

Review

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Review

Xanthine Oxidase: A Longstanding Target in Acute Pancreatitis with New Ways

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Abstract: Acute pancreatitis (AP) is a potentially fatal organ disease that is widespread globally. Although significant progress has been made in the previous decade, the study of mechanisms and therapeutic strategies is still far from being completed. Xanthine oxidase (XO) is an enzyme that generating urate and also responsible to produce reactive oxygen species (ROS). Considerable clinical and mechanical research has been conducted over many decades to explore the function of XO in AP and its promising therapeutic applications. Unquestionably, XO is a key part of starting and progressing AP by ROS production. Not fully known, though, are XO's many other functions besides ROS resource in both local and global processes, such as its role in cellular signaling or metabolism regulation. This review seeks to summarize the function of XO in AP pathophysiology by analyzing the inherent biological and chemical properties of XO, and lastly provide fresh concepts for future research.

Keywords: Xanthine oxidase; acute pancreatitis; drug target; cellular mechanism; multiple organ failure

1. The Major Challenge: Mechanism, Target, and Drug Discovery of Severe AP

Acute pancreatitis (AP) is a prevalent gastrointestinal disorder marked by intense pain¹. The estimation of AP incidence ranges from 110 to 140 per 100,000 population, which has been increasing². Gallstones, alcohol and triglyceridemia rank as the top etiologies³. The clinical guidance highlighted the need of supportive therapy including goal-directed fluid resuscitation, early oral feeding, enteral nutrition fluid control, and nutrition as aspects of the current therapeutic approach⁴. It is well acknowledged that 80% of AP patients are mild cases, which are mostly self-limited. But there still remains 20% AP patients developed severe AP (SAP), with local or systematic complications⁵. The high mortality of SAP, ranging from 20% to 40%, has been regarded as a critical problem⁵. Nevertheless, the most recent Cochrane review⁶ indicates that medication development is generally impeded resulting in restricted clinical intervention for AP, particularly in severe cases⁷. Therefore, a key component in achieving therapeutic success is to hinder the transition from mild to SAP.

By now, pathological research based on laboratory studies of AP has become the foundation of efforts. In summary, the mechanisms can be categorized into two primary processes: disturbance of acinar cell homeostasis and the amplification of the inflammatory cascade⁷. The major pathological events in acinar cells including calcium overload, trypsinogen activation, mitochondrial dysfunction and autophagy failure⁸. When the local inflammatory damage progresses to systemic alterations, it will lead to the activation of multiple organ failure, primarily respiratory and circulatory failure, which in turn contributes to the high fatality rate in SAP⁹. Therefore, identifying the pathogenic processes and associated targets that contribute to and worsen the development of AP is the crucial step in achieving a substantial decrease in clinical mortality.

2. What Makes XO a Drug Target

2.1. General Profile of XO and Target Characteristics

XO is a molybdoflavo, dimeric protein with a net molecular weight of approximately 290 kDa¹⁰. Each monomeric unit is composed of 3 major domains in (Figure 1), a small Fe/S center (amino acid: 3-165, molecular weight: 20 kDa), an intermediate Flavin Adenine Dinucleotide (FAD) domain (amino acid: 226-531, molecular weight: 40 kDa), and the Molybdopterin (Mo-Pt) center (amino acid: 590-1332, molecular weight: 85 kDa), which is the most prominent domain, the domain where hydro-oxidation takes place¹¹.

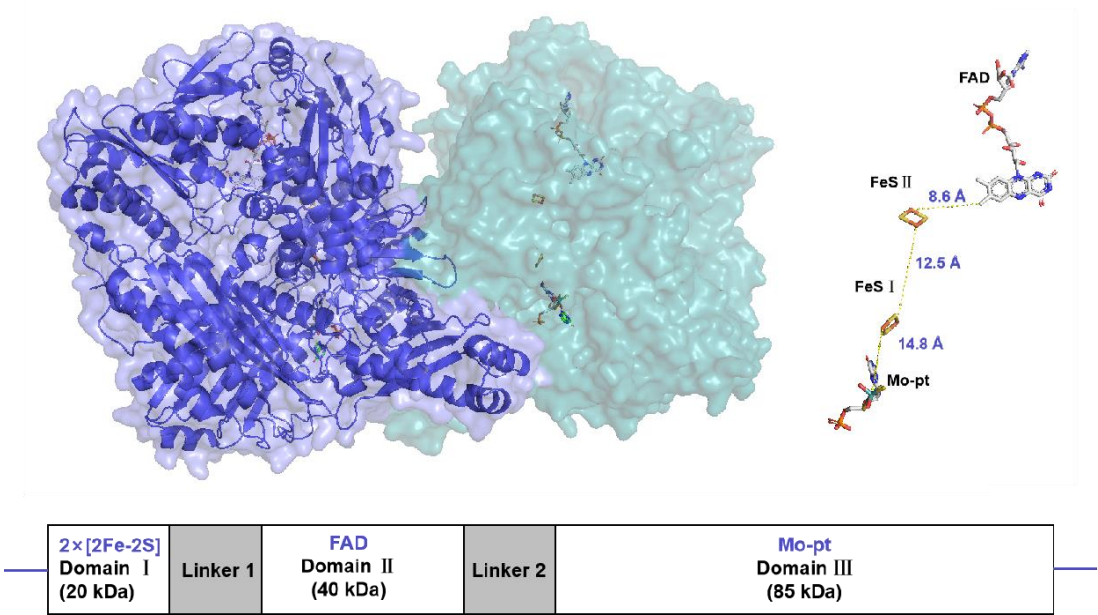


Figure 1. Surface representation of the XO crystal structure homodimer (PDB:3NRZ). Monomer A is in dark blue, while monomer B in light blue. The different protein cofactors (FAD, FeSII, FeSI, and Mo-pt), with the shortest distances between them marked, are represented on the right. The figure was created using PyMOL version 2.6.0.

