but strong evidence exists to support that allopurinol aids in resynthesis of ATP by inhibiting the breakdown of its catabolites, inhibiting the formation of ROS, and preventing mitochondrial membrane damage, therefore decreasing anaerobic respiration.

In many animal studies of liver IR injury, allopurinol has been shown to decrease damage, increase functional response after IR injury, and decrease oxidative stress. A study in humans cannot be evaluated at this point because of the surgical model utilized. Recommendations for improving the model are indicated.

Based on the results of liver function tests, experimental data indicated that 50 mg/kg of allopurinol was the ideal dose to achieve a consistent protective effect in animals with liver IR. The timing of administration of allopurinol was not uniform enough, but successful outcomes often centered around 1 h prior to ischemia. Finally, evaluation of further work on this compound will permit establishing more definitive conclusions in this area.

References

1. DeWall RA, Vasko KA, Stanley EL, Kezdi P. Responses of the ischemic myocardium to allopurinol. Am Heart J. 1971;82:362–70.
2. Crowell JW, Jones CE, Smith EE. Effect of allopurinol on hemorrhagic shock. Am J Physiol. 1969;216:744–8.
3. Siems W, Mielke B, Müller M, Heumann C, Räder L, Gerber G. Status of glutathione in the rat liver. Enhanced formation of oxygen radicals at low oxygen tension. Biomed Biochim Acta. 1983;42:1079–89.
4. Nordström G, Seeman T, Hasselgren PO. Beneficial effect of allopurinol in liver ischemia. Surgery. 1985;97:679–84.
5. Metzger J, Lauterburg BH. Effect of allopurinol on oxidant stress and hepatic function following ischemia and reperfusion in the rat. Liver. 1988;8:344–9.
6. Toledo-Pereyra LH, Cederna J, Choudhury S. Oxygen free radicals, allopurinol and the xanthine oxidase pathway during liver ischemia. Surg Res Commun. 1989;5:297–307.
7. Lee WY, Lee SM. Synergistic protective effect of ischemic preconditioning and allopurinol on ischemia/reperfusion injury in rat liver. Biochem Biophys Res Commun. 2006;349:1087–93.