XO is the oxidase form of xanthine oxidoreductase (XOR), and xanthine dehydrogenase (XDH) represents the dehydrogenase form of XOR. These two proteins are both encoded by the XDH gene and belong to the molybdenum hydroxylase flavoprotein family. Structurally, the main difference between XO and XDH is the FAD-binding domain. They can convert to each other during different conditions. Generally, XDH may be converted to XO via sulfhydryl side group oxidation reversibly, and via proteolysis by serine proteases irreversibly. The former situation, usually induced by hypoxia conditions, and the other transformation path is usually induced by prolonged ischemic conditions. XO transfers electrons to oxygen molecules and produces hydrogen peroxide (H₂O₂) and a superoxide anion, meanwhile, XDH transfers 2 electrons generated during the oxidation of the substrate in each reaction step to one molecule of NAD⁺(Figure 2). In this case, XO uses molecular oxygen as electron acceptor and consequently generates the superoxide anion, a molecule that participates in the generation of other ROS including hydrogen peroxide, hydroxyl radical and peroxynitrite¹². Under physiologic conditions, the enzyme exists mainly as XDH, which utilizes NAD⁺ as an electron acceptor and primarily functions as a dehydrogenase. Conversely, in pathological circumstances, cellular processes such as alterations in intracellular pH or calcium entry lead to the conversion of XOR to its oxidase form, XO, due to restricted proteolytic cleavage.

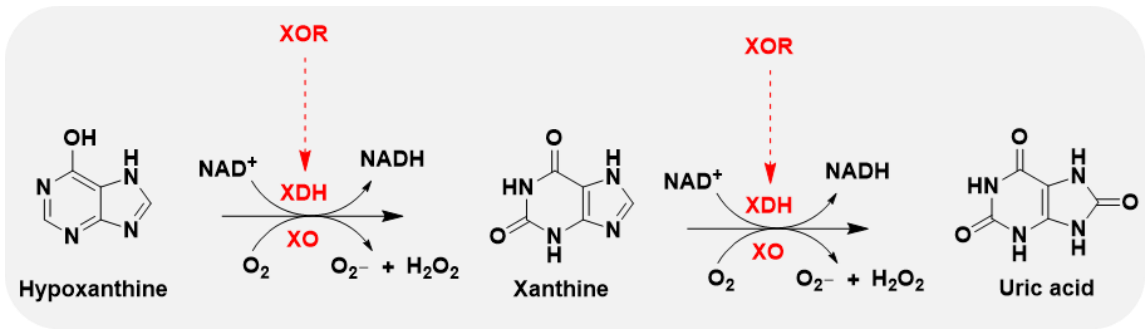


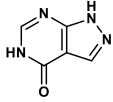
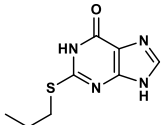
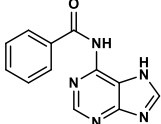
Figure 2. Schematic diagram of the oxidative hydroxylation of hypoxanthine through xanthine to uric acid, and reactive oxygen species generation, in the metabolism of purines.

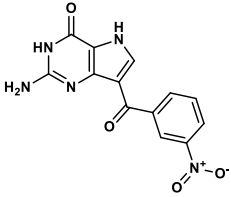
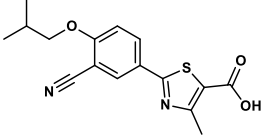
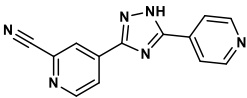
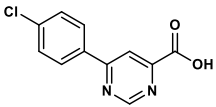
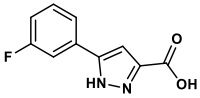
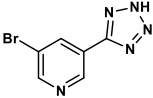
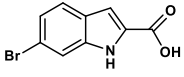
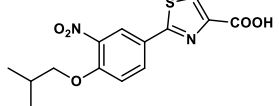
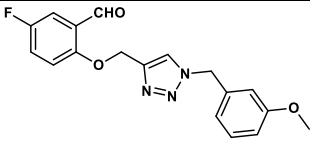
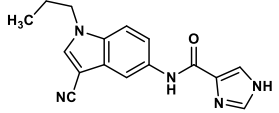
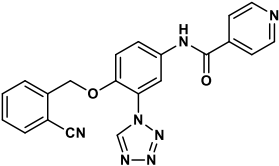
As a superoxide-producing enzyme, XO was found in serum and various organs. XOR is present in all cell types, acting mainly as dehydrogenase, and in most cases, with a low level of activity. The gene expression of XOR was found highest in the intestine and liver, with the highest enzyme activities correspondingly¹³ in other tissues including the brain, heart, lung, kidney, skeletal muscle¹⁴, and pancreas as well¹⁵. Additionally, it has been reported that plasma XOR activity is much lower in humans than in animals. XOR enzyme catalyzes the final 2 steps of purine degradation, namely the oxidation of hypoxanthine and xanthine to uric acid. Circulative XOR activity is associated with obesity, smoking, liver dysfunction, hyperuricemia, dyslipidemia, insulin resistance, and adipokines¹⁶. Two of the most significant contributions of XO to pathophysiology are the production of ROS and the buildup of uric acid. A recent prospective cohort study involving 124,316 people found a linear correlation between uric acid levels and the risk of AP¹⁷, suggesting an essential role of XO in AP progression.

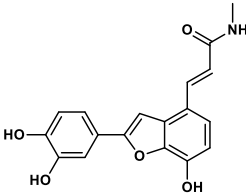
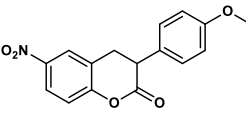
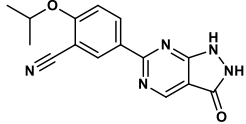
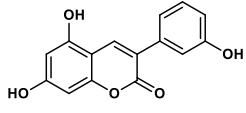
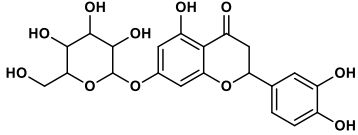
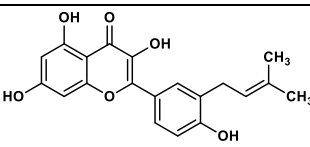
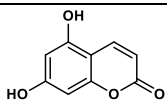
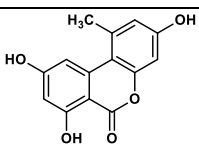
2.2. Inhibitors of XO

Due to the crucial role of XO in the development of acute pancreatitis, the focus has been on identifying potential therapeutic agents that can inhibit this enzyme. A multitude of different compounds have been discovered that can inhibit XO with varying degrees of selectivity and potency. These compounds can be classified into purine analogs, which closely resemble the structure of xanthine, and nonpurine analogs, which differ structurally¹⁸. Moreover, several synthetic and natural compounds with high selectivity are summarized and are presented (Table 1).

Table 1. General structural formulae of recently reported XO inhibitors.

Structure	Class	IC ₅₀ Value	Ref.
 1	Purine derivatives	0.2-50 μM	19
 2	Purine derivatives	0.115 μM	21
 3	Purine derivatives	0.45 μM	22

 4	Purine derivatives	0.065 μM	23
 5	Thiazole derivative	1.8 μM	25
 6	1,2,3-triazoles derivative	0.0053 μM	26
 7	Pyrimidine derivative	18 μM	28
 8	Pyrazole derivative	30 μM	28
 9	Isonicotinamide derivatives	64 μM	28
 10	Imidazole derivatives	82 μM	28
 11	Thiazole derivative	0.0486 μM	29
 12	1,2,3-triazoles	0.70 μM	30
 13	Imidazole derivatives	0.018 μM	31
 14	Isonicotinamide derivatives	0.022 μM	32

 15	Benzo[b]furan derivatives	4.45 μ M	33
 16	3-Phenylcoumarins derivatives	8.4 μ M	18
 17	Pyrimidine derivatives	0.039 μ M	34
 18	Coumarin analogues	2.13 μ M	35
 19	Flavonoid analogues	10.6 μ M	41
 20	Flavonoid analogues	8.45 μ M	42
 21	Coumarin analogues	10.91 μ M	42
 22	Ellagic acid analogues	0.23 μ M	43

(1) Purine analogs

Purine analogs, such as allopurinol (1), have been one focus of development for XO inhibitors. Allopurinol was the first US' Food and Drug Administration (FDA) approved purine based XO inhibitor. At low concentrations, allopurinol competes with the enzyme as a substrate and inhibitor, while at higher concentrations, it acts as a noncompetitive inhibitor, with an IC₅₀ ranging from 0.2-50 μ mol·L⁻¹. Its active metabolite, oxypurinol, binds to XOR and leads to enzyme inhibition. However, the use of allopurinol has been associated with Drug Reactions with Eosinophilia and Systemic Symptoms (DRESS syndrome) and inhibits other enzymes such as purine nucleoside phosphorylase (PNP) and orotidine-5'-monophosphate decarboxylase (OMPDC)²⁰. Other compounds with purine structures, such as, monosubstituted 2-(thioalkyl)purines²¹ (2), 6-(N-benzoylamino)purine²² (3), 9-Benzoyl 9-deazaguanines²³ (4) also been reported; However, these compounds have also been associated with severe side effects, including hypersensitivity with fever, skin rash and eosinophilia, renal toxicity, and gastrointestinal distress²⁴. Therefore, the broad

therapeutic use of these compounds has been limited, highlighting the need for novel, potent non-purine XO inhibitors with fewer adverse effects.

(2) Nonpurine analogs

Synthetic Nonpurine Analogs

Nonpurine analogs, such as febuxostat (5) and topiroxostat (6), have been widely studied as XO inhibitors^{25,26}. Febuxostat, a thiazole derivative, was one of the first non-purine XO inhibitors approved by the FDA. It acts as a highly potent non-competitive inhibitor of human XO²⁵. However, febuxostat has been found to have adverse side effects, such as acute liver dysfunction and an increased risk of heart-related deaths²⁷. Investigation into the inhibition mechanism of febuxostat founded that it exhibits a mixed-type inhibition, probably because of its tight binding to the molibdopterin cofactor of XO, which impedes the entry of the substrate into the active site of the enzyme²⁴. Likewise, topiroxostat also demonstrated a hybrid-type inhibition mechanism by forming a covalent linkage with the active site of the enzyme²⁸.

In response to the limitations of existing nonpurine XO inhibitors, researchers have explored the development of new molecules that are effective and specific in inhibiting XO. During a virtual screening study aimed at the discovery of novel inhibitors targeting another oxidoreductase, superoxide dismutase, four compounds with non-purine-like structures (compound 7, compound 8, compound 9, compound 10), were identified being capable of causing direct inhibition of XO²⁸. Following investigation of the structure–activity relationship around the thiazole core, compound 11, which was structurally related to febuxostat, was identified as a potent XO inhibitor with a novel 2-phenylthiazole-4-carboxylic acid scaffold, with an IC₅₀ value of 0.0486 μ M²⁹. 1,2,3-triazoles derivatives based on the structural features of topiroxostat are found effective inhibitors of XO resulted in reduced production of UA in the body. Zhang et. al. also investigated a series of 4-(phenoxymethyl)-1H-1,2,3-triazole derivatives, with compound 12 emerging as the most effective XO inhibitor with an IC₅₀ value of 0.70 μ M, approximately 14 times more potent than allopurinol. In vitro results, compound 12 showed potent hypouricemic effects at an oral dose of 20 mg/kg in a rat hyperuricemia model induced by potassium oxonate³⁰. They also conducted a study focusing on the potential of N-(1-alkyl-3-cyano-1H-indol-5-yl) aromatic heterocyclic carboxamide derivatives as XO inhibitors. They identified an optimized compound 13 with an IC₅₀ value of 0.018 μ M, demonstrating comparable potency to the known XO inhibitor topiroxostat³¹. Building on this work, they proceeded to design and synthesize a series of N-(4-alkoxy-3-(1H-tetrazol-1-yl) phenyl) heterocyclic aromatic amide derivatives based on the framework of a previously reported compound, identifying compound 14 with an IC₅₀ value of 0.022 μ M as the most promising inhibitor. This compound possesses a unique 1H-imidazole-5-carboxamide scaffold and comparable potency to topiroxostat³². Moreover, recent studies have reported the development of XO inhibitors based on benzo[b]furan scaffolds (15), 3-Phenylcoumarins (16), pyrimidine (17) and coumarin (18), further expanding the potential pool of efficacious XO inhibitors^{18,33–35}.