8. Liu PG, He SQ, Zhang YH, Wu J. Protective effects of apocynin and allopurinol on ischemia/reperfusion-induced liver injury in mice. World J Gastroenterol. 2008;14:2832–7.
9. Taha MO, Simões MJ, Noguerol EC, et al. Effects of allopurinol on ischemia and reperfusion in rabbit livers. Transplant Proc. 2009;41:820–3.
10. Jeon BR, Yeom DH, Lee SM. Protective effect of allopurinol on hepatic energy metabolism in ischemic and reperfused rat liver. Shock. 2001;15:112–7.
11. Karwinski W, Søreide O. Allopurinol improves scavenging ability of the liver after ischemia/reperfusion injury. Liver. 1997;17:139–43.
12. Durmuş O, Aricioğlu A, Güven T, Oğuz M, Yel M, Türközkan N. The effect of allopurinol on the liver ultrastructure, reduced glutathione and lipid peroxide levels during liver ischemia in guinea pigs. Gen Pharmacol. 1994;25:781–6.
13. Nauta RJ, Tsimoyiannis E, Uribe M, Walsh DB, Miller D, Butterfield A. Oxygen-derived free radicals in hepatic ischemia and reperfusion injury in the rat. Surg Gynecol Obstet. 1990;171:120–5.
14. Karwinski W, Drange A, Farstad M, Ulvik R, Søreide O. 60 min normothermic liver ischemia in rats: allopurinol improves energy status and bile flow during reperfusion. Eur Surg Res. 1990;22:27–33.
15. Rundles RW. The development of allopurinol. Arch Intern Med. 1985;145:1492–503.
16. Nobel Prize. 2010. http://nobelprize.org. Accessed 26 Jan 2010.
17. Product Information. 2000. Zyloprim, allopurinol. Bedminster: Faro Pharmaceuticals (PI revised 2/99). Reviewed Feb 2000.
18. AHFS Drug information. 1999. Bethesda: American Society of Hospital Pharmacists; 1999. pp. 3221–3224.
19. Product Information. 1999. Aloprim, allopurinol sodium for injection. Boca Raton: Nabi (PI revised 6/99). Reviewed Feb 2000.
20. Vasko KA, DeWall RA, Riley AM. Effect of allopurinol in renal ischemia. Surgery. 1972;71:787–90.
21. Toledo-Pereyra LH, Najarian JS. Total recovery of ischemic kidneys treated with allopurinol before transplantation. Surg Forum. 1973;24:302–4.
22. Lazarus HM, Owens ML, Hopfenbeck A. Allopurinol protection of hepatic nuclear function during hemorrhagic shock. Surg Forum. 1974;25:10–2.
23. Toledo-Pereyra LH, Simmons RL, Najarian JS. Protection of the ischemic liver by donor pretreatment before transplantation. Am J Surg. 1975;129:513–7.
24. McCord JM, Fridovich I. The reduction of cytochrome *c* by milk xanthine oxidase. J Biol Chem. 1968;243:5753–60.
25. Granger DN, Rutilli G, McCord JM. Superoxide radicals in feline intestinal ischemia. Gastroenterology. 1981;81:22–9.
26. Jaeschk H. Role of reactive oxygen species in hepatic ischemia-reperfusion injury and preconditioning. J Invest Surg. 2003;16:127–40.
27. Southard JH, Marsh DC, McAnulty JF, Belzer FO. Oxygen-derived free radical damage in organ preservation: activity of superoxide dismutase and xanthine oxidase. Surgery. 1987;101:566–70.
28. Kamiike W, Watanabe F, Hashimoto T, et al. Changes in cellular levels of ATP and its catabolites in ischemic rat liver. J Biochem. 1982;91:1349–56.
29. Karwinski W, Farstad M, Ulvik R, Søreide O. Sixty-minute normothermic liver ischemia in rats. Evidence that allopurinol improves liver cell energy metabolism during reperfusion but that timing of drug administration is important. Transplantation. 1991;52:231–4.
30. Atalla SL, Toledo-Pereyra LH, MacKenzie GH, Cederna JP. Influence of oxygen-derived free radical scavengers on ischemic livers. Transplantation. 1985;40:584–90.
31. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med. 1985;312:159–63.
32. Stripe F, Della Corte E. 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.
33. Engerson TD, McKelvey TG, Phyne DB, Boggion EB, Snyder SJ, Jones HP. Conversion of xanthine dehydrogenase to oxidase in ischemic rat tissues. J Clin Invest. 1987;79:1564–70.
34. Greenwalk RA, Cohen G, editors. Oxygen radicals and their scavenger systems. Cellular and medical aspects. New York: Elsevier; 1983.