Natural Nonpurine Analogs

Natural nonpurine analogs such as flavonoids, chalcones, terpenoids, alkaloids, coumarins, phenolic acids, and glycosides have been found to effectively inhibit XO. Flavonoids, a type of natural phenolic compound, have received the most attention, have been found to strongly inhibit XO in several studies³⁶. An integrated method combining enzyme kinetics, multi-spectroscopic spectra, and molecular simulations was used to investigate the structure-activity relationship and interaction mechanism between different flavonoids and XO. The findings suggest that active flavonoids can bind to XO's active center, block the entrance of substrates, and induce significant structural changes, leading to inhibition³⁷. Among them, flavanols including luteolin, quercetin³⁸, myricetin³⁹ and galangin⁴⁰ exhibited the remarkable inhibitory effect due to the hydroxylation pattern (hydroxylated number and position) on the core scaffold of flavonoids played a key role in their biological activity and affinity on XO, while the hydrogenation of the C2=C3 double bond were unfavorable for its

inhibition potency. An ethanolic extract obtained from *A. microcarpus* flowers containing luteolin 7-O-glucoside (19) displayed a higher XO inhibitory activity compared to the standard inhibitor allopurinol, with an IC₅₀ value of 4.8 µg/mL as opposed to 11.5 µg/mL, respectively in vitro studies⁴¹. A computational approach using molecular docking and free energy calculations led to the discovery of several natural compounds with XO inhibitory properties, including previously known inhibitors as well as newly identified substances like flavonol (20, IC₅₀ = 8.45 ± 0.68 µM) and 5,7-dihydroxycoumarin (21, IC₅₀ = 10.91 ± 0.71 µM) displayed the great potency as mixed-type inhibitors. Further analysis through docking studies and molecular dynamics simulations revealed that these hits interacted with key active site residues of XO, including ARG880, MOS1328, and ASN768⁴². Notably, some novel natural products with different chemotypes have been observed with XO inhibition, which provide novel scaffolds for developing new XO inhibitors. An extract from the endophytic fungus *Alternaria alternata* GDZZ-J6 showed the most potent inhibition of XO, with one of its secondary metabolites, alternariol (AOH, 22), a dibenzo- α -pyrone derivative, exhibiting strong inhibitory activity on XO. The potency of XO inhibition by AOH was more than 12 times higher compared to the standard inhibitor allopurinol⁴³. An integrated analytical model was developed for screening XO inhibitors, leading to the identification of polydatin, a stilbene compound, as a potential active ingredient for XO⁴⁴. These findings present promising potential for the development of natural XO inhibitors with improved efficacy.

3. Role of XO in AP Onset and Deterioration

3.1. Changes of XO Specifically Occur in Necrotic SAP Model Rather Than Edematous Model

To investigate the alterations in XO activity in AP, Devenyi⁴⁵ initially quantified the XO activity of dehydrogenase and oxidase in pancreatic homogenates of mice. The present study documented that following 3 injections of cerulein at a dosage of 50 µg/kg, the activity of pancreatic XO remained in its dehydrogenase form rather than its oxidative form. The study proposed that the oxidative form of XO could be transformed by intrapancreatic protease and concluded that, for the first time, this conversion may take place in other models of AP rather than cerulein-induced AP mice. It is reasonable to conclude that XO does not contribute to the development of mild edematous AP model⁴⁶.

The subsequent investigations demonstrated the obvious enhancement of XO in SAP models. The study conducted by D.Closa⁴⁷ revealed a notable increase in XO activity in rats with sodium taurocholate (NaT)-induced SAP in contrast to rats with cerulein-induced AP. Moreover, in NaT-induced cell death, the XDH was fully transformed into XO within the initial 5 minutes, thereby generating oxygen free radicals (OFRs). This observation implies that XO is the primary origin of OFRs that contribute to further damage of the pancreas⁴⁸. Other studied also reported that in NaT-SAP models, XO appears to undergo substantial alterations and is favorably correlated with pancreatic MPO levels⁴⁹. These dysregulations can directly lead to organ failure associated with AP⁵⁰. Therefore, it looks like XO has a distinct correlation with SAP pathogenesis. Moreover, it has been determined that XO-generated toxicity plays a significant role in AP models that are clinically relevant, such as fatty acid infusion (which corresponds to the cause of hyperlipemia in clinical settings), ischemia (shock), pancreatic duct blockage (gallstone), and acetaldehyde after ischemia (alcohol), rather than the cerulein AP model, which lacks a clear clinical equivalent⁵¹. However, the role of XO in ethanol-induced AP is still a subject of debate. Studies have shown that while ethanol played a significant role in the production of OFRs, the activity of XO in ethanol-induced AP was moderate⁵².

Our latest work⁵³ revealed that in a model of cerulein-induced edematous AP, the expression of the pancreas *Xdh* gene showed a substantial rise 8 hours after the initial injection. However, there was no significant increase observed in the XO protein level. The pancreatic mRNA level and serum enzyme activity of XO increased dramatically in an L-Arginine-induced severe necrotic AP model within 8 hours of modeling, while XO protein levels significantly increased at 72 hours. Furthermore, the severe AP model caused by NAT exhibited a notable rise in serum XO activity within 3 hours

after modeling. Taken together, these data unequivocally indicated that XO was particularly upregulated in the SAP model rather than in mild examples.

3.2. Targeting XO in Experimental AP

The classic XO inhibitors, allopurinol and its metabolite oxypurine, have been widely used in treatment and prevention of AP (Table 2). According to the current literatures, the effect of XO inhibitors on AP models seems controversial. However, this result is due to following reasons including the time and dose of drug administration, and most importantly, type of AP model.

Table 2. experimental studies on Inhibitors of XO.

Year	AP Model	Species	Drug	Dose	Administration	Effects	Drug effect	Ref.
1985	CDE	Mice	Allopurinol	24 mg/kg	Therapeutic	↓Serum amylase, pancreatic histological damage; survival rate	Positive	59
1985	FFA POSS ISCH	Dog	Allopurinol	100 mg	Prophylactic	↓Serum amylase, pancreas edema; pancreatic weight	Positive	60
1987	Cerulein	Mice	Allopurinol	0.7-7 mg/kg/h	Prophylactic	No effect on pancreas edema; no change in serum amylase level	Negative	45
1988	Cerulein	Rat	Allopurinol	20 mg/kg/h	Therapeutic	↓Serum amylase; pancreatic weight	Positive	69
1989	NaTC; CDE	Rat; Mice	Allopurinol	100 mg/kg for rats; 50 mg/kg for mice	Prophylactic	No change in serum amylase/lipase; pancreatic trypsin levels; no effect on histological damage, inflammation and survival	Negative	68
1991	ISCH+AA	Dog	Allopurinol	100 mg/kg	Therapeutic	↓Serum amylase; pancreas edema; pancreatic weight; hemorrhage	Positive	61
1992	PBDO + ISCH	Rat	Allopurinol	20 mg/kg	Prophylactic	↓Serum amylase; histological changes	Positive	58
1996	Cerulein	Rat	Allopurinol	50 mg/kg	Prophylactic	No change in serum amylase, lipase and trypsin level; no effect on pancreatic histological damage	Negative	71
1998	L-arginine	Rat	Allopurinol	100 or 200 mg/kg	Prophylactic	↓Serum amylase; pancreatic MDA; histological damage; catalase activity ↑SOD; GPx	Positive	56
1998	NaTC	Rat	Oxypurinol	10 mM; 0.066 ml/min	Therapeutic	↓Pancreatic MPO, MOD ↑GSH	Positive	50
1998	ERCP	Dog	Allopurinol	5 mg/kg	Prophylactic	↓Serum amylase and lipase; histologic changes	Positive	63

1999	NaTC	Rat	Oxypurinol	10 mM; 0.066 ml/min	Therapeutic	↓Plasma lipase, pancreatic MPO and MOD	Positive	98
2000	L-arginine	Rat	Allopurinol	200 mg/kg	Prophylactic	↓Serum amylase; pancreatic MDA; plasma CCK-like bioactivity; serum TNF-α and IL-6 levels; histological damage; ↑SOD; GPx;	Positive	57
2000	L-arginine	Rat	Allopurinol	200 mg/kg	Prophylactic	↓Serum amylase; histologic changes;	Positive	55
2000	NaTC	Rat	Oxypurinol	10 mM; 0.066 ml/min	Therapeutic	↓Plasma lipase; pancreatic MPO	Positive	100
2002	Ethyl alcohol	Dog	Allopurinol	10 mg/kg	Prophylactic	↓Pancreatic MPO	Positive	54
2004	NaTC	Rat	Oxypurinol	5 mM; 0.066 mL/min for 30 minutes	Therapeutic	↓Serum TNF-α; lung MPO activity	Positive	64
2006	NaTC	Rat	Allopurinol	200 mg/kg	Therapeutic	↓Serum amylase; plasma MDA; histological damage ↑ Plasma SOD and GSH-Px	Positive	67
2007	NaTC	Rat	Allopurinol	200 mg/kg	Therapeutic	↓Serum amylase; tissue MDA; histological damage ↑Tissue SOD and GSH-Px	Positive	65
2012	NaTC	Rat	Oxypurinol	5 mM, 0.066 ml/min for 30 min	Therapeutic	↓GSSH/GSH ratio; serum lipase ↑GSSH	Positive	66

CDE: cholinedeficient ethionine-supplemented diet. FFA: free fatty acid. POSS: partial duct obstruction combined with secretin stimulation. ISCH: ischemia. AA: acetaldehyde. PBDO: pancreatoco-biliary duct obstruction. NaTC: intraductal infusion of 5% sodium taurocholate. MDA: Malondialdehyde. SOD: Superoxide dismutase. GSHPx: Glutathione peroxidase. MLNs: Mesenteric lymph nodes. MPO: myeloperoxidase.

In severe necrotic AP models, both therapeutic and prophylactic administration of XO inhibition showed obvious beneficial effect on pancreatic injury and systematic inflammation. In cases of therapeutical administration, 10 mg/kg of allopurinol intravenous infusion successfully inhibited pancreatic SOD increasing in 15% ethyl alcohol-induced dog AP model⁵⁴. Similarly, in L-Arginine-induced SAP mice model, 200 mg/kg of allopurinol treatment could significantly ameliorated decease severity^{55,56} and cytokines levels including TNF-α and IL-6⁵⁷. The protective effect of allopurinol has been also confirmed in pancreatic duct obligation and ischemia with 20 mg/kg administration⁵⁸, and in choline-deficient ethionine-supplemented diet-induce-induced AP with 24 mg/kg⁵⁹, and in FFA, POSS and ischemia⁶⁰ and also ischemia with acetaldehyde⁶¹-induced AP with 100 mg administration. Meanwhile, prophylactic administration also showed beneficial effects on SAP models. Pretreatment with allopurinol could prevent pancreatic edema and the serum amylase in oleic acid induced-AP mice⁶², and could decrease histologic changes of the pancreatic parenchyma, serum amylase and lipase of pancreatography-induced pancreatitis in dogs⁶³. Inhibiting XO could significantly decrease 3.5% sodium taurocholate-induced pancreatic and lung injury⁶⁴, and systematic oxidative stress in AP rats^{50,65–67}. In addition, Lankisch⁶⁸ investigated the effect of allopurinol on 5% sodium taurocholate-

induced AP rats, and choline-deficient ethionine-supplemented diet-induced AP mice, with administration of allopurinol at 100 mg/kg bodyweight 1h before, and 12, 24, 36 h after surgery for rat, and 50 mg/kg bodyweight 1h before starting diet and every 24 h during diet up to 5 days for mice. This study did not achieve positive results, which may be attributed by the time and dose of drug administration, lower dose of therapeutic usage of inhibitors may be effective according to the previous study⁵⁹.

However, the effect of XO inhibitors in mild AP models did not achieve consistence. Pre-treatment of 5 mg/kg oxypurinol significantly decreased ERCP-induced pancreatitis in canine model⁶³. In cerulein-induced edematous AP, a continuous intravenous infusion of allopurinol (20 mg/kg/h) for 6 hours significantly improved cerulein-induced AP rats model⁶⁹. And in vitro cerulein model, pre-treatment of allopurinol (1 μ M) also showed beneficial effect⁷⁰. On the contrary, Devenyi⁴⁵ reported that 0.7-7mg/kg allopurinol did not improve pancreas edema. Similarly, Takafumi⁷¹ reported that 50 mg/kg allopurinol did not improve serum amylase, lipase, trypsin or pancreatic histological damage.