35. Jaeschke H. Glutathione disulfide formation and oxidant stress during acetaminophen-induced hepatotoxicity in mice in vivo: the protective effect of allopurinol. J Pharmacol Exp Ther. 1990;255:935–41.
36. Godin DV, Bhimji S. Effects of allopurinol on myocardial ischemic injury induced by coronary artery ligation and reperfusion. Biochem Pharmacol. 1987;36:2101–7.
37. Karwinski W, Ulvik R, Farstad M, Svardal A, Berge R, Søreide O. Effect of allopurinol on the concentration of endogenous glutathione in hepatocytes after an hour of normothermic liver ischemia. Eur J Surg. 1993;159:355–9.
38. Moorhouse PC, Grootveld M, Halliwell B, Quinlan JG, Gutteridge JM. Allopurinol and oxypurinol are hydroxyl radical scavengers. FEBS Lett. 1987;213:23–8.
39. Peterson DA, Kelly B, Gerrard JM. Allopurinol can act as an electron transfer agent. Is this relevant during reperfusion injury? Biochem Biophys Res Commun. 1986;137:76–9.
40. Castillo M, Toledo-Pereyra LH, Prough D, Sharpiro E, Gordon D, Choudhury S. Effective timing of allopurinol administration in the ischemic liver. Transplantation. 1989;47:727–30.
41. Rhoden E, Pereira-Lima L, Lucas M, et al. The effects of allopurinol in hepatic ischemia and reperfusion: experimental study in rats. Eur Surg Res. 2000;32:215–22.
42. Yildirim S, Tok H, Köksal H, Erdem L, Baykan A. Allopurinol plus pentoxifilline in hepatic ischaemia/reperfusion injury. Asian J Surg. 2002;25:149–53.
43. Adkison D, Höllwarth ME, Benoit JN, Parks DA, McCord JM, Granger DN. Role of free radicals in ischemia-reperfusion injury to the liver. Acta Physiol Scand Suppl. 1986;548:101–7.
44. Metzger J, Dore SP, Lauterburg BH. Oxidant stress during reperfusion of ischemic liver: no evidence for a role of xanthine oxidase. Hepatology. 1988;8:580–4.
45. Lu P, Liu F, Yao Z, et al. Nitrite-derived nitric oxide by xanthine oxidoreductase protects the liver against ischemia-reperfusion injury. Hepatobiliary Pancreat Dis Int. 2005;4:350–5.
46. Schauer RJ, Bilzer M, Kalmuk S, et al. Microcirculatory failure after rat liver transplantation is related to Kupffer cell-derived oxidant stress but not involved in early graft dysfunction. Transplantation. 2001;72:1692–9.
47. Jaeschke H, Farhood A. Neutrophil and Kupffer cell-induced oxidant stress and ischemia-reperfusion injury in rat liver. Am J Physiol. 1991;260:G355–62.
48. Caraceni P, Ryu HS, van Thiel DH, Borle AB. Source of oxygen free radicals produced by rat hepatocytes during postanoxic reoxygenation. Biochim Biophys Acta. 1995;1268:249–54.
49. Adams JD Jr, Lauterburg BH, Mitchell JR. Plasma glutathione and glutathione disulfide in the rat: regulation and response to oxidative stress. J Pharmacol Exp Ther. 1983;227:749–54.
50. Lauterburg BH, Smith CV, Hughes H, Mitchell JR. Biliary excretion of glutathione and glutathione disulfide in the rat. Regulation and response to oxidative stress. J Clin Invest. 1984;73:124–33.
51. Vriens MR, Marinelli A, Harinck HI, Zwinderman KH, van de Velde CJ. The role of allopurinol in human liver ischemia/

reperfusion injury: a prospective randomized clinical trial. Hepatogastroenterology. 2002;49:1069–73.
52. Karwinski W, Farstad M, Ulvik R, Sreide O. Sixty minutes normothermic ischemia in rat liver: the declining tissue concentration of hypoxanthine during reperfusion is not a washout phenomenon. Eur Surg Res. 1992;24:257–64.
53. Matsui N, Satsuki I, Morita Y, et al. Xanthine oxidase-derived reactive oxygen species activate nuclear factor kappa B during hepatic ischemia in rats. Jpn J Pharmacol. 2000;84:363–6.
54. Tsung A, Sahai R, Tanaka H, et al. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. J Exp Med. 2005;7:1135–43.
55. Kupcsulik P, Kokas P. Ischemic damage of the liver. Part I: In vitro investigation of the prevention of the ischemic lesion of the liver. Acta Hepatogastroenterol (Stuttg). 1979;26:279–83.
56. Kupcsulik P, Kokas P. Ischemic damage of the liver. Part II: In vivo investigation of the prevention of the ischemic lesion of the liver. Acta Hepatogastroenterol (Stuttg). 1979;26:284–9.