Collectively, in SAP models including L-Arginine and NaT models, prophylactic or therapeutic usage of XO inhibitors with a dose range from 20 to 200 mg/kg may largely be effective. For choline-deficient ethionine-supplemented diet-induced necrotic AP, therapeutic administration of lower dose around 24 mg/kg is recommended. This result is closely related to the XO increase during different models. Our study⁵³ revealed that XO significantly increased in L-Arginine-induced SAP as early as 8 h after modeling, and also increased in NaT-induced SAP at 3 h after modeling, with no significant changes was observed in cerulein-induced edematous AP model.

3.3. Role of XO in Pancreatic Injury During AP

As a longstanding target in AP, XO was recognized as a ROS resource in pancreatic acinar cells that activating inflammatory signals and oxidative stress-related cell death, and effectively increasing pancreatic enzymes⁷². However, the advance evidence revealed that, beside ROS, the process during oxygen consumption, like hypoxia conditions for pancreatic acinar cells, also triggers multiple signaling such as NLRP3 and composes the cellular mechanism of XO activation⁵³.

It has been well established that the XO molecule, functioning as a crucial redox response component, has the potential to cause harm to tissues and cells through oxidative stress. ROS are byproducts of aerobic metabolism. Under normal conditions, ROS are safely neutralized by the antioxidant defense system. However, when ROS production exceeds the capacity of the antioxidant defense system, the uncontained ROS cause cellular injury and dysfunction by attacking biomolecules, modulating redox-sensitive signal transduction pathways and transcription factors^{73,74}. The generation of oxygen radicals may occur through the action of XO that exists in the epithelial cells of pancreatic tissues. Claus Niederau⁷⁵ observed that an in vitro model exposed to exogenous XO (20 mU/mL) induces iron-catalyzed (50 μ M FeCl₃) reactions on hypoxanthine (500 μ M), leading to time-dependent damage in freshly isolated rat pancreatic acinar cells. The main cytotoxic oxidant species produced in this process is H₂O₂. Hence, the process by which XDH is converted to XO produces massive OFRs, which serve as a significant damaging resource. Neutralization of OFRs significantly ameliorated pancreatic injury caused by free fatty acid, pancreatic duct obstruction, and ischemia⁷⁶. During AP, multiple pathologies could trigger XDH convert to XO like ischemia⁵¹. It is worth to note that, OFRs produced by exogenous hypoxanthine/SOD alone does not induce the typical enzymatic and morphologic changes of AP, this is more like an aggregating factor rather than initial pathology⁷⁷. Oxygen radicals could induce tissue damage via lipid peroxidation during AP⁷⁸. Mechanisms of oxidative stress and redox status participating systematically in AP including glutathione depletion, xanthine oxidase activation, and thiol oxidation in proteins⁷⁹. Additionally, these alterations were partially influenced by the calcium excess established by oxygen radicals⁸⁰.

SAP patients generally suffered from deterioration of oxygen supply and consumption⁸¹. Hyperbaric oxygen could improve pancreatic micro circulation⁸² and inflammation⁸³, and could ameliorate pancreatic duct ligation-induced AP rats by inducing caspase-dependent apoptosis⁸⁴. In a dose-dependent way, the hypoxanthine-related oxidative system has been shown to activate trypsin

in pancreatic acinar cells of rats⁸⁵. During oxidative stress, the excessive ROS lead to hypoxia condition, which directly up-regulates HIF-1 α , and activated HIF-1 α subsequently induces multiple inflammatory signaling including NLRP3 inflammasome⁸⁶, and triggers glycolytic reprogramming by increasing LDHA⁵³. Thus, XDH activation-induced hypoxia and newly generated ROS could accelerate pancreatic injury by these inflammatory response and metabolic disorder during AP.

Apart from the oxidative stress or hypoxia that mediated by the conversion of XO, the metabolism disorder that triggered via XO activation also participates in AP. Another important role of XO in pancreatic function responding to purine metabolism. XOR is known as the rate-limiting enzyme in purine catabolism. It can also metabolize xenobiotics, including some anticancer compounds, which make XOR an important enzyme in inflammatory diseases. Nordback^{51,61} reported that, XO may participate in acute alcoholic pancreatitis by disturbing ethanol metabolism that triggers oxidizing acetaldehyde, the first product of and a substrate of XO.

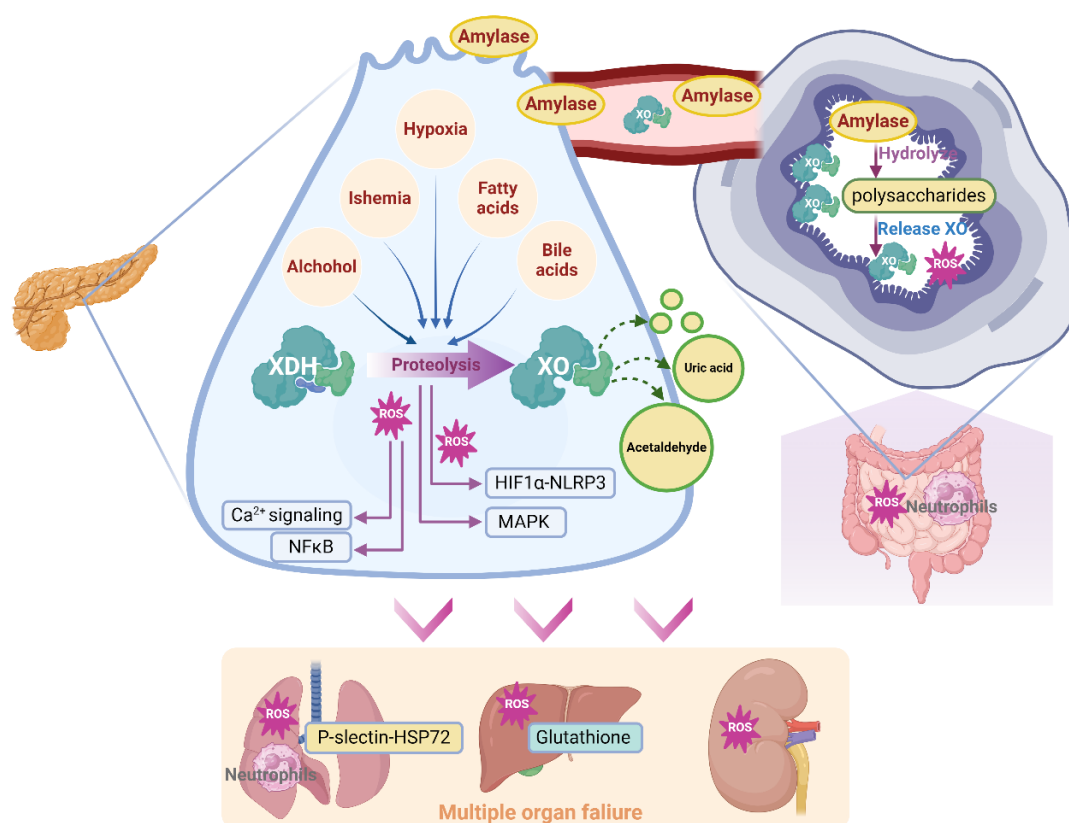


Figure 3. Schematic diagram of XO-mediated cellular mechanism during AP.

3.4. Effect of XO in AP-Associated Multiple Organ Failure

XO could be generated extracellularly as emission of damaged cells and into circulation⁸⁷. Thus, XO has been reported to participate in systemic pathology by ROS generating during AP progression^{79,88}. Due to the significant higher levels of antioxidants in mild AP patients than SAP patients, oxidative stress plays an important role in SAP rather than mild AP⁸⁹. Notably, since XO is the major source of circulating ROS, it may leave significant effect in the systematic influences of AP⁹⁰. ROS is an crucial pathology related to the onset of multiple organ failure in SAP⁹¹. ROS play an important role in ischemia-reperfusion injury, sepsis, acute respiratory distress, and multiple organ dysfunction syndrom⁹², by multiple mechanisms including affecting the rate of apoptosis in tissue and endothelial cells of varies organs⁹³. Additionally, oxidative stress immediately triggers ferroptosis via multiple mechanism like deubiquitinating and stabilizing Sirt3⁹⁴. And it has been widely accepted that ferroptosis participates in AP⁹⁵ through exacerbating lipid peroxidation⁹⁶.

The XO-mediated oxidative stress contributes to the multiple organ failure associated with SAP, and lung injury is the most common organ that be attacked during AP progression. A system

conducted by XO infusion with a dosage of 4 U/h and rate of 0.5 mL/h/100g body weight over a catheter inserted in the abdominal aorta, along with 10 mM hypoxanthine intravenous infusion, was previously reported to significantly recruit polymorphonuclear leukocytes in pancreas after 3 h, which may be attributed to the effect of oxygen radicals in releasing platelet activating factor and leukocyte B4⁹⁷. Correspondingly, inhibition of XO with 10 mM oxypurinol infusion significantly decreased SAP associated lung injury and P-selectin expression of lung, suggested that free radicals released by XO actions as mediators inducing lung P-selectin, which mediate neutrophil infiltration and organ inflammatory injury⁹⁸. Further evidence suggested that circulating XO might induce, in part, the synthesis of platelet activating factor, and subsequently mediate lung neutrophil infiltration by combination with P-selectin, subsequently triggered inflammatory injury and resulted in AP associated lung injury⁹⁹. Among polymorphonuclear leukocytes, neutrophil infiltration is a main group mediating lung injury during AP. XO-mediated free oxygen radicals participated in neutrophil recruitment in AP⁹⁸. Inhibition of XO by 10 mM oxypurinol decreased NaT-induced pancreatitis in SAP rats, as well as lung HSP72 protein expression, which was mediated by neutrophil infiltration in early stage of SAP¹⁰⁰. During physiological conditions, XDH/XO is bound to the polysaccharide chains of heparin-like proteoglycans, which are located on the cell membrane of endothelial cells of gastrointestinal system. Heparin could aggravate AP-induced lung injury by inhibiting the binding between XO and endothelial cells, which produces a free radical-generating system that induces an inflammatory response in the lungs⁴⁹. Susana Granell⁹⁰ reported that, during AP, massive amylase has been released in to circulative system, due to its ability to hydrolyze the internal α -1,4 linkages of polysaccharides, which may disrupt the binding-site of XDH/XOD and release XDH/XOD from endothelial cell surface in gastrointestinal system, amylase absorbed from ascitic fluid was involved in XO mobilization during AP. On the other hand, AP associated lung inflammation occurred due to the activation of proteolytic enzymes, which mainly contributed to the XDH-XO conversion and following free radical-dependent oxidative damage. During the early stage of AP, pulmonary dysfunction had generally occurred due to the high sensitivity of lung endothelium, and this process was at least partly mediated by XO-dependent free radicals. Notably, the oxygen in alveolar space is involved in XO-mediated lung inflammation, which use XO as substrate¹⁰¹.

Other organs including liver and kidney have also been associated with XO. Recent findings indicate that in L-Arginine-induced SAP model, the MDA activity significantly increased in pancreas and kidney. This improvement was notably achieved by administering 200 mg/kg of allopurinol. Furthermore, the oxidative stress in the kidney showed nearly the same level of evidence as in the pancreas, without any pathological changes. The pancreas and kidney exhibited a clear reduction in endogenous scavengers, while the liver showed a marginal decrease⁵⁵. Under the NaT-induced SAP paradigm, pancreatitis occurs simultaneously with the rise in XO activity in plasma and the reduction in glutathione levels in both plasma and liver⁵⁰. Collectively, the lung is the most likely affected organ during AP by XO-mediated injury, mechanisms involving ROS and circulative immune response. In case of liver and kidney, XO seems to injure these organs by accelerating local and systematic ROS. The effect of XO on other organs during SAP remains further research.

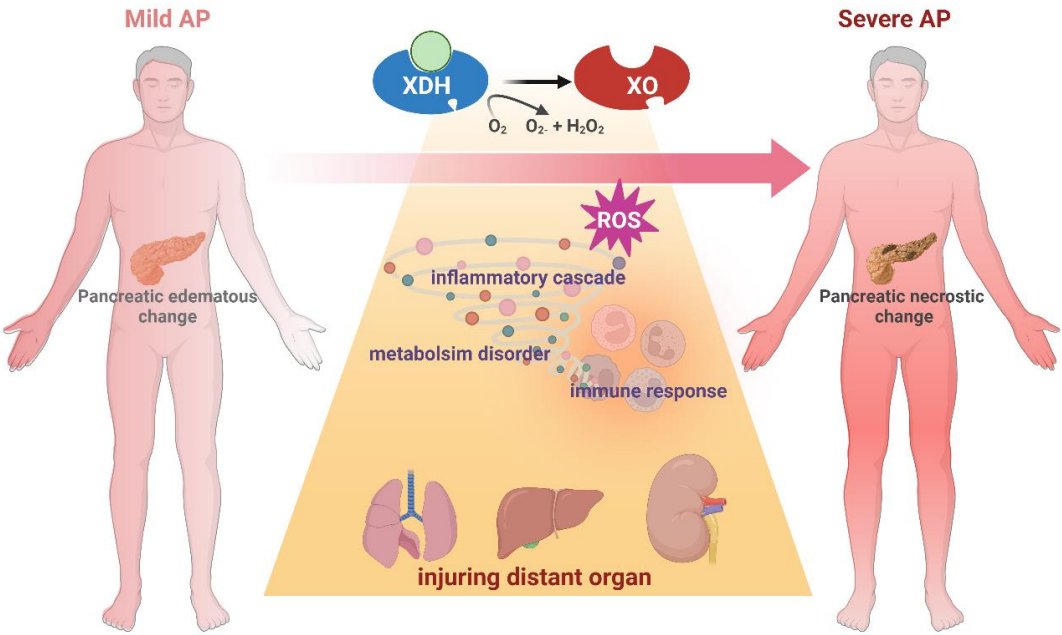


Figure 4. Schematic diagram of XO-mediated acceleration from AP to SAP.

4. Clinical Trials of XO Inhibitors in AP Patients

The clinical administration of XO inhibitors focused on ERCP-induced AP (Table 3). According to meta-analysis, prophylactic use of allopurinol failed to prevent ERCP-induced acute pancreatitis^{102,103}. Oral administration of 40, 200, or 300 mg of allopurinol 1-15 h prior to ERCP exhibited non beneficial effect on the incidence or severity of post-ERCP pancreatitis¹⁰⁴⁻¹⁰⁸. On the contrary, high-dose of 600 mg orally administration of allopurinol could protect ERCP-induced clinical AP, including promoting pancreatic injury and inhibiting incidences of complications¹⁰⁹. And another RCT using 300 mg showed protective effect on the incidences of hyperamylasemia and pancreatitis in patients submitted to high-risk procedures¹¹⁰. Thus, the diverse severity and etiology of AP, the dose and time window of drug administration, all contribute to the clinical outcomes of RCT. It cannot be concluded that if XO inhibition emerges beneficial effects on ERCP or other etiology-induced AP patients, more RCTs are needed in this field.

Table 3. Randomized placebo-controlled trials of allopurinol for acute pancreatitis.

Year	Sample size	Study design	Intervention	Clinical outcome	Ref
2001	300	RCT	Allopurinol, 40 mg, 15 h and 3 h before ERCP or prednisone, 200 mg, 15 h and 3 h before ERCP vs placebo, 15 h and 3 h before ERCP	Neither prednisone nor allopurinol showed a beneficial influence on the incidence and severity of post-ERCP pancreatitis	104
2005	243	RCT	Allopurinol, 600 mg, 15 h and 3 h before ERCP vs placebo, 15 h and 3 h before ERCP	Pretreatment with high-dose, orally administered allopurinol decreases the frequency of post-ERCP pancreatitis	109
2005	701	RCT	Allopurinol, 600 and 300 mg, 4 h and 1 h before ERCP vs placebo	Prophylactic oral allopurinol did not reduce the frequency or the severity of post-ERCP pancreatitis	105
2008	586	RCT	Allopurinol,300 mg, 1 h before ERCP vs placebo	Allopurinol does not appear to reduce the overall risk of post-ERCP pancreatitis	108

2009	170	RCT	Allopurinol, 300 mg, 15 h and 3 h before ERCP vs placebo	Oral allopurinol before ERCP decreased the incidences of hyperamylasemia and pancreatitis in patients submitted to high-risk procedures	110
2011	74	RCT	Allopurinol,300 mg, 3 h and just before ERCP vs placebo	Allopurinol does not reduce the occurrence and amylase rise of post ERCP pancreatitis	107

RCT: Randomized controlled trial. ERCP: Endoscopic retrograde cholangiopancreatography.

5. Limitations and Perspectives

Elevated evidence has revealed that XO functioned as an enhancer during the progression of SAP. Multiple risk factors during AP can activate XO including fatty acids, ischemia, hypoxia, alcohol and bile acids. These factors trigger XO conversion to the oxidative state, companied by massive ROS generation, which accelerate local injury. It has been concluded that, therapeutically administration of relative lower dose of XO inhibitors like allopurinol shows beneficial effect on severe AP models instead of mild cases. However, the regulatory variables in the diverse etiology and time course during AP progression, and the upstream of XO remain still unclear in terms of processes. Due to the lack of genetic tools, parallel comparison between diverse models regarding role of XO in pancreatic acinar cells and systematic circulation, the application of natural products that inhibiting XO, as well as the inconsistence in drug administration methods, the current research remains to resolve the question that how XO functions as in inflammatory response, immune activation, metabolism disturbance or other mechanisms. In this case, the future experimental should explore the cellular mechanisms of XO and the therapeutic effects of inhibitors with suitable dose using severe AP models. Regarding to clinical research, the current studies merely focused on the ERCP-induced AP, which probably failed to integrate the significant enhancement of XO in severe cases. Future studies with various etiology and large sample size are needed to testify the effectiveness of XO as a promising target in AP treatment.

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